Contract report for the Horticultural Development Council

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

PC 186a

December 2006

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* 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 three years. 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

- This project has shown that a very low inoculum of *Verticillium albo-atrum* can result in infection of tomato seedlings, that above- ground movement of the fungus is possible and that there can be many symptomless infected plants in a crop at the end of a season.
- Root drench treatment with carbendazim (eg Delsene 50 Flo or Cleancrop Curve) reduced symptom development in infected plants, including plants which already showed symptoms at the time of application and Unifect G (glutaraldehyde + QAC) was the most effective disinfectant in preventing growth of *V. albo-atrum* mycelium when tested in the presence of peat and soil.

Background and expected deliverables

HDC project PC 186 (O'Neill, 2002) forewarned UK growers of a new form of verticillium wilt that is a potentially 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 in to 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 disease becomes more widespread and damaging.

Key points from the 2002 review were:

- The causal fungus is slow-growing and not always easy to detect 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*).
- Growers estimated yield losses at 10 - 15%.
- The disease is difficult to eradicate from a nursery; it tends to occur in the same glasshouses each year.

- Sources of the disease and how it spreads are unknown.
- 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 best methods of disease control before the disease becomes very widespread. The associated Defra-funded project (HH3222SPC) investigated detection, molecular characterisation and natural suppression of *V. albo-atrum* in tomato (O'Neill, 2006).

Summary of the project and main conclusions

Literature review

The fungus *Verticillium albo-atrum*, its life cycle, the infection process, host resistance, disease epidemiology and diagnosis have been reviewed (Year 1 report, November 2004).

Symptoms and yield loss

The new verticillium wilt disease affecting resistant (*Ve*) varieties of tomato is essentially a 'slow wilt'. Symptoms differ from those of classic verticillium wilt. Thin stems, stunted growth and reduced leaf size are the most common manifestations of the problem; leaf wilting at this stage is rare. As the disease progresses, wilting may develop, and eventually, but not always, the plant may die. At this late stage the pathogen may sporulate on the stem. Grower estimates suggest a yield loss of 10-15% where wilting is obvious in a rockwool crop, with occasional dead plants; and 20-25% where plant death is widespread by September. The problem has been confirmed in rockwool, NFT and soil-grown crops.

Varieties affected

Many resistant (*Ve*+) varieties have been affected including Carousel, Cloe, Conchita, Eloise, Encore, Espero, Ferrari, Romalina, Rosa, Sarena and Solairo. Both Espero and Carousel grafted onto Beaufort and Maxifort rootstocks were found to be infected after

10 weeks but apart from slight vascular discolouration the scions did not show symptoms (i.e. these rootstocks are susceptible to *Verticillium* although in the presence of the pathogen they may offer advantages over ungrafted plants because of enhanced root vigour).

Causal fungus

The *Verticillium* species isolated most consistently from wilt-affected plants was *V. albo-atrum*. *V. dahliae, V. nigrescens* and *V. tricorpus* were isolated occasionally in recent outbreaks (isolate identity confirmed by DNA sequence analysis by D Barbara, Warwick HRI). Previous inoculation studies (O'Neill, 2002a) confirmed that *V. albo-atrum* isolated in 1999 from cv. Espero (*Ve*+) in the UK was equally damaging to susceptible (cv. Shirley) and resistant plants (cv. Trio and a numbered variety).

Occurrence of *Verticillium* in commercial crops

In October 2005 at the end of cropping, the incidence of *Verticillium* was determined in the stem bases of 14 crops. *Verticillium* was confirmed in 12 crops with up to 89% of stems infected. The mean incidence of stem infection was no greater in crops where verticillium wilt was considered to be present (7) than in crops where there were no symptoms of the disease (7). Affected plants were found in all counties where crops were examined (Isle of Wight, Kent, Lancs, Norfolk, Yorks), and in crops grown in the soil, on rockwool slabs and in NFT. These results suggest that infection of tomato stem bases by *Verticillium* is relatively common at the end of cropping, and that infection is often symptomless.

In November 2006 at the end of the growing season, the distribution of infected plants was examined in 10 adjacent rows of a crop grown on rockwool slabs. There were no obvious symptoms of verticillium wilt in the crop. *Verticillium* was found in the stem base of 45% of 120 plants. The number of infected plants on either side of the double rows of plants were similar (28/60 and 26/60), with no clear evidence of along-the-row spread. In only two of the 30 slabs were all four plants infected (there were two plants per propagation block and two blocks per rockwool slab). A further seven slabs had three positive, eight had two positive, none had one positive and four had no positives. These figures indicate that movement of *Verticillium* within a slab is not guaranteed even when there is more that one plant infected. Comparing the two plants in the same propagation

block (where roots are presumably intermingled), 12 out of 60 blocks had both plants positive, but 30 had only one and a further 18 had neither infected. These results strongly suggest that both plants did not become infected when one of them became infected. Possible explanations for this failure of both plants in a block to be infected (where one is infected) are a low inoculum, different times of infection or a means of spread other than through the roots. If there is a means of spread above the root zone, possible methods are by airborne spores, insects and through handling plants.

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

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.

A molecular method based on PCR (Polymerase Chain Reaction) for the specific and sensitive detection of *V. albo-atrum*, developed in a parallel Defra-funded project (HH3222SPC), was used to test various potential sources of the fungus. The detection limit of the PCR test is around 1,000 spores. The fungus was not detected on tomato seed, in rockwool taken from slabs, insects on sticky traps, or on drip pegs, all collected from an affected crop. Samples of crop debris, volunteer tomato seedlings, fallen leaves and fruit, and pathway sweepings were also negative. Further testing of samples (e.g. seed) is considered warranted when a detection method with a lower sensitivity is developed.

An inoculation experiment did not support the hypothesis that infected seed are a source of verticillium wilt in tomato. Tomato seeds inoculated with conidia of *V. albo-atrum* germinated normally and, when grown on for 4 weeks, no symptoms of verticillium wilt developed. However, further seed testing is needed before a firm conclusion can be drawn regarding seed transmission of *V. albo-atrum* in tomato.

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 *V. albo-atrum* spores in tomato crops. Nevertheless, there appears to be potential for above-ground spread. Stem

lesions bearing sporulating *V. albo-atrum* sometimes develop on tomato plants that have 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, and on tomato debris in soil. 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 onto Vaseline-coated slides, the Vaseline removed and tested for *V. albo-atrum* by PCR. *V. albo-atrum* was detected in the 2003/04 and 2004/05 crops (Table 1). *V. albo-atrum* was not detected in 2005/06 even though the end-of-season level of *Verticillium* in the crop was very similar to those of the earlier years. In 2003/04, the fungus was detected before symptoms were seen in the crop. These results indicate that above-ground spread of *V. albo-atrum* may account for outbreaks of the disease and spread within a glasshouse crop. The fungus was not detected in the air on a nursery in Norfolk where spore trapping was undertaken from March to May 2005 following occurrence of verticillium wilt symptoms in a few plants in February.

Table 1: Detection of *V. albo-atrum* in Vaseline on spore trap slides at monthly intervals in tomato crops on a nursery in Kent over three successive seasons.

	V. albo-atrum detected (+):		
Month	2003/04	2004/05	2005/06
December	+	-	-
January	+	-	-
February	+	+	-
March	-	-	-
April	-	-	-
May	+	-	-
June	+	+	-

July	+	-	-
August	-	+	-
September	-	-	-
October	-	-	NT
November	+	NT	NT

+ Vaa detected, - Samples tested and no *V. albo-atrum* found; NT – not tested
Each year the old crop was removed in early November and the new crop was planted in December.

Detection and monitoring of *V. albo-atrum* in commercial crops

Damp incubation and microscopic examination of leaf petioles slices was found to be a useful method for detection of *Verticillium* in tomatoes without destroying the plant. This method was used to examine the occurrence of the disease within commercial crops. In 2004, in a crop of cv. Encore where a few plants at a row end were visibly affected by verticillium wilt, 15% of apparently 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.

On a nursery in Kent with a history of verticillium wilt, rockwool crops were monitored for the disease at intervals throughout 2004, 2005 and 2006. Wilting was first seen in the crops, at a very low level, at the end of January 2004, and *Verticillium* was confirmed. There was little visual affect of the disease for most of the season, although the plants thinned in the head in midsummer. The incidence of infected plants of cv. Encore, determined by testing leaf petioles, increased from 6.5% in March to 21.2% in October. When stem bases were tested at crop pull-out in late October, 65% of stems were found to be infected. In 2005, the incidence of *Verticillium* in petioles of a crop of cv. Encore at this site increased from 1.8% in March to 50% by early August; 92% of stem bases were infected by crop pull-out on 15 October; verticillium wilt was not obvious in the crop. In 2006, the incidence of *Verticillium* in petioles of a crop of cv. Encore at this site was 22% in early September; 59% of stem bases were infected at crop pull-out in early November. Again, verticillium wilt was not obvious in the crop.

Inoculum level and plant age

An inoculum level of just 100 spores applied as a drench to tomato seedlings at two, four and six true leaves growth stages, resulted in development of wilt symptoms after around 5 weeks irrespective of seedling age. Seedlings inoculated with a higher inoculum of 1,000,000 conidia developed wilt symptoms after 4 weeks. Not all

inoculated plants had developed symptoms by 10 weeks after inoculation. The severity of symptoms increased with spore concentration from 100 to 1,000,000 spores/plant.

Detection of *V. albo-atrum* within stems following root and leaf scar inoculation

Ten weeks after inoculation of plants by drenching spores into the root zone, *V. albo-atrum* was confirmed by a specific PCR test in vascular tissue at the stem base and 50 cm above the stem base. Using this method, *V. albo-atrum* DNA (either mycelium or spores) was detected in plants inoculated with 10^6 spores/ml but not with the lower inoculum levels even though these treatments showed wilt symptoms.

Following inoculation of *V. albo-atrum* spores onto fresh leaf scars of tomato seedlings, the fungus was detected by PCR in surface sterilised stem tissue 1 cm above and 1 cm below the inoculation point. This result indicates *V. albo-atrum* is present within the stem to this extent.

Effect of plant growth and environmental factors on symptom expression

Measures which reduce water stress on a tomato crop appear to slow the development of verticillium wilt, and those which increase water stress enhance development of the disease. In the Netherlands, for example, removal of some fruit from trusses is suggested as a control measure. In chrysanthemum, verticillium wilt symptom expression is affected by growth stage and tends to show as plants come into flower. In field-grown tomatoes, verticillium wilt is reported to be worse when root growth is restricted (eg by a soil pan). A series of experiments was therefore undertaken to investigate the effect of various plant growth and environment factors on symptom expression of tomato wilt caused by *V. albo-atrum*.

Experiments on tomatoes grown in peat bags indicated that the severity of verticillium wilt symptoms is affected by fruit load and the extent of sideshoot growth. Wilt severity was reduced by reducing the fruit load and by regular removal of sideshoots.

Following drench inoculation with a standard inoculum of *V. albo-atrum* spores, plants grown in compost that was kept moist developed verticillium wilt symptoms whereas plants grown dry did not. Possibly this was due to death of conidia in the plants watered infrequently and allowed to dry out. A subsequent experiment on the effect of air-drying

on spore viability showed that wetted conidia of *V. albo-atrum* survived drying for 6h but not for 24h. Previous studies indicated that conidia of *V. dahliae* are capable of surviving for up to 2 weeks in soil (Green, 1969).

Eight weeks after inoculation, pot-grown plants simultaneously inoculated with *Phytophthora cryptogea,* a pathogen which reduces root function, and *V. albo-atrum,* were significantly reduced in height, by 14-20 cm, compared with plants inoculated with either fungus alone.

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. No consistent effect was observed.

Effect of rootstock on wilt development

The basis of resistance to *Verticillium* (the *Ve* gene) is the same in rootstocks as in F1 varieties. However, expression of resistance in rootstocks compared with F1 varieties may be affected by modifier genes, the genetic background or the greater vigour of rootstocks. The susceptibility of grafted and ungrafted plants was compared by drench inoculation of *V. albo-atrum* spores onto the roots of potted plants. No verticillium wilt symptoms had developed after 6 weeks. Surprisingly, a significantly greater incidence of stem base infection was detected in grafted than in ungrafted plants. Only a very low incidence of *Verticillium* infection was detected in stems of both grafted and ungrafted plants at 10 cm or more above the stem base. No conclusions on the effect of grafting onto a rootstock on *V. albo-atrum* in stems could be drawn because of the lack of symptoms and the low incidence of stem infection above the base.

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 (Fig 1). 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.

In a further experiment on cv. Shirley, the efficacy of carbendazim was evaluated as a curative treatment. Delsene 50 Flo applied as a drench significantly reduced symptom development in infected plants, including plants already showing symptoms at the time of application (Table 2).

Five isolates of *V. albo-atrum* obtained from tomato between 2000 and 2005 were tested for their sensitivity to carbendazim (as Delsene 50 Flo) in agar plate tests. Mycelial growth of all isolates was completely inhibited at concentrations of 2 mg/L or greater (i.e. there was no evidence of fungicide resistance).

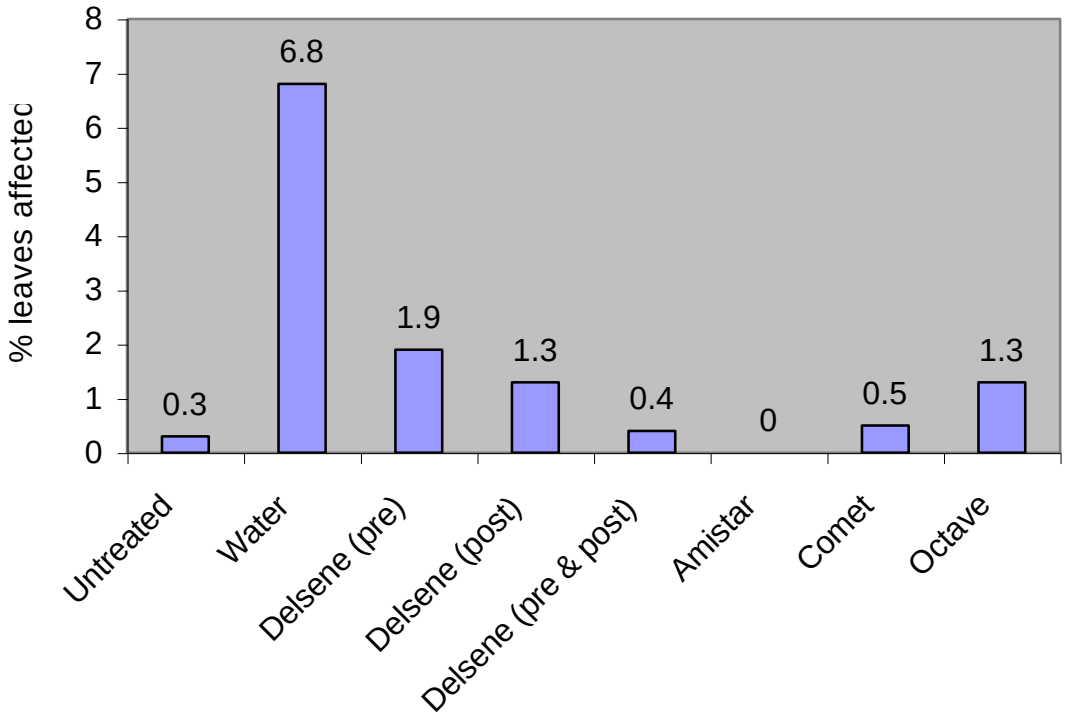


Fig 1. Effect of fungicide drench treatments on the percentage of leaves affected with verticillium wilt

Chemical disinfectants

Six disinfectants (Harvest Wash, Jet 5, sodium hypochlorite, Trigene, Unifect G and Virkon S) were all fully effective against conidia of *V. albo-atrum* when used at their

standard rate and with a contact time of 5 minutes or greater. Citric acid had no effect even after 8 hours.

When tested against *V. albo-atrum* mycelium grown on filter paper discs, only Panacide M and Unifect G eliminated the fungus following a 5 minutes contact time. Sodium hypochlorite was effective after 30 mins, Trigene after 1 hr and Jet 5 and Virkon S after 8 hours. Citric acid and Harvest Wash were ineffective. Unifect G was the most effective disinfectant at preventing growth of *V. albo-atrum* mycelium in the presence of peat and soil.

Table 2: Effect of fungicide treatment after inoculation with V*. albo-atrum* on the development of verticillium wilt symptoms.

	Inoculation with *V. albo-atrum* (15 Feb & 11 Apr)	Symptoms present on 5 May	Delsene 50 drench Applied	% plants affected 5 May	% plants affected 25 May
1.	-	-	-	0	0
2.	-	-	√	0	0
3.	√	√	-	23	62
4.	√	√	√	33	43
5.	√	-	-	0	31
6.	√	-	√	0	17

Contamination and disinfection of hands

Spores of *V. albo-atrum* were found to be surprisingly persistent on hands and were relatively difficult to remove by washing. Once contaminated by touching a sporulating stem lesion, a thumb was still transmitting *V. albo-atrum* after 160 sequential contacts with an agar plate. Even after washing with warm water and soap for 30 seconds, transmission of *V. albo-atrum* occurred for over 50 sequential contacts.

Bare hands were effectively cleaned of *V. albo-atrum* by rubbing with Med Gel for 1 minute; washing in warm water and soap for 1 minute was not effective. Latex gloves

also transmitted *V. albo-atrum* but were easier to clean; both Med Gel (1 minute) and warm water and soap (1 minute) were effective.

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

- Become familiar with the symptoms of 'slow wilt' caused by *V. albo-atrum* in resistant (*Ve*+) varieties. These are illustrated and described in HDC Factsheet 15/01.

- If you suspect plants are affected by verticillium wilt, have affected plants examined by a plant pathologist or tested at a diagnostic laboratory. The incidence of verticillium in a crop can be assessed in a non-destructive manner by tests on leaf petioles.

- Carefully and promptly remove severely wilted or dead plants that are infected by *V. albo-atrum*. Sporulation of *V. albo-atrum* can occur on stem lesions of dying plants late in the season, and there is evidence of aerial dissemination of these spores. Possibly sporulation may also occur on leaf debris, earlier in the season. Although no sporulation of *Verticillium* was found on leaves in affected crops we examined, abundant sporulation developed when affected leaves were incubated in a humid environment.

- Do not re-use rockwool slabs from an affected crop, either for tomato or another verticillium-susceptible crop.

- Do not handle stem lesions. After touching a sporulating *Verticillium* stem lesion, hands will be contaminated with spores of the fungus and they could spread the disease (e.g. via fresh de-leafing scars) to healthy plants

- Hands or latex gloves contaminated with *V. albo-atrum* spores can be disinfected by rubbing in Med Gel for 1 minute.

- Keep up with sideshoot trimming in an infected crop to reduce the risk of verticillium wilt symptom development.

- Apply measures to reduce stress on infected plants, such as use of shade screen in very bright conditions immediately after dull weather, adjusting heating and ventilation, and reducing a very high fruit load.

- Consider treatment with an approved carbendazim fungicide (eg Cleancrop Curve, Delsene 50 Flo) if verticillium wilt is confirmed in a crop. A root drench treatment reduced symptom development in infected plants, including plants already showing symptoms at the time of application.

- Take precautions to minimise the risk of other diseases affecting the crop. Grower observations, and research in the Netherlands, indicate rapid development of wilt and plant death may occur when a crop is affected by both Pepino mosaic virus and verticillium wilt. There is also evidence that severe reductions in growth occur when a plant is affected by both verticillium wilt and *Phytophthora* root rot.

- Clean and disinfect the glasshouse and all associated equipment after an outbreak of the disease. See HDC Factsheet 15/01 for more details.

- The disinfectants Harvest Wash, Jet 5, sodium hypochlorite, Trigene, Unifect G and Virkon S are all fully effective against conidia of *V. albo-atrum* when used at their standard rate and with a contact time of 5 minutes or greater.

- Only Panacide M and Unifect G eliminated *V. albo-atrum* mycelium grown on filter paper discs following a 5 minutes contact time. Sodium hypochlorite was effective after 30 mins. Consider using Unifect G to disinfect areas contaminated with *V. albo-atrum* and soil or organic matter.

SCIENCE SECTION (YEAR 3)

1. Occurrence of *Verticillium* in commercial crops

Introduction

Plants infected by *V. albo-atrum* may show few or no symptoms, or the symptoms may be mistaken for those resulting from infection by *Pythium*, or another root pathogen. In order to gain an appreciation of the extent of *Verticillium* in tomato crops, stem bases were examined at the end of cropping in 2005 from 14 commercial crops selected from the main cropping areas in England.

Methods

Fifty green stem bases were collected in October 2005 from each of 14 crops, seven known to be infected with verticillium wilt (from laboratory tests on plants where the grower was concerned about poor growth) and seven with no record of the disease (no prior crop testing). Ten plants were chosen at random in each of five rows from throughout an area of one variety, and a 20 cm length was cut from close to the stem base. After surface sterilisation, 10 transverse sections were cut from each length of stem, placed on damp filter paper and incubated. Stem sections were examined microscopically for *Verticillium* sporulation after 14 and 21 days.

Results and discussion

Verticillium was confirmed in 12 out of the 14 crops (Table 1.1). The mean incidence of infected stem bases was no greater in crops known to be infected by verticillium wilt (mean 27%; range 0-89%) than in crops with no symptoms (mean 35%; range 0-83%). Affected plants were found in all counties where crops were examined (Isle of Wight, Kent, Lancs, Norfolk, Yorks), and included crops of a wide range of varieties grown in the soil, on rockwool slabs, in NFT, and in rockwool slabs on hanging gutters (Tables 1.2-1.4). Both grafted (Beaufort and Maxifort) rootstocks and own-root plants were infected (Table 1.5). These results indicate *Verticillium* is relatively common in tomato crops at the end of cropping, and much of the infection is symptomless.

The last previous survey for vascular diseases of tomato, conducted by ADAS in 1979, recorded *V. albo-atrum* in 25% of 120 tomato crops, none of which had obvious symptoms of verticillium wilt (Fletcher & Harris, 1979)

Table 1.1. Occurrence of *Verticillium* in tomato stems at the end of cropping - October 2005

Verticillium known to be present in crop?	Number crops examined	Mean % stems infected	Levels of infection (%)
Present	7	26.6	0, 2, 2, 16, 54, 78, 89
No record of the disease	7	34.8	0, 6, 11, 20, 60, 64, 83
Total	14	30.7	

Table 1.2. Occurrence of *Verticillium* in stems (% infected) according to variety

Aranca	Claree	Classy	Conchita	Elegance	Encore	Ingar	Jester
0, 6	0	78	20	83	2, 2, 11, 64, 89	60	16, 54

Table 1.3. Occurrence of *Verticillium* in stems (%infected) according to location

Isle of Wight	Kent	Lancs	Norfolk	Yorks
0, 6	11, 20, 64, 83, 89	0, 2, 16, 54	2	60, 78

Table 1.4. Occurrence of *Verticillium* in stems (% infected) according to growing system

Hanging gutter (Rockwool)	NFT	Rockwool	Soil
78	11, 64	0, 0, 2, 2, 16, 20, 54, 60, 83, 89	6

Table 1.5. Occurrence of *Verticillium* in stems (% infected) according to rootstock

Non-grafted	Beaufort	Maxifort
0, 0, 2, 2, 11, 20, 60, 64, 78, 89	6	16, 54, 83

2. Sources of *V. albo-atrum* on a nursery

Introduction

The transmission of plant pathogenic *Verticillium* spp. via seed is accepted as a well established route of infection. Several experiments have shown that resting structures of plant pathogenic *Verticillium* spp. can persist on seeds for up to a year (Isaac & Heale, 1961). The objective of this study was to determine the ability of *V. albo-atrum* to infect tomato plants via seed-borne infection.

Materials and methods

Growth and maintenance of *V. albo-atrum*

Potato Dextrose Agar (PDA) was used throughout. The media was amended with sterilised antibiotics: penicillin, streptomycin sulphate and chloramphenicol. Mycelium (5 mm^2) was cut from the edges of plates of actively growing cultures of *V. albo-atrum* using a sterile scalpel. They were then removed using a sterile needle, inoculated in the centre of PDA plates and incubated at 20°C for 14-21 d before use.

Production of spore suspension

Conidial suspensions were made by flooding the surface of colonised PDA plates with sterile distilled water (SDW) and dislodging conidia using a sterile inoculation loop. The suspension was then pipetted out and filtered through four layers of sterile muslin to remove hyphal fragments and then centrifuged for 5 min at 3000 rpm. The supernatant was decanted into tubes filled with SDW shaken and centrifuged again for 5 min at 3000 rpm. The concentration of conidia was estimated using a haemocytometer and the suspension was diluted with SDW to reach a final concentration of 10^6 spores mL^{-1} in SDW. The stock spore suspension was then diluted with SDW to make suspensions of 10^2, 10^4 and 10^6 spores mL^{-1}. These were placed in universal tubes for use in the seed assay. If necessary they were refrigerated at 4°C overnight for later use.

Inoculation of tomato seeds

F1 hybrid tomato seeds (*cv.* Espero) were soaked in the spore suspensions for 1 and 10 min. This was done by immersing the five seeds in 1 mL of each of the dilutions of conidial suspension or SDW for 1 or 10 mins (Table 2.1). An additional control treatment (T9) with no immersion in water was included.

Table 2.1: Inoculation of tomato seed with *V. albo-atrum*.

Treatment	Exposure Time (min)	Spore suspension concentration (mL^{-1})
1	1	10^2
2	1	10^4
3	1	10^6
4	10	10^2
5	10	10^4
6	10	10^6
7	1	SDW
8	10	SDW
9	-	-

SDW – Sterile Distilled Water

Incubation and germination

After inoculation the seeds were placed on moist filter paper in a Petri dish. The tomato seeds were incubated at 22°C day and 20°C night in growth rooms with 18 h daylight for 7 d. After 7 d the seeds were scored by visual assessment for the emergence of a coleoptile from the seed coat.

Survival of *V. albo-atrum* on dried tomato seeds

The same procedure was followed as above except that after inoculation the seeds were left to dry completely and then stored in dry, sterile conditions for 7 and 14 d. The seeds were dried by placing on foil overnight in a laminar air flow cabinet. The seeds were stored in sterile universal tubes at room temperature. After 7 d and 14 d the seeds were placed on moist filter paper and incubated using the same procedure. There were 8 treatments including 6 inoculations and 2 controls as before.

Transfer of germinated seedlings

After 7 d the seeds were scored for germination and then transferred into pots to continue growing. The seeds were placed in standard 9 cm pots in a mixture of 6 parts John Innes No. 3, 6 parts Levington M1 compost mixed with 1 part Vermiculite and 1 part Perlite. The pots were placed in trays at random and then placed in the growing room and allowed to grow for 4 weeks.

Visual disease assessment

After 4 weeks each plant was measured for their height. Each plant was also assessed for any yellowing or wilting of leaves and the number of affected leaves was counted. Disease severity was calculated as a percentage using the following formula:

$$\text{Disease severity} = \frac{\text{Number of leaves with symptoms}}{\text{Total number of leaves on the plant}} \times 100$$

Results

Overall more than 80% of seeds in all treatments had germinated after 3-5 d and there was no noticeable treatment effect on the number of seeds germinated (Fig. 2.1).

The incidence of leaf yellowing and wilting was very low for all treatments (Fig. 2.2). Mean disease severity ranged from 2 to 5% and there was no noticeable difference in disease severity between treatments. For the plants germinated 14 d after inoculation, no disease symptoms were observed.

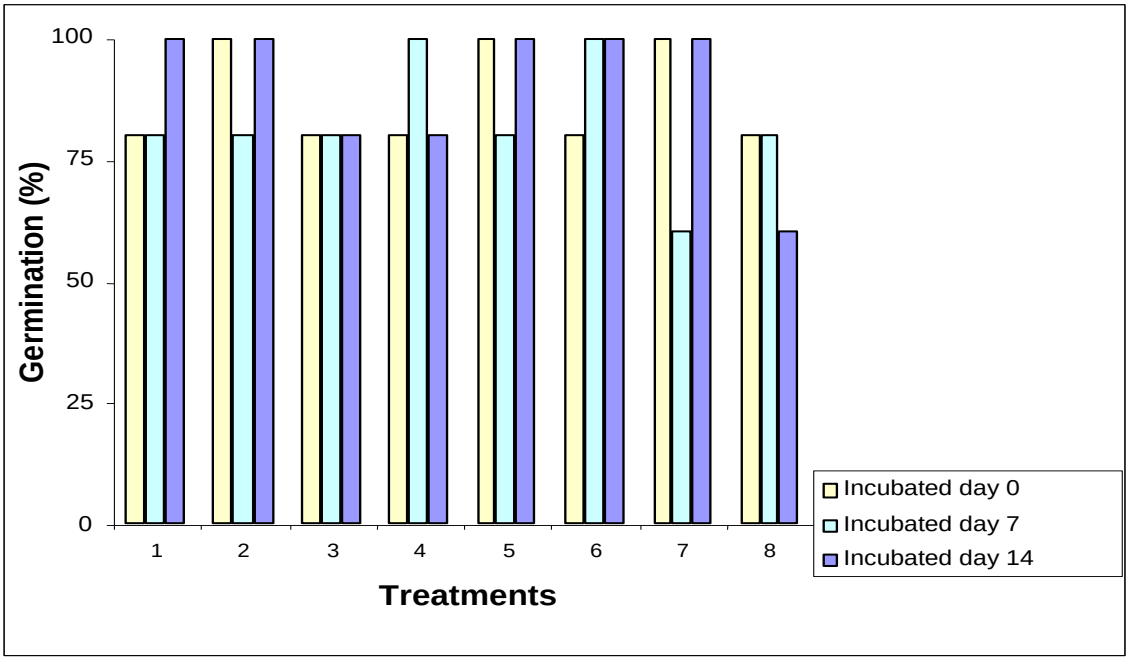


Figure 2.1: Percentage of seeds germinated after inoculation with *V. albo-atrum* . Seeds were placed on moist filter paper to start germination at 0, 7 and 14 days after inoculation.

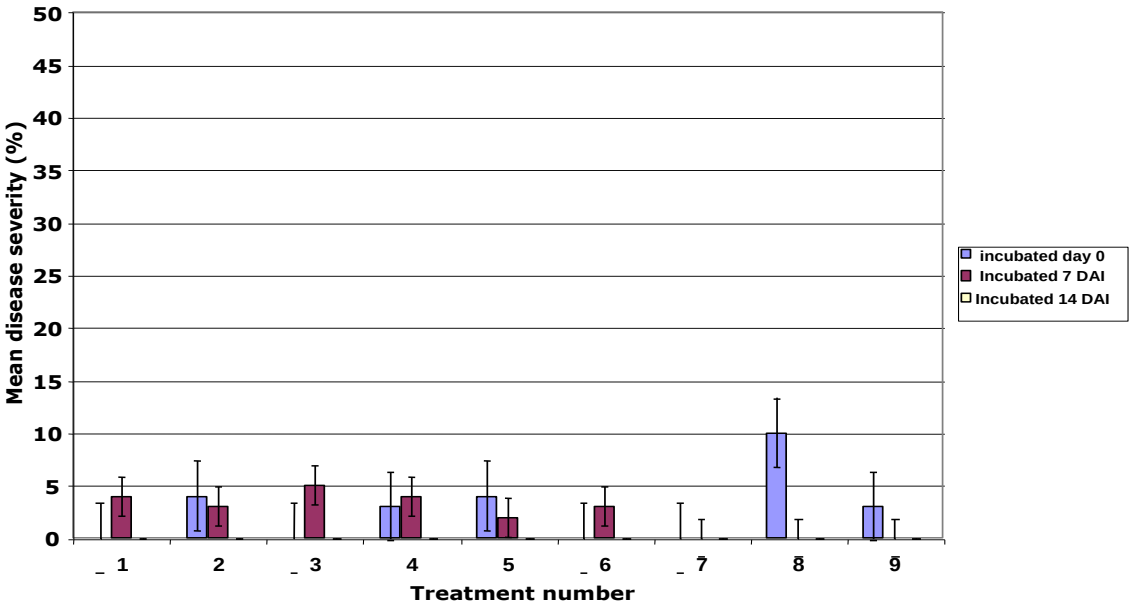


Figure 2.2: Mean disease severity for each treatment and germination time. (Value of standard error bar day 0 = 3.3; day 7 = 1.8; day 14 = 0).

Discussion

This experiment was designed to test the ability of *V. albo-atrum* to cause disease in tomato plants via seed-borne transmission. Inoculation of seeds with *V. albo-atrum* did not affect the germination of the seeds, the average germination percentage for all treatments and times was high (87.3%). The plants that grew from the germinated seeds appeared healthy. A few plants showed possible symptoms of verticillium wilt. However the fact that some of the control plants showed similar symptoms suggests that these symptoms may have been caused by other factors such as environmental stress and not by *V. albo-atrum* infection (plants were not examined for occurrence of *V. albo-atrum* within stems). Other work has shown that resting mycelium of *V. albo-atrum* can persist on seeds for up to 13 months (Isaac & Heale, 1961). The inoculum used in this study was a conidial suspension and not mycelium.

This experiment provides no evidence to support the hypothesis of seed transmission of *V. albo-atrum* in tomato. However, further work is needed to determine whether or not seed transmission of *V. albo-atrum* is important in the epidemiology of tomato verticillium wilt.

In earlier work at Nottingham University in this project, seeds from affected tomato plants were tested for *V. albo-atrum* using a PCR method with a detection limit of around 1,000 spores. The fungus was not detected. The PCR method used conventional PCR primers and SYBR green dye. The dye binds dsDNA and the fluorescence generated by the binding is measured over time to give the quantification. The sensitivity of PCR is determined by the efficiency of the primers in binding DNA and the primers used in our work were not optimal for real-time PCR (the fragment they generated was too large). It should be possible to increase the sensitivity of detection of *V. albo-atrum* by developing different primers and using real-time PCR. If this is done, it would be useful to test seeds from affected tomato plants using a PCR method with a low limit of detection (eg a few spores per seed).

3. Spread of *V. albo-atrum*

3.1 Spore trapping

Introduction

Sporulation of *V. albo-atrum* was demonstrated on colonised tomato debris in soil (Sewell, 1959). It is also 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 above-ground spread of the pathogen within a tomato crop. At present, however, there is no evidence to support this hypothesis. Rapid secondary spread of verticillium wilt in lucerne was reported to occur at cutting by dissemination of *V. albo-atrum* spores produced on infected stems, and by contact of these and transported fragments of diseased tissues with wounded surfaces of recently cut lucerne plants (Huang, 2003). Mechanical scattering of infected mint debris during harvest was suggested as a possible explanation for spread of verticillium wilt across rows in fields of mint (Johnson *et al*., 2006). Possibly de-leafing and other work in tomato crops results in movement of *V. albo*-atrum spores and infected tissues (e.g. leaf debris) that might infect recently created wounds on the stem, or contaminate roots. Conidia of *V. dahliae* are reported to be capable of surviving for at least 2 weeks in soil (Green, 1969). Season-long spore trapping on a tomato nursery with a history of verticillium wilt was undertaken to test for above ground spread of *V. albo-atrum*.

Materials and methods

A Burkard spore trapping machine was installed on a tomato nursery in Kent from November 2003 to September 2006, where it 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 *V. albo*-atrum DNA down to the equivalent of 1,000 spores per glass slide.

Spore trapping was undertaken from November 2003 to October 2004 (year 1), from December 2004 to October 2005 (year 2), and from December 2005 to September 2006 (year 3).

Results

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 3.1 and Table 3.1). 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, *V. albo-atrum* was detected in February, June and August. *V. albo-atrum* was not detected in samples collected in 2006.

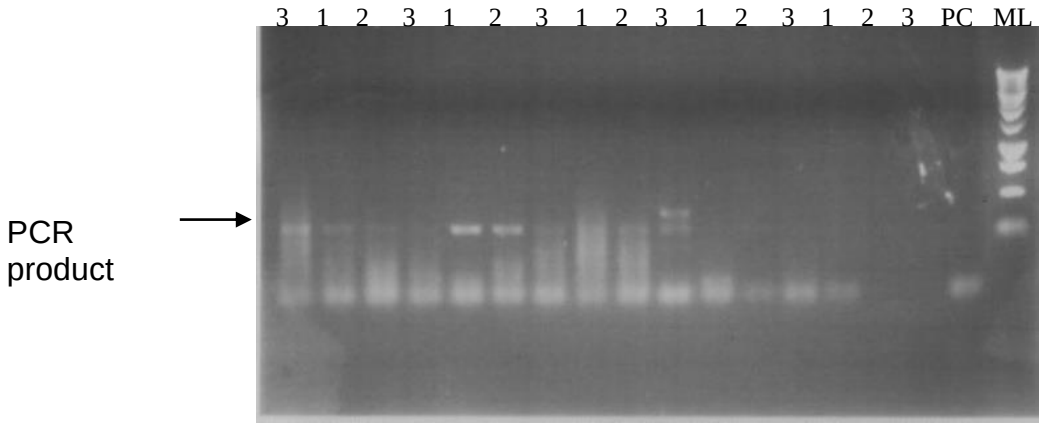


Figure 3.1: PCR results for DNA extracted from air samples collected from November 2003 until March 2004. 1, 2, 3 represent pooled samples collected from dates: 1st-10th, 11th-20th and 21st till end of the month respectively. PC- Primer control, ML- Mass Ladder. A white band opposite the arrow indicates occurrence of *V. albo-atrum* in the sample.

Table 3.1: Detection of *V. albo-atrum* in Vaseline extracts from spore trapping slides on a nursery in Kent.

	V. albo-atrum detected (+):		
Month	2003/04	2004/05	2005/06
December	+	-	-
January	+	-	-
February	+	+	-
March	-	-	-
April	-	-	-
May	+	-	-
June	+	+	-
July	+	-	-
August	-	+	-
September	-	-	-
October	-	-	NT
November	+	NT	NT

Discussion

These results are the first report indicating above ground movement of *V. albo-atrum* in a tomato crop. The origin of *V. albo-atrum* detected on spore traps (presumably as conidia; no insects were observed on the trap slides) 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 in either year. Sporulation of *V. albo-atrum* was shown to occur on affected leaves under humid conditions (in the laboratory) and possibly this may have occurred on leaves, or leaf debris, in the crops where *V. albo-atrum* was detected on the air-sampling spore trap.

3.2 Disease monitoring on nurseries by testing leaf petioles

Introduction

In 2004 and 2005, testing of leaf petioles proved to be an effective non-destructive method for determining occurrence of verticillium wilt in a crop. Further testing was undertaken in 2006. Petiole sections were incubated for 7-14 days and then examined microscopically for verticillate sporulation.

Methods

Samples of 50 lower leaves were collected at random, 1 leaf/plant, from visually healthy plants in glasshouse blocks with a history of verticillium wilt in Kent. The final sample in November consisted of a stem section around 0.5 m above the stem base. Ten transverse sections were examined from each petiole. The crop was ungrafted cv. Encore, grown on rockwool slabs on the floor in a glasshouse (B-block) where the disease had occurred in each of the last three years. The disinfectants Unifect G and Jet 5 were used to wash down between crops. Unifect G was used on concrete surfaces and over the whole floor after polythene and slabs were set out. Irrigation equipment was washed with water, acid and Jet 5.

Results

Plants were received in November 2005 and set out in December. Carbendazim drench treatments were applied to the crop twice, in early March and on 20 April. Verticillium wilt was not obvious on the nursery this year.

Verticillium was confirmed at each sample date, increasing from 4% in April (leaf petiole test) to 59% of plants in November (stem base test) (Table 3.2.1).

Table 3.2.1. Occurrence of Verticillium in leaf petioles and the stem base (November) of a tomato crop in Kent – 2006.

Sample date	% plants affected
April	4
June	10
July	12
September	22
November	59

Discussion

The results confirm those of 2004 and 2005, in that *Verticillium* can be present within a crop at a much greater level than is apparent from visible symptoms of the disease.

Although *Verticillium* was confirmed within plants at a similar level to previous years, no verticillium wilt symptoms developed this year. The absence of a grafted rootstock did not appear to result in an increased incidence of infection.

3.3 Pattern of infection in a crop

Introduction

Information on the location of plants infected by *V. albo-atrum* within a crop may provide clues as to how the disease spreads in a glasshouse. If there are runs of several adjacent plants affected, this suggests spread along a row, for example by root-root contact, in the run-off water or by crop handling. If there are predominantly single plants affected, in many different rows, this distribution supports above-ground spread of the fungus, possibly by movement of *V. albo-atrum* in the air. The distribution of infected plants was therefore examined in a crop known to be infected by the fungus.

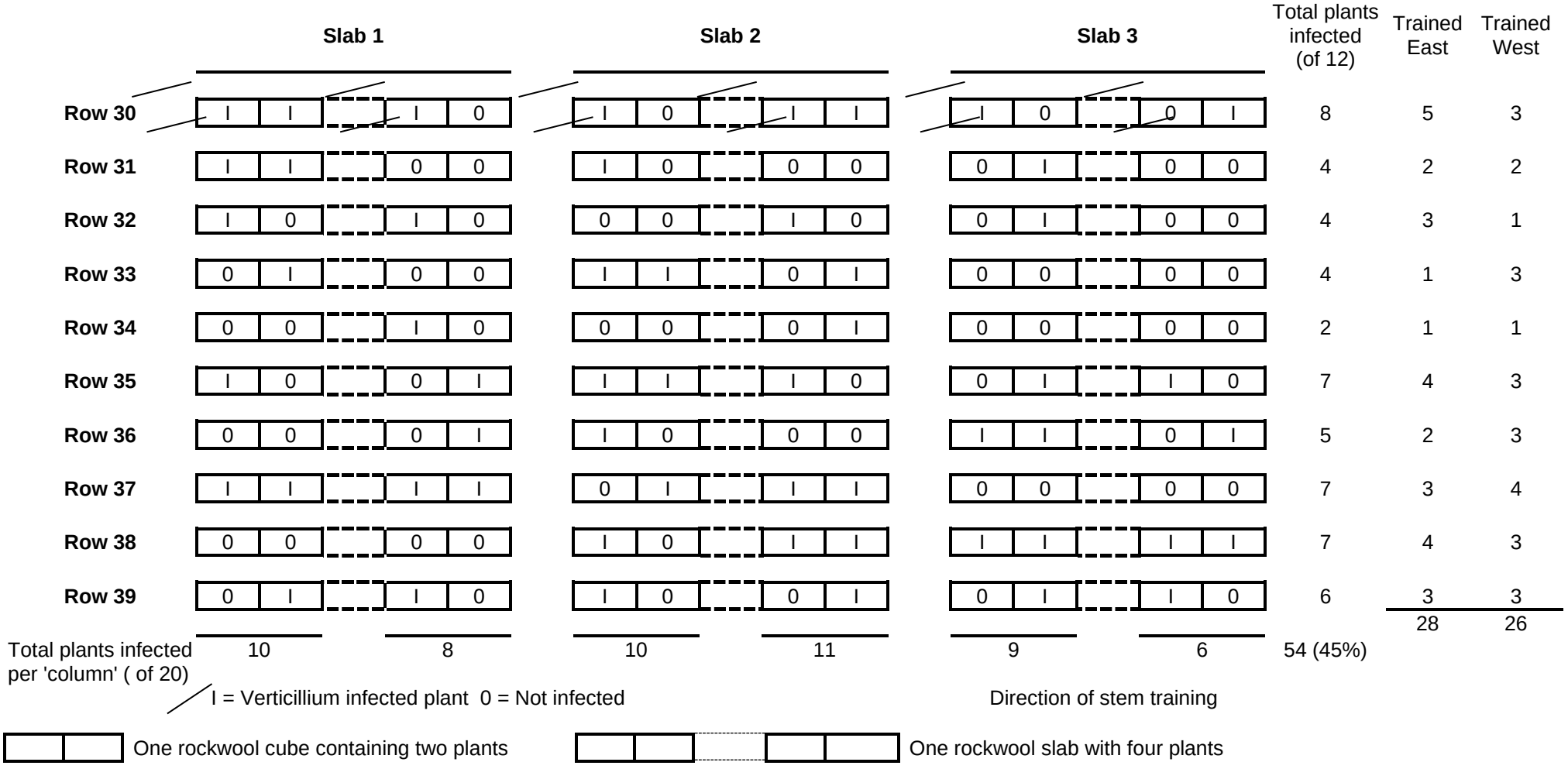
Methods

At the end of cropping in early November 2006, the occurrence of *Verticillium* was determined in 120 stem bases (12 adjacent plants in each of 10 adjacent rows) in an area of a tomato crop where previous testing of leaf petioles had shown *Verticillium* to be present in around 20% of plants. The location of each stem base in the crop was recorded. Plants were grown on rockwool slabs and drainage water was allowed to run to waste. There were two plants per propagation block and two blocks per rockwool slab. Each slab was an isolated entity. The plants on either side of the propagation block were trained in opposite directions (i.e. to form two rows facing onto adjacent pathways). A 30 cm length of stem was taken, starting at 50 cm above plant base. Stem bases were tested for *Verticillium* by damp incubation of 10 transverse sections per stem.

Results and discussion

Verticillium was detected in 45% of stem bases. The pattern of infected plants is shown in Figure 3.2. The number of infected plants on either side of the double rows of plants were similar (28/60 and 26/60), with no clear evidence of along-the-row spread (i.e. no long runs of infected plants, apart from row 30 which had 6 adjacent plants infected). Comparing individual slabs, in only two of the 30 slabs were all four plants infected. A further seven slabs had three positive, eight had two positive, none had one positive and four had no positives. These figures indicate that movement of *Verticillium* within a slab is not guaranteed even when there is more that one plant infected. Comparing the two plants in the same propagation block (where roots are presumably intermingled and share a common rhizosphere), 12 out of 60 blocks had both plants positive, but 30 had only one and a further 18 had neither infected. These results strongly suggest that both plants did not become infected when one of them became infected. Possible explanations for this failure of both plants in a block to be infected where one is infected are a low inoculum, different times of infection or a means of spread other than through the roots. If there is a means of spread above the root zone, possible methods are by airborne spores, by insects or through handling plants.

Figure 3.2: Pattern of infection by *Verticillium* in an area of a rockwool tomato crop – November 2006



4. Effect of daylength on symptom expression

Introduction

The objective of this study was to find out how daylength influences symptom development in verticillium resistant (*Ve*+) tomatoes, and if symptoms differ in short and long daylength conditions. An experiment in 2005 indicated that inoculated plants grown in short days developed symptoms before those of inoculated plants grown in long days. This experiment was conducted in growth rooms at Nottingham University.

Methods

Inoculation

Spore suspensions were prepared from 14-day-old culture of *V. albo-atrum* (isolate AR01/036) grown on PDA at 20-22°C. The concentration was adjusted to 10^6 and 10^4 spores/mL^{-1} and plants were inoculated with water as a control. A further treatment consisted of inoculation with a mixture of three isolates of *V. albo-atrum.* As an additional control, plants were grown with neither SDW nor spores applied. The full treatment list is shown below:

Daylength	Inoculum of *V. albo-atrum* (number conidia per plant)
1. Long	10^4
2. Long	10^6
3. Short	10^4
4. Short	10^6
5. Long	10^6
6. Short	10^6
7. Long	SDW
8. Short	SDW
9. Short	-
10. Long	-

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 growth rooms with an 8-hour or a 16-hour day length and at temperatures of 20°C day and 18°C night.

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. There were five plants per treatment.

Assessment

Yellowing and/or wilting of leaves were taken as symptoms of verticillium wilt. The number of affected leaves on each plant was assessed after 4 weeks. A disease severity score was calculated based on the proportion of leaves on a plant showing symptoms.

Results and discussion

The first symptoms were seen 3 weeks after inoculation on plants grown on long daylength (16 hours) and inoculated with the higher concentration of *V. albo-atrum* conidia. Plants grown on a short daylength showed symptoms after 4 weeks. There was a significant difference between the height of the plants grown in the long daylength and short daylength ($P< 0.001$). Plants were generally taller and healthier when grown on long daylength than on a short daylength. However, there was no significant difference between the heights of the plants inoculated with different inoculum levels (Figure 4.1).

Leaf yellowing was significantly greater ($P<0.001$) in plants grown in long days than in short days (Figure 4.2). However, both sets of control plants grown in the long daylength also showed symptoms suggestive of verticillium wilt; the reason for this is unclear. Possibly the plants had become infected during the experiment; or possibly some leaf yellowing symptoms were not due to verticillium wilt (plants were not tested to determine the occurrence of *Verticillium* within stems). Previously, in 2005, tomato plants grown in short days first showed symptoms at 2-weeks after inoculation, contrary to the results here. Because of these difficulties, it is not possible to say how daylength affects expression of verticillium wilt symptoms in tomato.

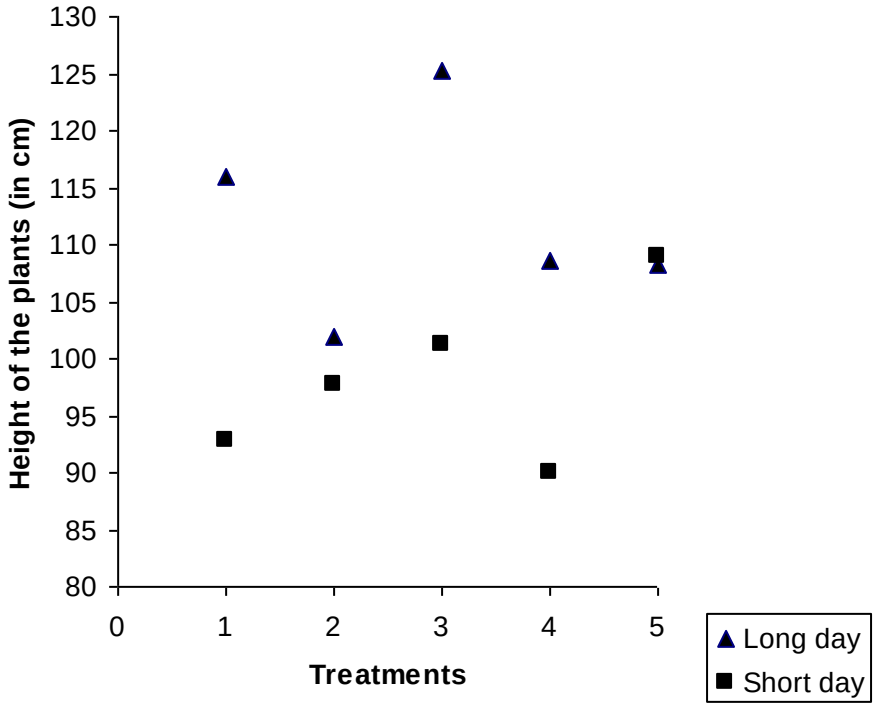


Figure 4.1: Effect of day length on height of the infected tomato plants. Treatment 1 plants infected with *V. albo-atrum* 1x 10^6 spores (ARO1/36); 2 plants infected with *V. albo-atrum* 1x 10^6 mixed isolate spores; 3 plants infected with *V. albo-atrum* 1x 10^4 spores (AR01/36); 4 Plants treated with sterile distilled water; 5 Untreated tomato plants.

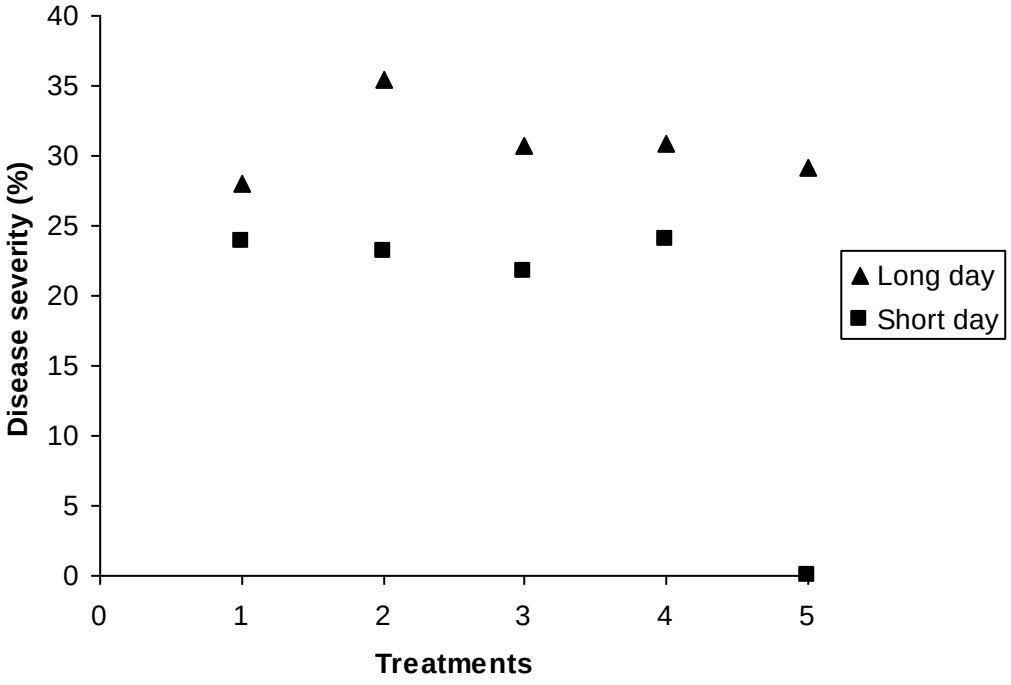


Figure 4.2 Effect of day-length and inoculation treatment on verticillium wilt symptoms development. Treatment; 1 plants infected with *V. albo-atrum* 1x 10^6 spores (ARO1/36); 2, plants infected with *V. albo-atrum* 1x 10^6 mixed isolate spores; 3, plants infected with *V. albo-atrum* 1x 10^4 spores (AR01/36); 4, Plants treated with sterile distilled water; 5, Untreated tomato plants.

5. Effect of Beaufort rootstock on infection in Espero stems

Introduction

Observations in commercial crops indicate that F1 hybrids such as Espero show less severe symptoms of verticillium wilt when grafted onto a rootstock, such as Beaufort, than when grown on their own roots. The resistance of both Espero and Beaufort is determined by the *Ve* gene; however, it is possible that the resistance of Beaufort to *V. albo-atrum* is influenced by modifier genes or by the greater plant vigour usually evident when growing on a rootstock. The objective of this experiment was to determine the susceptibility of one tomato rootstock (Beaufort), one F1 cultivar (Espero) and both graft combinations of the two, to *Verticillium albo-atrum*.

Methods

Treatments

Grafted and ungrafted plants were obtained from Delfland Nurseries Ltd. Treatment 1 was Tomato variety Beaufort ungrafted. Treatment 2 was tomato variety Espero ungrafted. Treatment 3 was Espero grafted onto Beaufort roots. Treatment 4 was tomato variety Beaufort grafted onto Espero roots. All four treatments were inoculated with *Verticillium albo-atrum*.

Experimental design and statistical analysis

There were a total of four treatments, with six tomato plants per plot, and five replicate blocks. Means and 95% confidence limits were calculated.

Spore inoculation

Plants were inoculated by drenching roots with a spore suspension of *V. albo-atrum* (isolate AR05/15), applying 1×10^7 conidia in 100 ml water. Plants were inoculated 3 weeks after sowing when they had 3-5 true leaves. Conidia were prepared as described previously.

Crop production

Tomato plants in 3 cm^2 peat blocks at the 3-5 true-leaf growth stage were potted on into 13 cm diameter pots containing Levington F1 compost. Plants were potted such that the graft union was at least 1 cm above the compost level. For each treatment, pots were placed in a gravel tray lined with capillary matting to aid daily watering. A gap of at least 30 cm was allowed between adjacent plots.

Plants were grown in a glasshouse at ADAS Arthur Rickwood. The glasshouse temperature was set at 18°C day and 16°C night with the maximum vent set at 28°C. Air temperature was recorded electronically. From 7 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 7 days after potting, and on a weekly basis, grafted plants were inspected for the presence of any adventitious roots growing out from the graft union. Any roots found were removed.

Disease severity

Each plant was assessed at 28 days and 43 days after inoculation and the maximum height to which symptoms extended (i.e. highest leaf number) and the total number of leaves affected by wilting and yellowing typical of *Verticillium* wilt, was recorded.

Stem infection by *Verticillium*

Each plant was cut using secateurs, at the base of the plant, leaving 3 cm of stem below the graft union, where appropriate. The plant was removed from the glasshouse and stripped of all its leaves and fruit leaving just the bare stem.

Stem pieces (3 cm long) were taken from 1 cm below the graft union and at 10 cm, 20 cm and 30 cm above the graft union, (for ungrafted plants, a 3 cm stem piece was taken at approximately 1 cm above the compost surface). Each 3 cm stem section was removed, taking care to note the position along the stem. All stem pieces were dipped in 70 % ethanol and allowed to dry, followed by 2 minutes in 0.5 % solution of sodium hypochlorite, to kill any *Verticillium* present on the outside of stems. The central 0.5 cm piece from each 3 cm stem piece was cut into slices and placed onto damp filter paper in a Petri dish, in sequence, so that the stem position of samples testing positive for

Verticillium could be identified. All Petri dishes were incubated on the bench surface at c. 20°C for 16 days. Each stem piece was examined for the presence of *Verticillium*.

PCR testing on stem samples

Two sets of stem samples, taken from the same sections tested for stem colonisation by ADAS, were sent to Nottingham University for PCR testing. Stem samples (1 cm long) were taken below the graft union and at 10 cm, 20 cm and 30 cm above the graft union. Thus, for each plot containing six plants, six 1 cm stem pieces taken below the graft union were placed into a universal tube and eighteen 1 cm stem pieces taken above the graft union were placed into a separate universal tube. All 40 sets of stems pieces were tested for *V. albo-atrum*, by PCR.

Results and discussion

No plants showed any visible symptoms typical of verticillium wilt at 28 or 43 days after inoculation. A low level of stem infection was found on microscopic examination of stem pieces at the end of the experiment (Table 5.1 – 5.2). The ungrafted plants (T1 and T2) had a significantly lower incidence of *Verticillium* than the grafted plants in the stem base. There was no significant difference between treatments above the stem base. The PCR test revealed no significant difference between treatments.

Table 5.1: Proportion of plants in which *Verticillium* was detected in stems 6 weeks after inoculation, determined by microscope examination of stem slices.

Treatment	Mean proportion of infected plants (and 95% confidence limits)
1. Beaufort on own roots	0 (0.0-0.0)
2. Espero on own roots	0.10 (0.00 -0.22)
3. Espero on Beaufort roots	0.33 (0.15-0.52)
4. Beaufort on Espero roots	0.38 (0.19-0.57)

Table 5.2: Occurrence of *Verticillium* at different heights in the stem following inoculation of roots.

	Total number of infected stem sections (of 30) at, and at distances above, the stem base			
Treatment	Below graft union or at 0 cm	10 cm	20 cm	30 cm
1. Beaufort on own roots	0	0	0	0
2. Espero on own roots	0	2	0	2
3. Espero on Beaufort roots	9	2	0	1
4. Beaufort on Espero roots	11	0	1	2

Table 5.3: Detection of *V. albo-atrum* in tomato stems by PCR test.

Treatment	Below graft or at 0 cm	Above graft at 10, 20, 30 cm
1. Beaufort on own roots	1/5	1/5
2. Espero on own roots	1/5	0/5
3. Espero on Beaufort roots	0/5	0/5
4. Beaufort on Espero roots	1/5	0/5

The PCR detection of *Verticillium* (Table 5.3) and the microscopic examination for *Verticillium* produced different results with regard to treatment, although both indicated a greater incidence in the stem base than in the upper parts of stems. Possibly the lack of consistency between test methods is due to a discontinuous distribution of *V. albo-atrum* within stems (the two methods used different pieces of stem).

It is difficult to draw conclusions from this experiment because of the lack of verticillium wilt symptoms and the low incidence of plant infection above the stem base. Based on the microscopic examination results, the greatest level of infection was detected in the stem base, with a lower incidence higher up the stem, consistent with previous experiments. Stem base infection was significantly greater in the two sets of grafted plants than in the ungrafted plants. Possibly either the grafting process, or the development of a grafted plant, influences the susceptibility of the rootstock.

6. Evaluation of fungicide treatments

6.1 Efficacy of carbendazim drench

Introduction

Currently the only fungicide permitted for treatment of verticillium wilt is carbendazim, which is applied as a drench to the roots. Two products (Cleancrop Curve and Delsene 50 Flo) are approved and available 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 carbendazim applied as a root drench for control of verticillium wilt after infection and after development of wilt symptoms.

Methods

Treatments

Details of individual treatments are given below (Table 6.1).

Experimental design and statistical analysis

There were a total of six treatments, with six tomato plants per plot in a complete randomised block. There were two replicate plots for treatments 1 and 2 (uninoculated controls) and five to seven replicate plots for all other treatments. Allocation of treatments 3-6 to plots was made after the appearance of symptoms in order to provide approximately equal numbers of plants in each treatment, and to spread treatments both along and across rows (see Table 6.1). The results were analysed either by ANOVA, or by regression analysis, as appropriate: ANOVA was performed on the change in % leaves affected; regression analysis was used for the number of plants affected.

Table 6.1: Detail of carbendazim fungicide treatments.

	Inoculation with Vaa	Symptoms present at fungicide treatment	Delsene 50 Flo applied	Number plants in category	Number replicate plots
1.	No	No	No	12	2
2.	No	No	Yes	12	2
3.	Yes	Yes	No	35	6
4.	Yes	Yes	Yes	30	5
5.	Yes	No	No	42	7
6.	Yes	No	Yes	36	6

Spore inoculation

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}. One ml of this spore suspension was added to 100 ml of water and applied as a drench to the roots, around the stem base.

Plants were inoculated with a drench of 1 ml of a mixture of two isolates (AR01/36 and AR05/15) at 1 x 10^6 conidia ml^{-1} in 100 ml water. Plants were inoculated 4 weeks after sowing when they had 2-3 true leaves (15 February) and again 8 weeks later (11 April).

Crop production

Tomato plants cv, Shirley in rockwool plugs at the 2 true-leaf growth stage were potted on into 13 cm diameter pots containing Levington F1 compost in February 2006. Plants were grown on for 14 weeks after the initial inoculation in a heated glasshouse at ADAS Arthur Rickwood. 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. Growth was slow for 3 weeks due to loss of heating in the glasshouse. From 35 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 28 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. Delsene 50 Flo was applied on 9 May and 23 May.

Disease severity

Each plant was assessed at 11 weeks (5 May) and 14 weeks (25 May) after inoculation for % leaf area affected by wilting or yellowing, on each of the first 16 leaves from the plant base. The number of plants affected was also recorded. The change in the proportion of leaves affected, and the mean % leaf area per plant affected, were calculated. Analysis was done on leaves above truss 1 in order to minimise confusion of age-related general yellowing of lower leaves with that due to verticillium wilt.

Results

Incidence and severity of verticillium wilt

Only occasional plants had developed symptoms of verticillium wilt by 8 weeks after the first inoculation, so plants were then re-inoculated. By 11 weeks after the first inoculation, the disease was more evident; angular areas of leaf yellowing, typical of verticillium wilt, were observed on a small proportion of inoculated plants in most treatments (Table 6.2).

Three weeks after the second inoculation, just before the first fungicide drench treatment was applied, verticillium wilt symptoms were present in 23-33% of plants in treatments 3 and 4, and none elsewhere. Three weeks later, shortly after the second fungicide drench, the uninoculated plants remained free of symptoms while 17-63% of plants in other treatments were affected (Table 6.2). The increase in incidence of plants with symptoms was less in T4 (fungicide applied) than T3 (no fungicide applied). Similarly, on inoculated plants that were not showing symptoms at the time of the first fungicide drench (T5 and 6), the increase in the incidence of plants with symptoms was reduced by the fungicide treatment.

In the three-week period after the first fungicide application, treatments differed significantly in the change in % leaves affected by verticillium wilt symptoms (Table 6.3).

On inoculated symptomatic plants, treatment with Delsene 50 Flo significantly reduced the increase in % leaves affected from 11.4% to 4.3%. There was a similar trend on inoculated asymptomatic plants.

Table 6.2: Effect of Delsene 50 Flo treatment on development of verticillium wilt symptoms.

Vaa Inoculation		Symptoms Present?	Delsene Drenches	Mean % plants affected [a] 5 May	25 May
1.	No	No	No	0	0
2.	No	No	Yes	0	0
3.	Yes	Yes	No	23 (5.4)	63 (9.6)
4.	Yes	Yes	Yes	33 (6.6)	43 (10.6)
5.	Yes	No	No	0	31 (8.4)
6.	Yes	No	Yes	0	17 (7.3)
Significance				<0.001	0.002

[a] Based on symptoms in leaves above truss 1. ( ) – standard error

Table 6.3: Effect of Delsene 50 Flo treatment on % leaves and leaf area affected.

Vaa Inoculation	Symptoms Present?	2 x Delsene Flo drenches	Change in % leaves affected[a] (5-25 May)	Change in % leaf area affected per plant[a] (5 – 25 May)
1. No	No	No	0	0
2. No	No	Yes	0	0
3. Yes	Yes	No	11.4	1.8
4. Yes	Yes	Yes	4.3	-0.1
5. Yes	No	No	6.9	0.7
6. Yes	No	Yes	2.1	0.2
Significance			<0.001	0.005
SED			2.86	0.62

[a]Based on symptoms in leaves above truss 1

Discussion

These results confirm that carbendazim as a drench treatment provides some control of verticillium wilt. Treatment reduced the increase in symptom development on plants that were already showing wilt symptoms at the time of application, as well as on infected but symptomless plants.

6.2 Sensitivity of *V. albo-atrum* to carbendazim

Introduction

A number of fungal pathogens have developed resistance to carbendazim or related MBC fungicides following repeated use. This includes *Verticillium tricorpus* associated with tomato (Locke & Thorpe 1976) and *Verticillium fungicola,* the cause of dry bubble disease in mushroom. Isolates of *V. albo-atrum* from tomato tested in 2000 were found to be sensitive to carbendazim (Bavistin DF) (O'Neill, 2002b). In order to determine the current efficacy of carbendazim, the sensitivity of five isolates recently obtained from tomato was examined.

Methods

Six isolates of *V. albo-atrum* from tomato were tested for their sensitivity to carbendazim by agar plate tests. Carbendazim (as Delsene 50 Flo) was incorporated into potato dextrose agar (PDA) at 0, 2, 20 and 100 mg/L of active ingredient. Mycelial plugs (6 mm diameter) of *V. albo-atrum* taken from the leading edge of a culture on PDA were placed in the centre of a PDA plate, incubated at around 20ºC and the diameter of mycelial growth measured after 7 and 14 days. There were three replicate plates per isolate and two diameters were measured at 90º to each other. Inhibition of mycelial growth was calculated by comparison with growth on unamended agar.

Results and discussion

All of the isolates grew on unamended agar and were completely inhibited by carbendazim at concentrations of 2 mg/L and greater (Table 6.3). Thus, there is no evidence of carbendazim resistance in the isolates tested.

Table 6.3. Growth of *V. albo-atrum* isolates on carbendazim-amended agar after 14 days.

Isolate	Year	Location	Mycelial growth (mm) on carbendazim at:			
	obtained	Of crop	0	2	20	100 mg/L
T179	1974	-	58	0	0	0
AR01/36	2001	Kent	48	0	0	0
AR01/140a	2001	Kent	50	0	0	0
PD2000/4186a	2000	Netherlands	42	0	0	0
PC3361*	2004	Yorks	50	0	0	0
AR05/15	2005	Norfolk	48	0	0	0

*Subsequently identified by a molecular test as *Verticillium nigrescens.*

7. Efficacy of chemical disinfectants

Introduction

The objective of the study was to test a range of commercially available disinfectants for their ability to control *V. albo-atrum in vitro.* The efficacy of the disinfectants was tested against spores and mycelium of *V. albo-atrum* in water alone and in water contaminated with peat and clay.

Materials and Methods

Preparation of spore suspensions and mycelium of *V. albo-atrum*

V. albo-atrum was grown on PDA prepared as described before. The PDA was poured into Sterilin 90 mm plates and square 25 well grid plates. Conidial suspensions for disinfectant assays were prepared as stated earlier. Sterile filter disc were placed on the surface of actively growing *V. albo-atrum* PDA plates for 3-4 days for the mycelia to colonies. These filter discs were then removed and used for the disinfectant assay.

Preparation and dilution of recommended doses of the disinfectants

Six commercially available disinfectants were tested for their ability to prevent the growth of *V. albo-atrum in vitro*. For each of the disinfectants the recommended dose or field application concentration was used (Table 7.1). The dilution was done using sterile pipettes in the laminar airflow cabinet using SDW to dilute the disinfectants. Each disinfectant's recommended dose was then further diluted to a 1:2, 1:4 and 1:8 concentrations and stored in labelled bottles for later use. To test the effectiveness of the disinfectants against conidia and mycelium of *V. albo-atrum* when mixed with soil, solutions were added to 500 mg peat, 500 mg clay (kaolin), and 250 mg each of peat and clay together, to 1 mL of the various concentrations of disinfectant (recommended dose, 1:2, 1:4 and 1:8 dilutions).

Addition of *V. albo-atrum* spores or mycelium to disinfectants

A 10^6 conidia/mL spore suspension was centrifuged for 5 min at 13,000 rpm to form a spore pellet. The pellet was then re-suspended in the disinfectant and allowed to react with the disinfectant for differing times (5 min, 30 min, 1 h, 4 h and 8 h) at room temperature. Filter paper discs colonised by mycelium of *V. albo-atrum* were mixed the disinfectant and treated in same manner as the spore pellets.

Table 7.1: Detail of disinfectant treatments.

Product	Active ingredient	Rate tested
Jet 5	5% peroxyacetic acid, 10% acetic acid, 25% hydrogen peroxide	1:125
Trigene	Halogenated tertiary amine	1:50
Unifect G	Glutaraldehyde <15%, ammonium compounds	4% (by volume)
Virkon S	Potassium peroxymonosulphate, surfactant, organic acids	2% (by weight)
Mossicide	30% dichlorophen Na salt	1:60 (by volume)
Sodium hypochlorite	Hypochlorite (8% available chlorine)	25,000 ppm

Unifect G is recommended at 2% for greenhouse cleaning; a 4% treatment is for very dirty places.

Further details of treatments can be found in Appendix 1 and 2.

Inoculation and scoring of PDA plates

Once the disinfectant and *V. albo-atrum* spores had incubated for the allotted time, 200 µL of the disinfectant with spores was pipetted off. This was centrifuged for 2 min at 13,000 rpm; the pellet was then re-suspended in 200 µL of SDW. Following this, 50 µL of the solution was placed in the middle of freshly prepared PDA in 100x100x20.8 mm 25 square well, labelled Sterilin plates. Treatment time was in addition to the cleaning time. There were three replicate plates per disinfectant. Mycelial discs were removed and washed twice in SDW before plating onto square wells. After inoculation the plates were incubated for 4 d at 20°C, then visually scored for signs of *V. albo-atrum* growth. Results were examined by analysis of variance.

Results

Effect on *V. albo-atrum* conidia

There were significant differences between virtually all factor combinations (disinfectants, exposure time and the presence of peat and clay contaminations) on the viability of *V. albo-atrum* ($p<0.01$). One main effect that was not significant was exposure time for conidia, but the interaction between disinfectant and exposure time was highly significant (Table 7.4).

Table 7.2 summarises the results of all the disinfectants against *V. albo-atrum* conidia. The most effective disinfectants against spores of *V. albo-atrum* in water were Jet 5, Mossicide, Trigene, Virkon S and Unifect G at all doses and immersion times used (Table 7.2). Trigene appeared less effective in the presence of peat, while Jet 5, Trigene, Virkon S and hypochlorite all showed reduced activity in the presence of clay. Overall, at the recommended dose, Mossicide, Jet 5, Virkon S and Unifect G were found to kill *V. albo-atrum* spore even in the presence of peat and clay after 30 minutes exposure.

Effect on *V. albo-atrum* mycelium

Due to the poor performance of sodium hypochlorite and Trigene against *V. albo-atrum* conidia, these disinfectants were not tested against mycelium.

Table 7.3 summarises the results of all the disinfectants against *V. albo-atrum* mycelium and Table 5 illustrates the effect of various factors and their efficacy . The most effective disinfectant was Unifect G, fully effective against mycelium in water at all doses and immersion times. The recommended dose of Unifect G was also found to kill *V. albo-atrum* mycelium even in the presence of peat and clay, with a short exposure.

Table 7.2: Summary of the effect of rate (expressed as a fraction of the standard rate tested), exposure time and soil contamination on disinfectant efficacy against *V. albo-atrum* conidia.

Product	Contamination	Lowest rate and least exposure that is effective [a]		Highest rate and longest exposure that is ineffective [b]	
Mossicide	Nil	**0.125**	**5 mins**	-	-
Jet 5		**0.125**	**5 mins**	-	-
Virkon S		**0.125**	**5 mins**	-	-
Unifect G		**0.125**	**5 mins**	-	-
Trigene		**0.125**	**5 mins**	-	-
Hypochlorite		**0.125**	**5 mins**	(0.5	4 h)
Mossicide	Peat	**0.125**	**5 mins**	-	-
Jet 5		**0.125**	**5 mins**	(1	8 h)
Virkon S		**0.125**	**5 mins**	-	-
Unifect G		**0.125**	**5 mins**	-	-
Trigene		1	30 mins	0.5	4 h
Hypochlorite		**0.125**	**5 mins**	-	-
Mossicide	Clay	**0.125**	**5 mins**	-	-
Jet 5		0.5	5 mins	(1	8 h)
Virkon S		0.5	5 mins	0.125	4 h
Unifect G		**0.125**	**5 mins**	-	-
Trigene		0.25	8 h	(1	4 h)
Hypochlorite		-	-	(1	8 h)

[a] Where no values are shown, this indicates the treatment was not fully effective at any of the rates or durations tested.
[b] Where no values are shown, this indicates the treatment was fully effective at all rates and exposure durations tested.
( ) – results appear to contradict the lowest rate and least exposure found to be effective.

The most effective treatments are shown in bold.

Table 7.3: Summary of the effect of rate (expressed as a fraction of the recommended rate), exposure time and soil contamination on disinfectant efficacy against *V. albo-atrum* mycelium.

Product	Contamination	Lowest rate and least exposure that is effective [a]		Highest rate and longest exposure that is ineffective [b]	
Mossicide	Nil	**0.5**	**5 mins**	(1	30 mins)
Jet 5		0.5	1 h	(1	8 h)
Virkon S		1	8 h	1	8 h
Unifect G		**0.5**	**5 mins**	-	-
Mossicide	Peat	**0.125**	**30 mins**	(1	8 h)
Jet 5		1	1 h	1	30 mins
Virkon S		0.5	4 h	1	30 mins
Unifect G		**0.5**	**5 mins**	(0.5	8 h)
Mossicide	Clay	-	-	1	8 h
Jet 5		1	1 h	(1	8 h)
Virkon S		1	1 h	(1	8 h)
Unifect G		**1**	**5 mins**	(1	8 h)

[a] Where no values are shown, this indicates the treatment was not fully effective at any of the rates or durations tested.
[b] Where no values are shown, this indicates the treatment was fully effective at all rates and exposure durations tested.
( ) – results appear to contradict the lowest rate and least exposure found to be effective.

The most effective treatments are shown in bold.

Table 7.4: Analysis of variance on the effect of various factors on efficacy of disinfectants against *V. albo-atrum* conidia (higher order interactions used to estimate error).

Source of variation	df	MS	F probability
Disinfectant (D)	5	30.650	<0.001
Exposure time (E)	3	26.546	<0.001
Contamination (C)	4	0.335	0.443
Rate used (R)	4	119.444	<0.001
D x E	15	6.578	<0.001
D x C	20	3.234	<0.001
E x C	12	0.786	0.013
D x R	20	3.758	<0.001
E x R	12	2.634	<0.001
C x R	16	0.336	0.525
D x E x C	60	1.140	<0.001
D x E x R	60	1.276	<0.001
D x C x R	80	0.343	0.582
E x C x R	48	0.240	0.950
Residual	240	0.358	-

Table 7.5: Analysis of variance on the effect of various factors on efficacy of disinfectants against *V. albo-atrum* mycelium (higher order interactions used to estimate error)

Source of variation	df	MS	F probability
Disinfectant (D)	3	18.215	<0.001
Exposure time (E)	4	13.978	<0.001
Contamination (C)	3	19.909	<0.001
Rate used (R)	4	44.472	<0.001
D x E	12	2.288	<0.001
D x C	9	3.902	<0.001
E x C	12	1.048	0.004
D x R	12	2.183	<0.001
E x R	16	1.669	<0.001
C x R	12	1.784	<0.001
D x E x C	36	1.147	<0.001
D x E x R	48	0.739	0.004
D x C x R	36	0.538	0.134
E x C x R	48	0.637	0.025
Residual	144	0.410	-

Discussion

Surfaces in glasshouses may be treated with disinfectant to try and minimise the potential spread of pathogens, such as *V. albo-atrum.* These surfaces may also be contaminated with organic matter, such as peat, or soil. Hence the experiment was designed to ascertain whether the selected disinfectants were effective at killing the spores and mycelium when they were mixed with peat and/or clay. There were significant interactions between virtually all combinations of factors tested (disinfectant product, rate, exposure time, contamination with peat or clay) making it difficult to summarise the results.

The most effective disinfectants against spores of *V. albo-atrum were* found to be Jet 5, sodium hypochlorite, Trigene, Virkon S and Unifect G. However, the presence of clay seemed to cause the biggest reduction of disinfectant effectiveness. The products

Jet 5, sodium hypochlorite, Trigene and Virkon S all failed to control *V. albo-atrum* in the presence of clay at dilutions greater than the recommended rates. In work carried out by Avikainen *et al.* (1993), it was shown that sodium hypochlorite and Virkon S were effective against spores of *Verticillium*. This result was based on the action of each disinfectant against a spore solution alone. The presence of peat at the level used in our work (500 g/L) did not seem to affect the efficacy of the disinfectants, with the exception of Trigene at greater dilutions.

Contact time and concentration of the disinfectants both affected the efficacy of the disinfectants against both mycelium and spores. As expected, lower concentrations of disinfectant were less effective. The different contact times showed that in some cases (e.g. Trigene) the disinfectant seemed to lose effect after extended contact. This result is surprising and the cause unknown.

The nature of *V. albo-atrum* mycelium cells makes them more resistant than conidial cells. In most cases 30 min exposure time was sufficient to kill spores while a four-hour exposure time was necessary to kill mycelium.

From the results, Unifect G was the most effective disinfectant to kill spores and mycelium of *V. albo-atrum.*

8. 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 seed, leaf debris, nursery sweepings, volunteer seedlings or fallen fruit taken 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 glasshouse tomato crop. The fungus was detected on spore trap slides 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). It was again detected in 2005.

3. This work confirmed that *Verticillium* may symptomlessly infect the stem base of many plants in a crop at the end of a season.

4. The pattern of infected plants with a crop grown on rockwool slabs indicates that plant-to-plant movement of *Verticillium* within a slab or propagation block is not guaranteed when one plant is infected.

5. The severity of verticillium wilt was significantly reduced by drench treatment with carbendazim (Delsene 50 Flo).

6. There was no evidence of resistance to carbendazim (in Delsene 50 Flo) in five isolates of *V. albo-atrum* obtained from tomato between 2000 and 2005.

7. Unifect G was found to be the most effective disinfectant to kill *V. albo-atrum* spores and mycelium in the presence of peat and clay contamination.

9. Technology transfer (Year 3)

Meetings

1. Project review meeting, Warwick-HRI, 27 January 2006.
2. Project review meeting, ADAS Arthur Rickwood, 27 June 2006.
3. Final project meeting, University of Nottingham, 17 November 2006.

Articles

O`Neill TM (2006). Wake up call for tomato wilt. *HDC News* **127***,* 23-25.

Shaddick C (2006). Wrestling with new wilts*. Commercial Greenhouse Grower,* October 2006, 40-41.

Presentation

Tomato Verticillium wilt project update. Wight Salads Seminar, Isle of Wight, 30 June 2006 (Tim O'Neill).

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Appendix 1: Effect of rate, exposure time and soil contamination of efficacy of disinfectants against conidia of *V. albo-atrum* (number of replicates showing growth: n= 3)

Disinfectant	Exposure Time	Rate			
		Recommended rate	0.5x	0.25x	0.125x
Mossicide - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Mossicide + peat	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Mossicide + clay	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Mossicide + peat + clay	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Jet 5 - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Jet 5 + peat	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	3	3	3	3
Jet 5 + clay	5 min	0	0	3	3
	30 min	0	0	3	3
	1 h	0	0	3	3
	4 h	0	0	3	3
	8 h	3	3	3	3
Jet 5 + peat + clay	5 min	0	0	3	3
	30 min	0	0	3	3
	1 h	0	0	3	3
	4 h	0	0	3	3
	8 h	3	3	3	3
Virkon S - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Disinfectant	Exposure Time	Rate			

		Recommended rate	0.5x	0.25x	0.125x
Virkon S + peat	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Virkon S + clay	5 min	0	0	3	3
	30 min	0	0	0	0
	1 h	0	0	0	3
	4 h	0	0	0	3
	8 h	0	0	0	0
Virkon S + peat + clay	5 min	0	3	0	0
	30 min	3	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Unifect G - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Unifect G + peat	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Unifect G + clay	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Unifect G + peat + clay	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Trigene - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Trigene + peat	5 min	3	1	3	3
	30 min	0	2	3	3
	1 h	0	3	3	3
	4 h	0	3	3	3
	8 h	0	0	0	0
Trigene + clay	5 min	3	3	3	3
	30 min	3	3	3	3
	1 h	3	3	3	3
	4 h	3	3	3	3
	8 h	0	0	0	3

Disinfectant	Exposure Time	Rate			
		Recommended rate	0.5x	0.25x	0.125x

Trigene + peat + clay	5 min	3	3	3	3
	30 min	3	3	3	0
	1 h	3	3	3	3
	4 h	3	3	3	3
	8 h	0	0	0	3
Sodium Hypochorite - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	3	3	3
	4 h	0	3	3	3
	8 h	0	0	0	0
Sodium Hypochorite + peat	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Sodium Hypochorite + clay	5 min	3	3	3	3
	30 min	2	3	3	3
	1 h	2	3	3	3
	4 h	3	3	3	3
	8 h	3	3	3	3
Sodium Hypochorite + peat + clay	5 min	3	3	3	3
	30 min	0	3	3	3
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	3	3	3

Appendix 2: Effect of rate, exposure time and soil contamination of efficacy of disinfectants against mycelia of *V. albo-atrum* (number of replicates showing growth: n= 3)

Disinfectant	Exposure Time	Rate			
		Recommended rate	0.5x	0.25x	0.125x
Mossicide - in water	5 min	0	0	3	3
	30 min	1	2	0	0
	1 h	0	0	3	1
	4 h	0	0	0	0
	8 h	0	0	0	2
Mossicide + peat	5 min	0	1	3	2
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	3	3	2	2
Mossicide + clay	5 min	3	3	3	3
	30 min	3	3	3	3
	1 h	3	2	2	3
	4 h	1	1	3	3
	8 h	3	3	3	2
Mossicide + peat + clay	5 min	3	3	3	3
	30 min	2	2	3	3
	1 h	1	1	3	3
	4 h	0	2	1	2
	8 h	2	2	3	3
Jet 5 - in water	5 min	2	1	3	3
	30 min	2	1	2	2
	1 h	0	0	1	3
	4 h	0	0	0	3
	8 h	2	0	2	1
Jet 5 + peat	5 min	3	3	3	3
	30 min	3	3	3	3
	1 h	0	2	3	3
	4 h	0	0	3	3
	8 h	0	1	1	3
Jet 5 + clay	5 min	3	3	3	3
	30 min	1	1	3	3
	1 h	0	1	1	3
	4 h	0	0	0	3
	8 h	3	3	3	3
Jet 5 + peat + clay	5 min	3	3	3	3
	30 min	2	3	3	3
	1 h	0	2	3	3
	4 h	2	2	3	3
	8 h	2	1	3	3
Virkon S - in water	5 min	2	1	3	3
	30 min	2	1	2	2
	1 h	0	0	1	3
	4 h	0	0	0	3
	8 h	2	0	2	1
Virkon S + peat	5 min	3	3	3	3

Disinfectant	Exposure Time	Rate			
	30 min	3	3	3	3
	1 h	0	2	3	3
	4 h	0	0	3	3
	8 h	0	1	1	3
Virkon S + clay	5 min	3	3	3	3
	30 min	1	1	3	3
	1 h	0	1	1	3
	4 h	0	0	0	3
	8 h	3	3	3	3
Virkon S + peat + clay	5 min	3	3	3	3
	30 min	2	3	3	3
	1 h	0	2	3	3
	4 h	2	2	3	3
	8 h	2	1	3	3
Unifect G - in water	5 min	0	0	0	0
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	0	0	0	0
Unifect G + peat	5 min	0	0	1	2
	30 min	0	0	1	0
	1 h	0	3	0	1
	4 h	0	0	0	0
	8 h	0	3	3	3
Unifect G + clay	5 min	0	1	3	3
	30 min	0	3	3	3
	1 h	3	2	3	3
	4 h	0	0	0	0
	8 h	3	3	3	3
Unifect G + peat + clay	5 min	0	0	2	1
	30 min	0	0	0	0
	1 h	0	0	0	0
	4 h	0	0	0	0
	8 h	3	3	3	3