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# AUTHENTICATION

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

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# PRACTICAL SECTION FOR GROWERS

# Commercial benefits of the project

This project forewarns UK growers of a new form of Verticillium wilt, which is a potential serious risk to the economic production of tomatoes. It is caused by a new strain of *Verticillium albo-atrum* which is able to infect the current Verticillium resistant tomato cultivars. The project reviews the problem, details best practice on how to manage it and identifies research requirements.

## **Background and objectives**

Since 1998 there have been an increasing number of outbreaks of *Verticillium* wilt in UK tomatoes in varieties claimed to be *Verticillium* resistant. Both hydroponic (rockwool and NFT) and soilgrown (organic) crops have been affected with significant yield reductions reported. Prior to these outbreaks, *Verticillium* wilt in tomato crops has been rare for over 20 years. Recent tests suggest that a new aggressive strain of *V. albo-atrum* is present in the UK. Because of the atypical *Verticillium* symptoms and the confusion caused by other root rots (eg *Pythium*), the disease may be much more widespread, in all systems of tomato culture, than is appreciated. We have assessed the risks posed to the production of protected tomatoes from *Verticillium* wilt. Current and potential control measures for both conventional and organic crops have been outlined. Priorities for R&D to devise effective, long-term disease management strategies have been determined.

## Summary of results and conclusions

This project has critically reviewed Verticillium wilt in protected tomatoes and assessed the risks posed to UK production from what appears to be a new, aggressive strain. Potential control measures are outlined. Priorities for R&D to aid effective and sustainable disease control are identified.

## 1. Symptoms and yield loss

The new Verticillium wilt affecting Verticillium (Ve) resistant varieties of tomato is essentially a 'slow wilt'. Symptoms differ from those of classic Verticillium wilt. Thin stems, stunted growth, wilting of the youngest leaves and reduced leaf size are the most common manifestations of the problem. As the disease progresses, wilting becomes more common, 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.

## 2. Varieties affected

Many Ve-resistant 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 some slight vascular discolouration the scions did not show symptoms (ie these rootstocks are susceptible to the new Verticillium strains although in the presence of the pathogen they may offer advantages over ungrafted plants because of enhanced root vigour).

## 3. Causal fungus

The fungus isolated most consistently from wilt-affected plants is *V. albo-atrum*. *V. dahliae* has been isolated occasionally, while *V. tricorpus* has not been detected in recent outbreaks. In several

countries, outbreaks of wilt in Ve-resistant tomato cultivars have been ascribed to *V. dahliae* race 2, a strain of the fungus which is not controlled by the Ve gene. This cannot be the explanation for outbreaks in the UK (or Holland), as the fungus involved is predominantly *V. albo-atrum*. Inoculation studies confirmed that *V. albo-atrum* isolated in 1999 from cv. Espero (a Ve-resistant variety) in the UK was equally damaging to susceptible (cv. Shirley) and resistant (Trio and a numbered cultivar) plants. The new strain of *V. albo-atrum* is slow growing (1-2 mm/day at 20°C) and appears to be more difficult to isolate from tomato plants than the 'classic' wilt pathogen.

## 4. <u>Dutch experience</u>

A slow wilting disease of Ve-resistant tomato cultivars, caused by a new strain of *V. albo-atrum*, is reported to be widespread in the Netherlands. Where plants are affected by both Verticillium wilt and pepino mosaic virus, the combined disease, not unexpectedly, were more damaging than either disease alone.

# 5. Sources and spread of Verticillium

Whilst seed transmission of *V. albo-atrum* on lucerne, and of *V. dahliae* on lettuce and sunflower, is documented, transmission of *Verticillium* species on tomato seed has not been reported. We plated out small batches (200) of Espero and Carousel seeds onto agar in 2001, and no Verticillium was recovered.

Soil is the usual source of *Verticillium* in many crops and it is possible that dust and soil contamination of a glasshouse are potential sources of Verticillium in hydroponic tomato crops. Although unlikely to multiply in water, irrigation water contaminated with *Verticillium* spores could also be a source of the disease.

Dispersal of *Verticillium* spores (conidia) in the air has been recorded (eg conidia were detected 10m above infected potato crops), but from the sticky nature of the spores dispersal by water splash or insects is more probable. In Holland the fungus has been found on millipedes in tomato glasshouses. Drip pegs were demonstrated in Holland to be a potential means of *Verticillium* carryover between tomato crops. The pathogen was detected in plants 3 months after insertion of the contaminated drip pegs.

Many weed species are hosts of *V. dahliae* and/or *V. albo-atrum*. Although unlikely to be the direct cause of a problem in hydroponic tomato crops, they could allow the fungus to persist on the nursery. With soil grown tomato crops, infected weeds and tomato seedlings could be a major risk to tomato cropping.

The extent of Verticillium wilt in affected crops was surveyed at the end of the 2001 season. This was judged by the presence of extensive vascular staining and the absence of other obvious causes (eg botrytis, fusarium crown and root rot). Generally 5-10% disease was found, with 50-70% in some severely affected crops.

# 6. Control - resistant varieties and rootstocks

The Ve gene, incorporated into most European tomato cultivars since 1975, has provided very effective control of Verticillium wilt until recently. No alternative source of Verticillium resistance is currently available in European tomato varieties (a new type of resistance, effective against *V. dahliae* race 2, was reported in the USA in 1987, but appears not to have been utilised commercially so far). The commonly used resistance of rootstocks Beaufort and Maxifort also have the Ve-gene. These rootstocks are susceptible to the new Verticillium strain but may still be useful as the extra vigour provided by the rootstocks may further slow the development of wilt compared

with ungrafted plants. Similarly, varieties which differ in vigour (especially of root growth) may differ in their symptom expression.

There is evidence that homozygous Ve-resistant cultivars (VeVe) are slightly more resistant to Verticillium than heterozygous (Veve) cultivars. However, there appear to be no homozygous Ve cultivars available at present in the UK.

With tomato seedlings, the Ve resistance gene was shown to be less effective at high temperatures  $(28^0 \text{ C})$ , than at low temperatures  $(20^0 \text{ C})$ .

# 7. Control - fungicides

Bavistin DF is permitted as a root drench treatment on tomatoes (SOLA 0965/2000) and grower reports indicate that it provides some benefit. There is a risk that with repeated use the fungus will develop resistance to this fungicide. Tests carried out by us in 2000 demonstrated that all six isolates of *V. albo-atrum* (ex tomato) we tested, were currently very sensitive to the fungicide.

# 8. <u>Control - cultural measures</u>

Measures which reduce water stress on the crop appear to slow the development of wilt. Encouraging vegetative growth early in the season, by placing plants straight into the planting hole, was found to reduce wilt severity in rockwool crops. Removal of some fruit from trusses is suggested as a control measure in Holland.

For soil grown crops, it is important that the soil structure is good, with no pan restricting root growth. A chargeable soil test is available to check for and quantify soil-borne *V. dahliae* preplanting, but not *V. albo-atrum*.

# 9. <u>Control - nursery hygiene and disinfection</u>

Plants affected by Verticillium wilt should be removed as soon as they become unproductive. If allowed to remain *in situ* and die, sporing lesions of *Verticillium* can develop on the stem base and contaminate the nursery, increasing the risk for the next crop. Where Verticillium wilt is obvious at crop termination, it is recommended that after the final pick the stem bases are sprayed with a suitable disinfectant immediately prior to the crop pull-out.

Ensure that trimmings and dead plants are placed in a covered skip or container. Verticillium can sporulate profusely on dead tissue.

Do not re-use rockwool slabs from an affected crop, either for tomatoes or any other Verticillium wilt susceptible crop (eg strawberries, cucumber).

Disinfectants demonstrated to have activity against Verticillium, and available in the UK, include: Formalin, Jet 5, sodium hypochlorite, glutaraldehyde (e.g. Glu-Cid), Horticide, Unifect G, Jet 5 and Virkon S. The nature of the surface being treated, and other considerations (eg health and safety cost; spectrum of activity) need to be considered in choosing the most suitable product.

## 10. Risks to other crops

Cucumber, lettuce, peppers and especially aubergine may be at risk from the new tomato strain of *V. albo-atrum*. All of these crop species are known to be affected by Verticillium wilt diseases, cucumber quite commonly. Sweet pepper following tomato was affected by Verticillium wilt in Holland. A Verticillium wilt disease of lettuce, caused by *V. dahliae*, was recently described in

America. Further work is needed to determine the exact host range of the new strain and the damage caused to different crops.

# 11. <u>R&D priorities</u>

Areas where there are significant gaps in our knowledge of tomato Verticillium wilt are identified, and prioritised.

# Action points for growers

- Become familiar with the symptoms of Verticillium wilt in Ve-resistant varieties. If you suspect the problem, have affected plants tested for *Verticillium*.
- Remove plants affected by wilt including the stem base, as soon as they become unproductive.
- Ensure that crop trimmings and dead plants are promptly placed in a covered skip or container, or deposited well away from the glasshouse.
- Root drench treatment with Bavistin DF can reduce disease severity. Treatment in the early stages of the disease is likely to be more effective than later treatment.
- Do not re-use rockwool slabs from an affected crop, either for tomatoes or any other Verticillium susceptible crop.
- Clean and disinfect the glasshouse and all associated equipment, especially drips pegs, after an outbreak of the disease. See HDC Factsheet 15/01 for more details.
- For soil grown crops, ensure there is no soil pan restricting root growth before you plant.
- Where wilt is present, consider encouraging vegetative growth in order to reduce disease severity; adjust heating and ventilation to reduce stress on plants.
- Where wilt is present, spray layered stems with a suitable disinfectant after the final pick at crop termination.
- Take care to minimise root damage, for example at layering.
- Check for and rectify waterlogged (un-slit) slabs.
- Control weeds in the crop, particularly self-sown tomatoes in soil crops.
- Do not handle stem lesions.
- Control sciarids.

# Anticipated practical and financial benefits

- As a result of this project tomato growers will be aware of the symptoms, and the potentially serious yield losses which may occur, if a crop becomes affected by Verticillium wilt caused by the new strain of *V. albo-atrum*.
- Growers will be able to reduce the rate of disease spread and minimise its impact on yield by adopting the practical measures detailed here. It is unlikely, however, that complete disease control will be possible until new genetic resistance is identified and incorporated into European tomato varieties.
- Verticillium wilt in Ve-resistant varieties is estimated to have caused yield losses of 10-20% on at least nine nurseries in 2001. On some nurseries the losses resulting from Verticillium are considered to have been greater than those from botrytis. Adoption of the measures outlined here will reduce these losses.

## **SCIENCE SECTION**

# 1. Introduction

Verticillium wilt of tomatoes has been an uncommon disease in the UK since the introduction of resistant cultivars in the mid-1970s. Outbreaks have occurred sporadically in susceptible cultivars which lack the Verticillium wilt resistance (Ve) gene (e.g. Gardener's Delight). Since 1998, however, Verticillium wilt has occurred increasingly in hydroponic and soil-grown crops of claimed resistant cultivars. At least nine nurseries were affected in 2001. Outbreaks in hydroponic crops are unusual as plants are grown out of the soil, the usual source of Verticillium. The objective of this review is to explain these unusual occurrences, determine the future risk to UK tomato production, and to propose potential disease management strategies.

## 2. Verticillium species causing wilt in tomato

There are two species of Verticillium capable of causing wilt in tomato:

# *Verticillium albo-atrum Verticillium dahliae*

Both species occur very commonly in the UK. *V. albo-atrum* is generally considered the more aggressive pathogen, though all can cause wilting and yield reduction. In the last survey of wilt diseases in UK tomato crops (Fletcher *et al.*, 1977), *V. dahliae* predominated in northern England and *V. albo-atrum* in the south. The usual method of identifying which species is present in a crop is to isolate from affected plants and culture the fungus on potato dextrose or other agars (Talboys, 1960). The morphological features and growth characteristics distinguishing the species are summarised below from descriptions of the fungi published in 1970 (Hawksworth & Talboys, 1970 a, b; Hawksworth, 1970).

**Table 1.** Characteristics of V. albo-atrum and V. dahliae in culture

	V. albo-atrum	V. dahliae
Conidial size (µm)	3.5-10.5 x 2.5	2.6-6 x 1.4-3.2
Conidiophore base	Dark	Clear
Resting bodies*	DM	MS
Colony reverse	White to cream	-
Growth at 30 <sup>0</sup> C	Nil	Good
		11 1

\*DM - dark mycelium; MS - microsclerotia; CS - chlamydospores

A third species, *Verticillium tricorpus* has also, very occasionally, been associated with a vascular disease of tomatoes in the UK.

# 3. Symptoms of the disease

In crops where Verticillium wilt has been confirmed in Ve-resistant varieties, the main symptoms are as follows:

- Reduced growth (stunting), very thin stems in the plant head
- Slow progression of symptoms to severe wilting and plant death ('slow wilt')
- Extensive vascular browning in the stem (extending up to 3 m above the plant base)

- Transient wilting around midday, usually of youngest leaves
- Increased blossom end rot (occasionally)
- Slight or no obvious root rotting (blocks remain firmly attached to slabs)
- Basal and/or stem lesions
- Smaller fruit (reduced yield)
- Smaller leaf size

These symptoms have been observed in crops grown on rockwool slabs, in NFT and in soil. In a few crops, occasional plants have also shown leaf chlorosis and desiccation, usually as wedge-shaped sectors, a symptom of classic Verticillium wilt.

These symptoms are less severe than those of classic Verticillium wilt, which are:

- Rapid wilting of leaves, progressing from the base of the plant upwards
- Wilting sometimes confined to a succession of leaves on one side of the plant
- Leaves develop yellow blotches and sectors, wither and die
- Plants die after several weeks
- Extensive vascular browning in the stem

The gross symptoms observed in tomato crops affected by *Verticillium* are explicable in terms of the effect of a vascular pathogen on the plant physiology. Vascular occlusion by the fungus, by cell wall degradation, and by the plant's response to restrict fungal spread, result in water stress and reduced transpiration. Water stress leads to reduced photosynthesis and consequent reductions in leaf area and fruit yield. Occlusion of xylem vessels on one side of the stem results in wilting of leaves on that side. Cell wall degrading enzymes and toxins produced by the fungus may also be involved (Durrands & Cooper, 1988).

## 4. Observations on recent outbreaks in resistant varieties

## Outbreaks in the UK

A summary of the incidence of tomato Verticillium wilt confirmed by the authors, or by the ADAS Plant Clinic, is shown in Table 2. The annual number of outbreaks has increased from one nursery affected in 1996 to at least nine in 2001. Both *V. albo-atrum* and *V. dahliae* were recovered from affected plants but *V. albo-atrum* was isolated most commonly. *V. dahliae* did not occur on its own at any of the sites but was found with *V. albo-atrum* on four sites out of the nine where the disease was found.

Date	Location <sup>a</sup>	Variety	Species	Frequency of confirmation <sup>b</sup>
<u>1996</u>				
Feb & Oct	West Sussex	Solairo	Vt and Vaa	2
<u>1997</u>				
	Nil			
<u>1998</u>				
May	Kent	Espero	Vaa	1
July	Kent	Eloise	Vaa	1
1999				
Apr & Sep	Kent	Espero	Vaa	5
Sep	Kent	Christina	Vaa	1
Sep	Kent	_	Vaa	1
Oct	W Sussex	_	Vaa and Vd	1
Oct	W Sussex	-	Vaa	1
<u>2000</u>				
Mar	Kent	Espero	Vd	1
Apr, May, July	Kent	Espero	Vaa	4
Jun	Cardiff	-	Vert	1
July	Kent	_	Vaa	1
Oct	West Sussex	Rosa	Vd	1
May	Kent	Eloise	Vaa	2
2001				
May & Jun	Kent	Espero	Vaa & Vd	1
Jun	Kent	Encore	Vaa	1
Jun	Cardiff	-	Vd	2
Jun	W Sussex	Espero	Vaa	1
Jun	W Sussex	Carousel, Ferrari	Vert	4
Jun	I of Wight	Cloe, Sarena Conchita,	Vaa & Vd	2
Jul	Kent	Cloe, Espero	Vaa	1
Aug	Lancs	Spirit??	Vert	1
Aug	Gloucs	Espero, Encore	Vert	1
Oct	I of Wight	Carousel on Beaufort,		3
	C	Carousel on Maxifort,		
Oct	Somerset	Romalina -	Vaa	1

## Table 2. Occurrence of tomato Verticillium wilt in the UK - 1996 to 2001

<sup>a</sup> For some nurseries, samples for confirmation were received twice or more during a year, usually as plants in different glasshouse blocks developed symptoms.

<sup>b</sup> Number of plant samples on which the pathogen was confirmed. Note

Vd = *Verticillium dahliae*; Vaa = *Verticillium albo-atrum*; Vt = *Verticillium tricorpus*; Vert = *Verticillium* spp.

# Effect of temperature on growth of *V. albo-atrum* and *V. dahliae* isolates cultured from Ve-resistant tomatoes

Four isolates of *V. albo-atrum* and two of *V. dahliae* obtained from Ve-resistant tomatoes were grown on PDA at 15, 20, 25 and  $30^{\circ}$  C. Both *V. dahliae* isolates grew well at  $30^{\circ}$  C, while two of the *V. albo-atrum* isolates showed no growth and two showed little growth at this temperature. (Table 3). The optimum temperatures for mycelial growth were: *V. albo-atrum* 20-21° C; *V. dahliae* 25-26°C. These results are in accordance with the effect of temperature previously described for the two species (Hawksworth & Talboys, 1970 a, b).

<i>Verticillium</i> species and reference number	Isolated from	Mean growth rate (mm/day) $\pm$ SE)				
		15	20	25	30 <sup>0</sup> C	
V. albo-atrum						
01/036	Tomato	0.96 <u>+</u> 0.02	1.27 <u>+</u> 0.12	1.08 <u>+</u> 0.06	0	
01/040	Tomato	1.37 <u>+</u> 0.04	$1.78 \pm 0.10$	$2.50 \pm 0.01$	0.37 <u>+</u> 0.19	
01/067a	Tomato	0.97 <u>+</u> 0.16	1.26 <u>+</u> 0.04	$0.98 \pm 0.04$	0	
01/067c	Tomato	1.53 <u>+</u> 0.04	2.05 <u>+</u> 0.12	1.80 <u>+</u> 0.55	$0.23 \pm 0.08$	
V. dahliae						
01/51	Tomato	1.14 <u>+</u> 0.04	1.21 <u>+</u> 0.02	1.41 <u>+</u> 0.06	0.49 <u>+</u> 0.19	
01/156	Tomato	$1.00 \pm 0.20$	1.11 <u>+</u> 0.04	$1.20 \pm 0.01$	0.59 <u>+</u> 0.06	
99/121	Chrysanthemum	0	2.00 <u>+</u> 0.21	2.60 <u>+</u> 0	2.28 <u>+</u> 0.16	

**Table 3.** Effect of temperature on mycelial growth of *Verticillium* spp. isolated from tomato -2001

# Pathogenicity of V. albo-atrum isolated from a Ve-resistant variety

A glasshouse pathogenicity test, in which seedlings of resistant (Trio and a numbered variety) and susceptible (Shirley) cultivars were inoculated with a spore suspension of *V. albo-atrum*, isolated from tomato in 1999, demonstrated that the isolate was equally damaging to Verticillium wilt resistant and susceptible cultivars (O'Neill & Scrace, unpublished). Five weeks after inoculation, vascular browning and severe wilt symptoms were evident in both susceptible and resistant varieties. The fungus was re-isolated from stained stem tissue.

## Extent of Verticillium wilt in affected crops - 2001

Examination for vascular browning during the season indicated that, where Verticillium wilt was present in a crop, it was sometimes more widespread than apparent from wilting symptoms. In order to estimate the extent of disease occurrence in wilt affected crops, several crops were examined in October 2001 by cutting the stem at and above the base and checking for vascular browning in a minimum of 50 plants per crop (Table 4). Stem lesions with *Verticillium* sporing on them were found at one site where the crop was severely affected by wilt; the species was cultured and confirmed as *V. albo-atrum*. Additionally, two crops of Espero in Yorkshire, with no known history of Verticillium wilt, were checked for vascular staining; the disease was not found at these sites.

On two nurseries (sites A and B, Table 4) where we confirmed Verticillium wilt, we observed that Espero grafted onto Beaufort and Maxifort and placed on infested rockwool slabs, and Carousel grafted onto the same rootstocks and planted in infested soil, did not show wilt symptoms after 10 weeks, although at both sites we isolated *V. albo-atrum* from the scion stem tissue. Possibly this indicates that varieties grafted on these rootstocks tolerate the disease better than ungrafted plants. However, at neither site were there ungrafted Espero or Carousel plants which had been planted at the same time. it is possible that ungrafted Espero and Carousel would not show symptoms within 10 weeks.

Sample	County	Cultivar	% stems	Verticillium <sup>a</sup>	Severe wilt	Crop age
and		and nursery code	with	wilt	evident	at
month		(A - D)	vascular	confirmed		sampling
examined			browning			(weeks)
1. Sep	Kent	Espero (A)	51	Yes	Yes	>40
2. Sep	Kent	Rosa (A)	12	No	No	>40
3. Oct	Isle of Wight	Cloe (B)	59	Yes	Yes	14
4. Oct	Isle of Wight	Carousel (on Beaufort)	0	Yes (1)	No	10
		(B)				
5. Oct	Isle of Wight	Carousel (on Maxifort)	6	Yes (1)	No	10
	-	(B)				
6. Oct	Kent	Espero (on Beaufort) (A)	20	Yes (9)	No	10
7. Oct	Kent	Espero (on Maxifort) (A)	20	Yes (10)	No	10
8. Oct	Kent	Espero (A)	70	Yes	Yes (17%)	>40
9. Oct	Kent	Rosa (A)	26	Yes	No	>40
10. Oct	Kent	Conchita (A)	16	Yes	No	>40
11. Oct	Kent	Cloe (A)	50	Yes	No	>40
12. Nov	Yorks	Espero (C)	7 (slight)	No	No	>40
13. Nov	Yorks	Espero (D)	3 (slight)	No	No	>40
14. Oct	W Sussex	- (Ê)	11	No	-	>40
15. Oct	W Sussex	- (F)	7	Yes	-	>40
16. Oct	W Susses	- (G)	5	Yes	-	>40
17. Oct	Kent	- (H)	3	Yes	-	>40
18. Oct	Kent	- (J)	5	Yes	-	>40
910 1				2001		

**Table 4.** Occurrence of vascular browning and confirmation of Verticillium wilt in UK tomato

 crops - Autumn 2001

<sup>a</sup>10 weeks after planting in an area where wilt occurred in summer 2001

() - number positive out of 10 stems tested

#### Effect of Verticillium wilt on fruit yield

Discussion with growers suggest a yield loss of up to 10-15% where wilt is obvious in a rockwool crop from May/June; and 20-25% where the disease has caused plant death by September. These figures are in broad agreement with published data for soil-grown field tomatoes affected by race 2 of *V. dahliae*. Losses of 30-69% occurred with *V. dahliae* race 1 in susceptible varieties (i.e. without the Ve-gene) (Bender & Shoemaker, 1984); smaller losses of 23-25% occurred with *V. dahliae* race 2 in race 1 resistant Ve varieties. The yield reduction is due to reduced fruit number, reduced fruit size and an increase in unmarketable fruit.

# Outbreaks in Holland

A new aggressive strain of *V. albo-atrum* affecting Verticillium resistant cultivars of tomato was reported in the Netherlands in 1991 (Paternotte & van Kesteren, 1993). Rockwool-grown crops of the varieties Calypso and Criterium were affected. This new strain was found to be less virulent on susceptible cultivars (i.e. those lacking the Ve gene) than a control isolate of *V. albo-atrum* (i.e. one which did not cause symptoms on varieties containing the Ve gene). The symptoms described in Dutch crops ('slow wilt') are similar to those recently observed in UK crops.

Since the first report of serious losses due to Verticillium wilt in resistant cultivars, the number of outbreaks has increased steadily each year with at least 40-50 holdings affected in 2001 (Paternotte, pers. comm.).

# Outbreaks caused by V. dahliae race 2

Race 2 of *V. dahliae*, by definition, is not controlled by the tomato Ve resistance gene (Pegg, 1974). Outbreaks of tomato Verticillium wilt in Ve-resistant tomato cultivars in Australia (O'Brien & Hutton, 1981), Greece (Ligoxigakis & Vakalounakis, 1992), Italy & Southern France (Pegg, 1974), South Africa (Ferreira *et al.*, 1989) and the USA (Bender & Shoemaker, 1984; Alexander, 1962) have all been associated with the occurrence of *V. dahliae* race 2.

This cannot be the explanation for the recent outbreaks of Verticillium wilt in the UK as the predominant cause of wilting has been *V. albo-atrum* rather than *V. dahliae*.

# 5. Possible sources of the pathogen

Seed

Seed transmission of Verticillium has been reported in lucerne and sunflower (Hawksworth & Talboys, 1970) and more recently in lettuce (Subbaro et al., 2001). In 1976, outbreaks of Verticillium wilt in lucerne in the USA, a disease previously unknown in the country, was considered to have been due to the introduction of an European strain of *V. albo-atrum* on seed (Christen *et al.*, 1983). Similarly in the 1960s. outbreaks on lucerne in the UK were attributed to introduction of *V. albo-atrum* on Belgian seed. Although seed transmission of Verticillium on tomato has not been reported, the possibility of a seed-borne origin for the recent outbreaks in the UK cannot be excluded. There is generally a period of several weeks between infection and symptom development, so the disease would not necessarily be apparent during propagation.

As a preliminary examination of this possibility, unopened packets of Carousel and Espero, were obtained in October 2001 from a plant propagator. 200 seeds of each variety were cultured on agar and the resultant fungal growth examined. No growth of Verticillium was obtained.

## Young plants

Contamination of young plants during propagation by spores of *V. dahliae* or *V. albo-atrum* could potentially result in wilt after planting out, even though no symptoms may be evident on receipt of plants. Experimental work on transmission from drip pegs indicates a potential latent period of several months (Paternotte, 1999).

# Drip pegs and other glasshouse equipment

Experimental work in Holland (Paternotte, 1999) demonstrated that drip pegs, taken from close to infected tomato plants led to infection by *Verticillium* when placed in the rockwool pots of new plants. The plants were found to be infected 3 months after the start of the experiment although no wilt symptoms were showing at this time. The fungus was also found on plastic covering the soil (Paternotte, 2001).

# Soil

Soil is a common source of Verticillium wilt outbreaks in many crops, including soil-grown tomatoes. *V. dahliae* microsclerotia can survive in soil for at least 13 years (Wilhelm, 1955). Although *V. albo-atrum* conidia can survive in soil for only a few weeks, the fungus is able to colonise roots of immune crop or weed hosts and persist for long periods (Pegg, 1974). Windblown dust, and soil brought into a glasshouse on shoes or wheels are potential sources of Verticillium in hydroponic crops. Additionally for soil-grown crops, organic matter and composed waste added to the soil are also potential sources.

# Water

Irrigation water and pooled run-off water contaminated with *V. albo-atrum* or *V. dahliae* conidia are potential sources of wilt in tomatoes. Run-off water from a Verticillium wilt affected potato crops was found to contain conidia of *V. dahliae* (Pegg, 1974). It seems possible that run-off water from wilt affected tomato crops may lead to disease spread within a house, although Paternotte (2001) reported he did not find *V. albo-atrum* in drain water from infested rockwool slabs, nor did he observe spread of the pathogen by recirculation of the nutrient solution.

# Air

Conidia of *V. albo-atrum* and *V. dahliae* are produced in sticky masses and most commonly are spread by water-splash or insects. *Verticillium* was confirmed on millipedes in wilt affected tomato glasshouses in Holland (Paternotte, 2001). Dispersal in air-currents is probably uncommon, although *V. dahliae* conidia have been detected 10 m above an infected potato crop (ref.). There is little information on insect dispersal of conidia but it would seem likely that fungus gnat flies and mites would readily acquire conidia from stem lesion.

# Alternative crop and weed hosts of Verticillium in the UK

Both *V. albo-atrum* and *V. dahliae* have very wide host range (Table 5) (Greenwood, 1983; Hawksworth & Talboys, 1970 a,b). Additionally, *V. dahliae* can persist for many years in the rhizosphere of some non-hosts.

Table 5.	Alternative crop	and weed hosts	of Verticillium	species
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V. albo-atrum	V. dahliae
Crops	Potato
Potato	Strawberry
Hops	Linseed
Lucerne	Dahlia
Strawberry	HONS (Acer) spp.
Vicia bean	Aubergine
Phaseolus bean	Pepper
Pea	Cucumber
Clover	
Cucumber	
Weed hosts of V. albo-atrum	
Shepherd's Purse (Capsella bursa-pastoris	
Groundsel (Senecio vulgaris)	
Ribwort ( <i>Plantago lanceolata</i> )	
Chickweed (Stellaria media)	
Fat hen (Chenopodium album)	
Small nettle ( <i>Urtica urens</i> )	
Stinging nettle (Urtica dioica)	
Dandelion (Taraxacum officinale)	
Black nightshade (Solanum nigrum)	
Wild chamomile (Matricaria chamomilla)	
Scentless mayweed ( <i>Tripleurospermum inodoratum</i> )	
Ox-eye daisy (Chrysanthemum leucanthemum)	
Creeping thistle (Cirsium arvense)	
Canadian fleabane (Erigeron canadensis)	
Gallant soldier (Gallinsoga parviflora)	
Curled dock ( <i>Rumex crispus</i> )	

# 6. Control

## Resistant cultivars and rootstocks

A high level of resistance to Verticillium wilt was first reported in a small fruited wild tomato (*L. pimpinellifolium*) from Peru (Schaible *et al.*, 1951). This resistance, conferred by a single dominant gene (Ve) was incorporated into European tomato cultivars from around 1975 (Paternotte & van Kesteren, 1993) and proved very effective. It is reported effective against *V. albo-atrum* and race 1 isolates of *V. dahliae*. However, it is not effective against some isolates of *V. dahliae*, and these are classed as race 2. Race 1 and race 2 isolates of *V. dahliae* are morphologically indistinguishable. Information on which varieties carry the Ve gene is given in Table 6, and for older varieties in Fletcher (1992).

Now that there are two strains of *V. albo-atrum* differing clearly in their pathogenicity to tomato, the original strain which does not cause wilt in tomato varieties carrying the Ve gene, and the new strain which does cause wilt in such varieties, it would seem logical that they are called race 1 and

race 2 respectively, in parallel with the nomenclature used for *V. dahliae* on tomato. However, this has not yet been proposed in the scientific literature.

Field observations and glasshouse tests indicate that the dominance exerted by the Ve gene is incomplete. This means that tomato hybrids heterozygous for the gene (Veve) are less resistant than hybrids which are homozygous (VeVe). In a glasshouse test using a race 1 isolate of *V*. *dahliae*, the mean wilt score (0-9 index with 9 being severe wilt) for heterozygous resistant hybrids (1.3 - 2.9) was significantly higher than that of homozygous resistant hybrids (0.6 - 1.0). Susceptible hybrids lacking the Ve gene scored considerably less (5.3 - 6.9) (Okie & Gardner, 1982).

From discussions with seed companies, it is reported that most European varieties recently affected by Verticillium wilt carry the Ve gene in the heterozygous condition. However, it is also reported that older varieties in which Verticillium wilt was not a problem before the mid-1990s(e.g. Solairo) were also heterozygous for Ve-resistance gene. This suggests that the current lack of homozygous Ve-resistance in tomato cultivars is not the explanation for recent outbreaks. Studies are needed to determine the susceptibility/resistance of homozygous Ve-resistant varieties (if available) in comparison with heterozygous Ve-resistant varieties with the same genetic background (i.e. isogenic differentials, to the *Verticillium* strains now affecting UK tomato crops in order to determine if homozygous Ve varieties would offer reduced risk of severe wilt disease.

Expression of the Ve resistance gene may be influenced by modifier genes. The variation in susceptibility of heterozygous Ve-hybrids found by Okie & Gardner, and the apparent greater tolerance of some rootstocks (e.g. Beaufort) to the current Verticillium wilt, may be due to presence or absence of different modifier genes alongside the Ve gene, or greater vigour in the root system. If there has been a gradual loss of Ve-expression during the breeding of new varieties due to a change in associated modifier genes, this might explain the current outbreaks of Verticillium wilt. However, there is no experimental evidence that this has happened. To the contrary, inoculation studies by Parernotte & van Kesteren (1993) demonstrated that the Ve gene in Criterium and Calypso remained effective against pre-1991 isolates.

On the evidence available, it seems likely that the cause of Verticillium wilt in Ve-resistant varieties is due to a change in the fungus, rather than a change in varietal resistance. The puzzling feature which remains to be explained is how a new strain has apparently spread widely in a relatively short time.

The Ve gene appears to operate against race 1 isolates by limiting, rather than excluding, vascular colonisation (O'Garro & Clarkson, 1988). When *V. albo-atrum* infects tomato plants that have the Ve gene, the xylem is quickly protected by a suberin coating that prevents the fungus from colonising (Robb *et al.*, 1989). The fungus is restricted to the vascular tissue of the roots and the lower stem, principally by the formation of tyloses (in-growths from neighbouring cells into the xylem vessel) which occlude xylem vessels in the tap root and hypocotyl (Bishop & Cooper, 1984). Recently, elemental sulphur has been identified as a phytoalexin produced in resistant tomato cultivars as a defence mechanism against *V. dahliae* (Williams *et al.*, 2002).

In growth room tests with young tomato seedlings the Ve resistance gene was found to be less effective against *V. albo-atrum* at high temperatures, favouring development of Verticillium wilt (Jones & Overman, 1976; Jones *et al.*, 1977). Increasing the temperature from 20 to  $28^{\circ}$  C increased the proportion of infected plants from 40 to 79%, and of wilted plants from 10 to 61%.

Genetic resistance to race 2 of *V. dahliae* was identified in 1987 (Berry & Oakes), and reported to act in a dominant, monogenic character. The race 2 resistant varieties, Ohio 11 and Ohio 12, were also resistant to tomato mosaic virus (Tm2<sup>2</sup>) and fusarium crown and root rot. It appears that this resistance gene has not been incorporated into European tomato cultivars.

Variety	Туре	Source	Resista	nces				
	<b>7</b> I		TMV	Vert	Fus	FCRR	Clad	PM
Aranca	Cocktail	Enza	Tm	V	F2	-	C5	-
Bandita	Truss	Samuel Yates	Tm	V	F2	Fr	-	-
Beaufort	Rootstock	PDR	TM	V	F2	?	C5	-
Bloody	Mini-truss	Syngenta	Tm	V	F2	-	C5	-
Mary								
Campari	Cocktail	Enza	Tm	V	F2	-	C5	-
Celine	Plum	Syngenta	Tm	V	F2	-	C5	-
Cheetah	Classic	Syngenta	Tm	V	F2	Fr	C5	-
Claree	Cherry	Enza	Tm	-	F2	-	C5	-
Classy	Truss	Syngenta	Tm	V	F2	Fr	C5	-
Cloe	Truss	Syngenta	Tm	V	F2	Fr	C5	-
Clotilde	Truss	Syngenta	Tm	V	F2	Fr	C5	-
Ellery	Cherry	Enza	Tm	-	F2	-	C5	-
Groove	Beef	Syngenta	Tm	V	F2	Fr	C5	-
He-Man	Rootstock	Syngenta	Tm	V	F2	Fr	C5	-
Maggy	Classic	Syngenta	Tm	V	F2	Fr	C5	Oi
Mona	Midi-plum	Syngenta	Tm	V	F	Fr	C5	-
Lisa	-							
Maxifort	Rootstock	PDR	Tm	V	F2	Fr	C5	-
Nectar	Cherry	Enza	Tm	-	F2	-	C5	-
Pannovy	Classic	Syngenta	Tm	V	F2	-	C5	-
Rapsodie	Beef	Syngenta	Tm	V	F2	Fr	C5	-
Romalina	Mini-plum	Samuel Yates	?	?	?	?	?	-
Romana	Truss-plum	Samuel Yates	?	?	?	?	?	-
Shirley	Round	Enza	Tm	-	F2	-	C5	-
Triton	Round	Enza	Tm	V	F2	-	C5	-

**Table 6.** Resistance of some currently-grown tomato cultivars and rootstocks to *Verticillium wilt* and other diseases

Samuel Yates - UK distributor for Western Seeds

PDR - Pinetree de Ruiter

See Fletcher (1992) for information on the genetic resistance of older varieties.

## Fungicide treatment

Fungicides reported active against *Verticillium* species are those belonging to the MBC group (benomyl, carbendazim, thiophanate-methyl) and prochloraz. At present carbendazim (Bavistin DF) is the only product permitted as a root drench treatment to tomatoes for control of Verticillium wilt. Treatment is permitted under a Specific Off Label Approval (0965/2000), with a maximum of four treatments to rockwool and NFT crops and just one to a soil-grown crop. The approval has no stipulated expiry date. Grower comments indicate that Bavistin DF helps to manage the disease in rockwool tomatoes, but does not control it completely. Experimental work with Bavistin DF applied as a drench treatment for control of Verticillium wilt in cucumber found a 40% reduction in wilt incidence where four drenches were used (HDC report PC 39).

In a previous instance in tomato, the MBC fungicide benomyl (Benlate) became ineffective after repeated use because of the development of resistance in the causal fungus, *V. tricorpus* (Locke & Thorpe, 1976). *Verticillium fungicola*, the cause of dry bubble disease of mushroons, developed resistance to carbendazim after three years of intensive use of MBC fungicides on the crop. There is a relatively high risk that resistance will develop in isolates of *V. albo-atrum* and *V. dahliae* to carbendazim (Bavistin DF) with continued or intensive use of the fungicide.

In Holland, application of a fungicide (not named, but probably Bavistin DF or a similar MBC fungicide) at planting a rockwool crop was reported to be phytotoxic, though four treatments at monthly intervals gave a large reduction in the incidence of wilt (Paternotte & Kaarsmaker, 2000).

# Cultural measures

Where Verticillium wilt is widespread in a crop, it has been advocated that crop production is modified to reduce stress on plants (Paternotte & Kaarsmaker, 2000). Measures include:

- growing plants more vegetatively (planting on arrival rather than placing plants adjacent to the rockwool planting hole; copious watering).
- trimming fruit trusses (reduction in number of fruit per truss).
- adjusting heating and ventilation to reduce water stress.

Trials were undertaken at Naaldwijk in 2001 to determine how effective such measures were at permitting economic crop production in the presence of Verticillium wilt. Ferrari showed severe, symptoms when cultivated 'generatively', while the disease was largely suppressed with 'vegetative' cultivation, resulting in a large yield increase and reduced blossom end rot. However this approach was not successful with the naturally vegetative cultivar Aromata.

Not unexpectedly, Verticillium and pepino mosaic virus together are far more damaging than infection by either disease alone (Stijger & Paternotte, 2001). Combined infection resulted in a greater incidence of plant wilting and death, blossom end rot and poor fruit colour development.

In soil grown field tomatoes in the USA, yield loss to *Verticillium dahliae* was greater (71%) where root growth was restricted by a soil pan, than where root growth was unrestricted (40%) (Ashworth *et al.*, 1979). Attention to soil structure will reduce the risk of a damaging attack of Verticillium wilt in organic and other soil-grown crops in the UK.

## Nursery hygiene and disinfection

In Holland it is reported to be extremely difficult to get rid of *Verticillium* from commercial glasshouses by hygiene measures (Patermotte, 2001). Observations to date in England lead to a similar conclusion. Nevertheless, it would appear sensible to attempt to reduce the inoculum both during cropping and at the end of a crop in order to reduce the risk of an early and severe attack in the following crop. At present, information on the efficacy of various disinfectant is largely based on work with *V. dahliae*, particularly with respect to cucumber crops. Published work is summarised below. It should be noted that many of the tests below refer to disinfection of media, or where the fungus has been grown on wood or cloth and penetrated beyond the surface. The greatest requirement for a hydroponic tomato nursery is likely to be for surface disinfection rather than a more penetrating treatment, and some of the products which performed poorly here may be sufficient for surface disinfection in a glasshouse.

Of eight disinfectants tested against *V. dahliae* growing on cloth pieces and wooden discs, only one (Disinfectol EL; 60% ethanol used undiluted) was effective (Koponen *et al.*, 1992). The other seven products tested were partially effective or ineffective after 60 minutes exposure. The products were based on iodine, QAC, glutaraldehyde, sodium hypochlorite or potassium peroxysulphate. On metal and plastic surfaces 10% hypochlorite was fully effective after 60 minutes; efficacy was reduced to around 50% kill by the presence of peat and clay.

In a further study (Avikainen *et al.*, 1993), it was found that cucumber root infection by *V. dahliae* in a peat substrate could be reduced with a 60 minute exposure to formalin, sodium hypochlorite, an iodine based product (Iobac P) and potassium peroxysulphate (2% Virkon S). The degree of reduction of *V. dahliae* in cucumber, the test plant species, was 82, 81, 83 and 84% respectively. Two QAC compounds (Menno Ter Forte and Talosept) were ineffective at the rates tested. No product gave 100% control.

In a laboratory test (Avikainen *et al.*, 1993), *V. dahliae* was mixed with peat and used to contaminate plastic pots. The pots were then soaked in disinfectant for 15 and 60 minutes before assessing survival of the fungus. Three disinfectants resulted in complete kill of *V. dahliae*: 10% sodium hypochlorite after 15 minutes; 1% Korsolin (glutaraldehyde) after 60 minutes, and 3% Iobac P (1.8% iodine) after 15 minutes.

In a further experiment, the effectiveness of disinfectants against *V. dahliae* in sand was investigated. Disinfectant was sprayed ( $4 \text{ l/m}^2$ ) onto sand contaminated with *V. dahliae* on plastic pots. The sand was washed with water after 60 minutes, and cucumber seedlings grown as bait plants over the treated sand. Formalin (82-90% control), sodium hypochlorite (79-90%) and Iobac P (61-95%) were the most effective treatments, significantly more effective than 2% Virkon S, two QAC's and 60% ethanol. No treatment gave 100% control.

Experimental work carried out by Interox (unpublished; data supplied by Certis) using the disinfectant Proxitane 0510, a product based on peracetic acid (PAA and equivalent to Jet 5), demonstrated activity against *Verticillium nigrescens*. The disinfectant was tested against the fungus by *in vitro* suspension tests using mycelium suspended in clean or potato-soil Ringer's solution. In clean solution the disinfectant was effective after 15 mins at 500 mg/l PAA and after 5 mins at 1500 mg/l PAA. Slightly longer contact times were required under soiled conditions. These results indicate that Jet 5 should have activity against Verticillium. jet 5 at the standard dilution (1:125) contains 400 mg/l PAA.

We tested Unifect G (glutaraldehyde + QAC) against V. *albo-atrum* isolated from Ve-resistant tomatoes and grown on filter paper squares, and found it to be completely effective. A 2% solution was effective against both spores and mycelium after a 2 minute exposure, even in the presence of a small amount of organic matter.

These results, and the observed persistence of Verticillium wilt on nurseries as a recurring problem after an initial outbreak, demonstrate that the fungus is not so readily reduced by disinfection as some other pathogens of tomato. In summary, the above results suggest the following general guidelines on relative efficacy of different disinfectant types against *Verticillium*. Whichever product is used, it is important to read and follow the instructions carefully with respect to rate, optimum conditions of use, and health and safety precautions.

Effective

Sodium hypochlorite (on relatively clean surfaces only) Glutaraldehyde (e.g. Unifect G, Horticide) Iodine based products (not commonly used in UK; staining) jet 5 (slight reduction in activity when PAA was tested in soiled water)

Less effective QACs

Variable results Virkon S

Effective but impractical/no longer accepted Ethanol Formalin

<u>No information</u> There is no information available on a number of specific disinfectants that are used in the UK.

# 7. Risks to tomato and other glasshouse crops

Verticillium wilt was recently discovered in sweet pepper in Holland (Paternotte, 1999), and the pathogen was believed to have originated from the previous tomato crop. Whilst monitoring Verticillium wilt in a tomato crop in the UK, young wilting cucumber plants, grown in a house alongside affected tomatoes, were found to be infected with *V. albo-atrum*. Table 5 in this report lists crops which are known to be susceptible to Verticillium wilt and are commonly affected. Further work is needed to determine more precisely the effect of the new tomato strain of *Vertcillium* on important crop hosts.

# 8. Priorities for R&D

The following topics were identified as ones where there are knowledge gaps in our understanding of tomato Verticillium wilt. Priority areas for research to help develop a sustainable strategy for management of the disease are indicated: \*\*\* - high; \*\* - medium; \* - low.

## 1. Financial loss

Estimate the current losses to Verticillium wilt in the UK by examining a representative sample of crops in mid-summer, and again immediately prior to crop termination, and assessing incidence of the disease. (\*)

# 2. Varietal/rootstock resistance

Determine the relative susceptibility of current commercial varieties and rootstocks to Verticillium wilt i) by inoculating plants with a range of isolates under controlled conditions; ii) by growing plants on known infested slabs under commercial conditions. (\*\*\*)

# 3. Characterisation

Determine if the strains of *V. albo-atrum* and *V. dahliae* currently infecting Ve-resistant tomato varieties can be readily distinguished from older isolates, unable to infect such varieties, by molecular methods. (\*\*)

# 4. Rapid detection

Validate a commercial rapid diagnostic, or if necessary develop, a low-cost rapid, sensitive assay for reliable detection of *V. albo-atrum* and *V. dahliae* in tomato roots and stems. Utilise a molecular diagnostic on bulk samples to determine if seed and young plants delivered to production nurseries are infected or contaminated with Verticillium. (\*\*)

## 5. Disease sources and spread

- a) Investigate the locations and possible means by which Verticillium spreads on a nursery (e.g. in irrigation tanks, in run-off solution/pooled water on floors; associated with pests or biocontrol agents; in the air; in green waste). (\*\*\*)
- b) Determine if seed and/or young plants are a source of Verticillium wilt, by conventional isolation and baiting techniques. (\*\*\*)

## 6. Risk to other crops

Determine the pathogenicity of current isolates of *V. albo-atrum* and *V. dahliae* from tomato to other protected crops, particularly cucumber and pepper but also lettuce and chrysanthemum, in comparison with pre-1991 tomato isolates (if available) and isolates sourced from each host crop. (\*\*\*)

## 7. Control - fungicide efficacy

- a) Evaluate the effectiveness of Bavistin DF against tomato Verticillium wilt, including an evaluation of number of treatments and timing of treatment in relation to infection. (\*\*)
- b) Monitor the sensitivity of Verticillium isolates to carbendazim on a regular basis to ascertain early detection of any resistance development. (\*\*)

## 8. <u>Control - alternative fungicides</u>

Determine the effect of Amistar, Scala, and any other fungicide newly approved for use on tomato, on tomato Verticillium wilt, subject to initial enquiries with the manufacturer on likely approval for drench treatment being not automatically excluded. (\*\*)

## 9. Control - biocontrol

Maintain an overview of development in Verticillium control on other crops, particularly biocontrol, and if appropriate, test candidate biocontrol organisms on tomato Verticillium. (\*\*)

## 10. Control - soil amendments

Investigate the effect of organic amendments (e.g. seaweed, crustacean waste, green-waste compost) on the long-term development of Verticillium wilt in soil grown/organic crops. (\*\*\*)

# 11. Control - nursery hygiene

Determine the efficacy of chemical disinfectants against Verticillium on drip pegs, and other suspected or proven areas of pathogen carryover, using appropriate methods (\*\*\*)

# 9. Conclusions

# 1. Nature of the problem

Tomato Verticillium wilt is affecting a small but increasing number of nurseries in the UK. At least nine nurseries were affected in 2001. The disease has occurred in a wide range of varieties all previously resistant to wilt.

## 2. Symptoms

Symptoms differ from those of classical Verticillium wilt. The current problem results in a 'slow wilt', initially expressed as wilting of upper leaves, thin stems in the plant head and reduced growth.

## 3. Yield reduction

Outbreaks of Verticillium wilt in rockwool crops have caused yield reductions of 15-20%, sometimes greater.

## 4. Cause of wilt

Both *V. albo-atrum* and *V. dahliae* have been found associated with wilting plants. *V. albo-atrum* more commonly. *V. dahliae* was not found on its own at any of the sites. An isolate of *V. albo-atrum* obtained from tomato in 1999 was shown to be capable of causing wilt in Verticillium resistant varieties, as well as in susceptible varieties.

## 5. Resistant varieties

Since around 1975, the Ve gene has been used in most European tomato varieties to provide resistance to Verticillium. It has been very effective until now. There are no alternative types of resistance currently available in European tomato varieties.

## 6. Race 2 of V. dahliae

The Ve gene initially provided control of *V. albo-atrum* and of race 1 isolates of *V. dahliae*. Soon after the Ve gene was first used, some tomato crop became infected with *V. dahliae* and these

isolates, able to overcome the Ve gene, are known as race 2 isolates. We are unaware of any previous documented case of *V. dahliae* race 2 occurring in the UK.

# 7. Situation in Holland

A problem with Verticillium wilt affecting resistant tomato varieties was observed on two nurseries in Holland in 1991 and now affects many crops there. The disease was shown to be caused by a new, strain of *V. albo-atrum*.

# 8. Is heterozygous Ve less effective than the homozygous condition?

The Ve gene may be present in a variety in the homozygous (VeVe) or heterozygous (Veve) condition. Information from seed companies indicate that most UK varieties and rootstocks are heterozygous for Ve. Experimental work and field observations in the USA indicate that heterozygous Ve hybrids are not quite as resistant to *V. dahliae* as homozygous hybrids.

## 9. Temperature and Ve expression

The Ve gene is reported to be less effective at high temperatures and under low light conditions.

# 10. Why is there a problem now?

The current outbreaks of Verticillium in tomato may result from (i) a change in *V. albo-atrum*; (ii) a change in the Ve-resistance condition within tomato varieties or (iii) a combination of both. On the information now available, it seems likely that a new strain of the fungus has been selected rather than varietal resistance has changed. Further work is needed to clarify this. It seems logical that the new strain of *V. albo-atrum*, able to cause wilt in varieties carrying the Ve-gene, be called *V. albo-atrum* race 2.

# 11. Not all Ve-resistant varieties are the same

Although the Ve gene is the only source of Verticillium resistance used in commercial tomato varieties, resistant varieties do not necessarily behave the same when challenged with the fungus. The speed at which a variety succumbs to wilt can differ. This has been observed in recent outbreaks in the UK, and noted when varieties and rootstocks were tested under standard conditions in Holland. Possible explanations for these differences are (i) presence/absence of modifier genes which influence the expression of the Ve gene (ii) effect of differences in root vigour on symptom development.

## 12. Performance of rootstocks

Although we have shown that the rootstocks Beaufort and Maxifort are both susceptible to the new Verticillium, it is reported that varieties grafted on these rootstocks tolerate the disease better than ungrafted plants. On one nursery we observed that Espero grafted onto these rootstocks, and placed onto Verticillium infested rockwool slabs showed no wilt after 10 weeks; on a second nursery, Carousel grafted onto these rootstocks and planted in naturally infested soil showed no wilt after 10 weeks. On both nurseries *V. albo-atrum* was recovered from the scion stem tissue. Unfortunately, there were no ungrafted Espero and Carousel plants which had been planted at the same time, and it is possible that ungrafted plants also would not show symptoms within 10 weeks.

## 13. Fungicide treatment

Grower reports indicate that treatment with Bavistin DF can reduce losses to the new Verticillium. A maximum of four root drench treatments can be applied to hydroponic crops (one to soil-grown crops) under Specific Off Label Approval 0965/2000.

# 14. Risk of fungicide resistance

The selection of Verticillium pathotypes resistant to carbendazim (Bavistin DF) is a real risk. In 1975, resistance was confirmed in *Verticillium tricorpus*, affecting soil grown tomatoes, following intensive use of the related fungicide benomyl (Benlate). Severe wilt occurred even where the fungicide was used. *Verticillium fungicola*, the cause of dry bubble disease on mushrooms, developed resistance to carbendazim after three years of intensive use of MBC fungicides.

## 15. Possible sources of the problem

The source of Verticillium currently causing wilt in UK and Dutch tomato crops, and how it has spread between nurseries is unknown. Possibilities include: seed, young plants, soil, wind-blown dust, infested water, and insects. A soil test is available to quantify *V. dahliae*, but not *V. alboatrum*.

## 16. Possible means of spread on a nursery

The mechanism of spread of Verticillium around a nursery is unknown. Possibilities include insects, debris from an infected crop, contaminated equipment (e.g. drip pegs), water splash, irrigation water, run-off solution.

## 17. Persistence

Once Verticillium has occurred in a glasshouse, there is a tendency for it to re-occur in the following tomato crop. Work in Holland suggests contaminated drip pegs are one possible means of carryover between crops.

## 18. Disinfectants

Work on Verticillium diseases of other crops indicates a number of disinfectants which are likely to be effective against Verticillium from tomato.

These include: ethanol

formaldehyde (Formalin) glutaraldehyde (e.g. Glu Cid) glutaraldehyde + QAC mixtures (e.g. Horticide, Unifect G) sodium hypochlorite potassium peroxysulphate (Virkon S) hydrogen peroxide/PAA (Jet 5)

## 19. Disease management strategy

Where Verticillium wilt has occurred, it is recommended that the following measures are considered in order to minimise losses in the following tomato crop:

- removal of unproductive plants before Verticillium sporulates on them
- spray layered stems with a suitable disinfectant after the final pick at crop termination
- do not re-use rockwool slabs from an affected crop
- thoroughly clean and disinfect drip pegs and other glasshouse equipment
- promptly identify the cause of any wilting
- treat with Bavistin DF if *Verticillium* is confirmed
- take care to minimise root damage (e.g. at layering)
- check for and rectify waterlogged slabs
- if Verticillium is confirmed, adjust heating/ventilation to reduce water stress on plants

## 20. Research needs

Gaps in our knowledge of this disease have been identified and research avenues recommended to fill them. Research areas are prioritised.

## **10. Technology transfer**

- 1. Verticillium wilt: an old threat renewed. Presentation and summary paper at the 2001 National Tomato Conference, 11 October, 2001 (Tim O'Neill).
- 2. Act now to control Verticillium wilt. HDC Factsheet 15/01, November 2001 (John Fletcher and Tim O'Neill).
- 3. Super wilt. Grower, 13 September, p 19 (Tim O'Neill).
- 4. Tomato Verticillium risks assessed. HDC News 79, 6.

## **11. Acknowledgements**

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#### **12. REFERENCES**

The following is a list of references about Verticillium which were consulted during this review. Although they are not all quoted in the text, they are listed here as a reference source for those who wish to do further reading on the subject.

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