Contract report for the Horticultural Development Council

Sweet pepper: aspects of the epidemiology of a stem and fruit rot caused by *Fusarium oxysporum*

PC 260

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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. 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

Although not commonly found as an aggressive pathogen on growing plants, *Fusarium oxysporum* is frequently present in UK sweet pepper crops. It has been confirmed as the cause of limited lesions at stem nodes, spreading lesions on stems and a fruit rot and can result on post harvest loses of 5-10%. It can be eliminated by treatment of seed with hypochlorite and has been found to pose little risk to crops of cucumber, tomato and lettuce.

Background and expected deliverables

A Fusarium stem and fruit rot of sweet pepper has occurred on at least one UK nursery for several years in succession. Fruit losses of over 20% have been reported on some picking occasions. In 2005, *F. oxysporum* was identified as the fungus consistently associated with affected tissues (PC 232a). A stem and fruit rot of pepper associated with *F. oxysporum* has been reported in the Netherlands in recent years. This project aims to increase understanding of the biology of Fusarium fruit and stem rot of pepper in order to enable rational strategies for disease control to be devised. The expected deliverables are:

- Confirmation that *F. oxysporum* is the cause of fruit and stem rot on pepper.
- Increased knowledge of disease epidemiology, including seed transmission, infection points on the plant and disease spread.
- Knowledge of whether *F. oxysporum* from pepper can infect cucumber, lettuce and tomato; and whether isolates of *F. oxysporum* from other hosts can infect pepper.
- Development of practical methods for disease management based on knowledge of pathogen biology.

Summary of the project and main conclusions

Pathogenicity of Fusarium oxysporum *to pepper*

The ability of *F. oxysporum* to infect and cause disease in sweet pepper was investigated in a series of experiments. Detached pepper fruit and fruit stalks rapidly developed a soft rot when inoculated with mycelium of *F. oxysporum* from cucumber, *Hebe*, *Lisianthus*, stock, sweet pepper and tomato. Isolates obtained from pepper and cucumber stems appeared more damaging than other isolates.

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When young pepper plants, cv. Special, were inoculated at fresh de-leafing scars and over the roots with conidia of *F. oxysporum,* using isolates obtained from sweet pepper, no stem lesions or permanent wilting had developed 8 weeks later. However, the fungus was re-isolated from the roots and leaf scars of these inoculated plants, and to a lesser extent from uninoculated plants, at the end of the experiment. When young pepper plants, cv. Fiesta, were inoculated at fresh de-leafing scars with mycelial inocula of a *F. oxysporum,* using an isolate obtained from a pepper stem lesion, small, pale brown stem lesions developed after eight days. After six weeks the lesions remained small in size (around 10-20 mm long) and none had girdled the stem or caused plant wilting.

The growth rate of three isolates of *F. oxysporum* from pepper was determined at 5, 10, 15, 20, 25 and 30°C. Mycelial growth was greatest at 25°C, with little growth below 10°C or above 30°C. There was evidence that stem lesion development was greater when inoculated plants were incubated for 5 days at 25°C and 100% RH than at a lower temperature (20°C) and humidity (70% RH).

From these results it was concluded the *F. oxysporum* is a relatively weak pathogen of sweet pepper. The conditions under which limited stem node lesions develop into spreading lesions have not been identified.

Occurrence of *F. oxysporum* on pepper seed, in glasshouse irrigation run-off water and in glasshouse air

A *Fusarium* sp., most probably *F. oxysporum*, was recovered from seeds of cvs Fiesta and Kelly. This result is in accordance with that previously found (HDC Project PC 232a, February 2006) where *Fusarium* was recovered from seed of cvs Britney, Fiesta and Special at rates of 0.8-3.4%. In this study, *Fusarium* was not recovered after hypochlorite treatment of seed (1% hypochlorite for 5 mins).

A range of *Fusarium* types were recovered from irrigation run-off water and from passive spore sampling of the air of glasshouses containing pepper crops. Some isolates of *Fusarium* from seed, air and water were identified as *F. oxysporum*.

Investigation of systemic infection

Isolation from the stem base of pepper plants taken from a commercial crop at the end of a season (November 2006) revealed a very low incidence of infection with *F. oxysporum*. The incidence of internal stem base infection was no greater in plants that had shown Fusarium

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stem node lesions during the season than in plants without this symptom. This result suggests that systemic vascular infection is not the source of Fusarium stem node lesions.

Isolation from internal tissues of visibly healthy pepper fruit revealed a low incidence of *Fusarium* on seed, the tissue supporting seeds, the fruit wall and within the fruit stalk. This lack of a consistent association of fruit stalk infection with internal fruit infection does not support the hypothesis that *Fusarium* enters fruit via the stalk. Possible alternative entry points are through the flower, blossom end rot damage sites and around the calyx.

Susceptibility of some protected vegetable crops of F. oxysporum isolated from pepper

Inoculation of roots and fresh leaf scars of young cucumber, lettuce, pepper and tomato plants with a conidial suspension of *F. oxysporum* obtained from pepper did not result in any permanent wilting or stem lesion development in any of the crops within 9 weeks. This result indicates that the *F. oxysporum* associated with fruit and stem rot of pepper is not strongly pathogenic to any of the crops.

Occurrence of Fusarium fruit rot and stem rot on pepper crops in the Lee Valley – 2006

Six nurseries in the Lee Valley growing glasshouse crops of sweet pepper were examined for evidence of Fusarium fruit and stem rot in April and September 2006. Sporulating *Fusarium* was found on all nurseries on attached or fallen fruit, most commonly on small, aborted fruit. A wide range of varieties was affected. When examined microscopically in the laboratory, the spores on most samples were consistent morphologically with *F. oxysporum*. Fusarium stem node lesions and fruit stalk browning were found in one glasshouse on one nursery. This result indicates the *F. oxysporum* is relatively common in pepper crops in the Lee Valley but causes stem rot only very rarely.

In June 2006, a sample of cv. Fiesta fruit that had developed pale brown spots and dimples during storage in a packhouse was received. *F. oxysporum* was isolated from the inside of fruit beneath visible symptoms. The problem was reported to have developed on around 5-10% of packed fruit during cool storage in a packhouse. This result indicates the *F. oxysporum* can cause a post-harvest fruit rot.

Occurrence of Fusarium stem rot and internal fruit infection in one crop – 2006

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On a nursery with a history of Fusarium stem and fruit rot, over 1,200 stems in a crop of cv. Special were examined for stem node and fruit stalk lesions at 7-28 day intervals between 29 March and 11 July. Lesions were cut out as they were found and examined for fungi by isolation onto agar. Stem node lesions (108 in total) were more common than fruit stalk lesions (30). *Fusarium*, most probably *F. oxysporum* was recovered from 52% of the stem node lesions and from only 11% of fruit stalk lesions. The cumulative number of stem node lesions over the monitoring period represented 8.9% of stems affected, assuming one lesion per stem. Single affected stems were more common than clusters of affected stems. Lesions occurred more commonly in April-May (1.7 - 1.9% of stems recorded affected at each visit) than in June – July (0 – 1.2% of stems), few lesion were reported to have developed after July. Only one *Fusarium* spreading stem lesion (c. 1 m long) was found, and this occurred outside of the monitoring area.

Samples of 50 visibly healthy mature fruit were collected in March, May, June, July and September and examined for *Fusarium* on seed or internal tissues. *Fusarium* was confirmed in 13, 6, 2, 8 and 6% of fruit at these times respectively.

Financial benefits

Stem and fruit rot of sweet pepper caused by *Fusarium oxysporum* has caused significant losses on one nursery in England for several years in succession. In 2005 the disease on this nursery was estimated to have caused losses in excess of £20,000 through a combination of staff costs in cutting out nodal lesions to prevent stem death and unmarketable fruit. In 2006 the causal fungus was found to be present on several nurseries in the Lee Valley and has caused stem lesions on two and a post-harvest fruit rot, resulting in the loss of 10-15% of packaged fruit during storage, on a third. The potential financial benefit to be gained from a greater understanding of Fusarium stem and fruit rot of sweet pepper is reduced losses during crop production, reduced losses between harvest and sale and reduced returns from supermarkets due to customer complaints.

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Action points for growers

- Note the symptoms of sweet pepper Fusarium fruit rot and stem rot caused by *F. oxysporum* (see the photographs in HDC report PC 232a, February 2006).
- Stem lesions appear to develop most commonly during March – May. Spreading stem lesions caused by *F. oxysporum* can appear similar to those caused by grey mould (*Botrytis cinerea*) and *Sclerotinia* (*S. sclerotiorum*). If in doubt about the cause of a fruit or stem rot, seek expert advice.
- Prompt removal of attached and fallen aborted fruit may reduce the risk of *Fusarium* infection in a crop. Such fruit are frequently colonised by *Fusarium* and could act as a source of infection.
- There is limited evidence that the disease is favoured by warm temperatures (20-25ºC) and a high humidity. Use of ventilation to reduce humidity may reduce risk of the disease.
- Growers should be aware *Fusarium* symptoms can develop on fruit during packing and should continue to monitor fruit during this stage of production.

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SCIENCE SECTION

1. Pathogenicity of *Fusarium oxysporum* to pepper

Introduction

In an initial study on this disease (PC 232a), *Fusarium oxysporum* was found consistently associated with an internal rot of pepper fruit. Occasionally the fungus was also found associated with limited lesions at stem nodes, with girdling stem lesions and with fruit stalk lesions. Sporulation of *F. oxysporum* was observed on aborted fruit indicating they may be a source of spores leading to fruit and/or stem rot. The fungus was generally isolated in pure culture from fruit and stem lesions indicating it is probably the cause of rot symptoms. However, inoculation of stem wounds and fruit stalks of mature pepper plants on a nursery with conidia of *F. oxysporum* from pepper, using a mixture of isolates, failed to cause rot after 9 weeks, although the fungus was recovered from tissue at the inoculation point. Inoculation of flowers and young fruit on mature plants failed to cause an increase in the incidence of Fusarium fruit rot. Further experiments were therefore devised to try and confirm that *F. oxysporum* is a cause of fruit and stem rot of pepper and to identify the conditions favourable to disease development.

1.1 Pathogenicity of *F. oxysporum* isolates to detached pepper fruit and fruit stalks using mycelial inocula

Objective

To determine the pathogenicity of mycelial inocula of *F. oxysporum* isolates from pepper and other hosts to detached pepper fruit.

Materials and methods

In May 2006 discs (1 cm diameter) were cut from the side of visibly healthy pepper fruit, cv. Boogie, laid on moist filter paper in plastic Petri dishes, and inoculated in the centre of the inner surface with a 6 mm plug of *F. oxysporum* on potato dextrose agar (PDA). The isolates are listed in Table 1.1. Transverse sections of pepper fruit stalks, around 5 mm thick, were similarly prepared and inoculated. There were 20 replicate fruit and stalk pieces per isolate. Control tissues were inoculated with PDA alone. Inoculated tissues were incubated at 20°C and examined daily for 5 days for evidence of rot. The extent of tissue rot was assessed on a 0-4 index:

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0 – no rot visible

1 – brown rot extending beyond inoculation point but not to the edge of the disc

2 – brown rot extending to the edge of the disc but not penetrating down the side

3 – rot extending to the disc edge and at least half-way through the thickness of the disc

4 – disc completely rotted

Results and discussion

In an initial experiment, all the isolates of *F. oxysporum* tested caused a rot of pepper fruit and fruit stalk tissues (Table 1.1); the uninoculated fruit and fruit stalk discs remained unrotted. One isolate (VGN) obtained from a pepper stem lesion was notably more damaging than the other isolates. In a second experiment, an isolate from a pepper stem lesion and an isolate from a cucumber stem lesion were both very aggressive to pepper (Table 1.2). These results confirm that some isolates of *F. oxysporum* are capable of rotting detached pepper fruit and fruit stalk tissue from a mycelial inoculum. Isolates from other hosts (cucumber, *Hebe, Lisianthus*, stock and tomato) also rotted pepper tissue in this test; there was evidence that some isolates from pepper and cucumber are more damaging to pepper than isolates from other hosts, but there was not a clear grouping into pathogenic and non-pathogenic isolates.

Table 1.1: Pathogenicity of *F. oxysporum* isolates to detached pepper fruit and fruit stalks from mycelial inocula – Experiment 1

No	Isolate code	Obtained from:	No. of inoculation sites of 20 (and standard error in brackets) with rot index >2 after 4 days on:			
			Fruit side		Fruit stalk	
1	Uninoculated	-	0	(0.00)	0	(0.00)
2	VGN	Pepper stem	20	(0.00)	19	(0.05)
3	AR06/54	Pepper fruit	4	(0.09)	4	(0.09)
4	K	Pepper petiole	11	(0.11)	-	-
5	H	Pepper stem	9	(0.11)	-	-
6	I	Pepper stem	13	(0.11)	-	-
7	J	Pepper calyx	12	(0.11)	-	-
8	AR05/195	*Hebe* stem	9	(0.11)	12	(0.11)
9	Lincs	*Lisianthus* stem	11	(0.11)	-	-
10	AR03/76	Stock stem	11	(0.11)	8	(0.11)
11	AR05/205	Tomato stem	8	(0.11)	-	-

- not tested

Table 1.2: Pathogenicity of *F. oxysporum* isolates to detached pepper fruit and fruit stalks from mycelial inocula – Experiment 2

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No	Isolate code	Obtained from:	No. of inoculation sites (of 20) (and standard error in brackets) with rot index >2 after 4 days on:			
			Fruit side		Fruit stalk	
1	Uninoculated	-	11	(0.11)	3	(0.08)
2	VGN	Pepper stem	20	(0.00)	11	(0.11)
4	AR06/67	Cucumber stem	2	(0.00)	20	(0.00)
5	AR06/68	Cucumber stem	17	(0.08)	8	(0.11)

1.2 Pathogenicity of *F. oxysporum* to young pepper plants using conidial inocula

Objective

To determine the pathogenicity of *F. oxysporum* to young pepper plants using conidia.

Methods

Pepper plants cv. Special at the 8-10 true leaf stage were inoculated with a conidial suspension of *F. oxysporum* using a mixture of isolates obtained from pepper fruit, stem lesions and seed. Plants were inoculated at fresh de-leafing scars (two/plant) or by drenching conidia over the rockwool cube (50 mL/plant of a 5 x 10^5 spores/mL suspension). Control plants were inoculated with sterile distilled water. Inoculated plants in rockwool cubes were placed on capillary matting in gravel trays and grown for 8 weeks in a heated glasshouse. There were six replicate plants per treatment. Plants were examined for wilt, stem lesions or other symptoms at intervals. At the end of the experiment in April 2006, isolations were made from vascular tissue within the stem base, from inoculated leaf scars and from roots.

Results and discussion

Transient wilting of some plants was observed at 1 and 4 weeks after inoculation especially of plants inoculated at a fresh leaf scar, but no permanent wilting developed (Table 1.3). No stem lesions developed and all plants appeared healthy at the end of the experiment. There was a much greater degree of root browning on plants that were inoculated on the roots with *F. oxysporum* (71% of visible root area) than on uninoculated and leaf-scar inoculated plants (6-9%).

F. oxysporum was recovered from all leaf scars inoculated with the fungus, and from none of the uninoculated leaf scars on control plants. *F. oxysporum* was recovered from within the stem base and from roots of both inoculated and uninoculated plants (Table 1.3). These results indicate that the *F. oxysporum* associated with pepper fruit and stem rot is not

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strongly pathogenic to pepper. No stem lesions, as occur occasionally in commercial crops, developed following inoculation. The re-isolation of *F. oxysporum* from leaf scars 8 weeks after inoculation indicates that infection has occurred. Possibly inoculum level, host condition and environmental variables influence symptom development.

Table 1.3: Occurrence of wilting and recovery of *F. oxysporum* from pepper plants following conidial inoculation of roots and leaf scars

Treatment (inoculation site)	No. plants (of 6) wilting		No of plants (of 6) from which *Fusarium* was isolated (standard error in brackets):		
	14 Feb	7 Mar	Roots	Stem base	Leaf scar
1. Leaf scar – control	1 (0.15)	1 (0.15)	2 (0.33)	1 (0.15)	0 (0.00)
2. Leaf scar – inoculated	4 (0.19)	1 (0.15)	5 (0.83)	2 (0.19)	6 (0.00)
3. Roots control	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
4. Roots – inoculated	2 (0.19)	0 (0.00)	3 (0.50)	2 (0.19)	-

1.3 Pathogenicity of *F. oxysporum* to young pepper plants using mycelial inocula

Objective

To determine the pathogenicity of mycelial inocula of *F. oxysporum* obtained from pepper to fresh stem wounds on young pepper plants under controlled temperatures and humidity.

Method

Potted pepper plants cv. Fiesta at the 6-8 true-leaf stage were inoculated at fresh leaf scars (two per plant) with mycelial plugs (6 mm diameter) of *F. oxysporum* (isolate I) from pepper. Plugs were held in place with cling film. Immediately after inoculation, half of the plants were loosely enclosed in a polythene bag to create a high humidity environment. The plants were grown in controlled environment (CE) cabinets at 20°C or 25°C and at around 70% (low) and 100% (high) RH for 5 days and then transferred to a heated glasshouse. There were 5 replicate plants per treatment and plants were arranged in a fully randomised structure within each CE cabinet and in the glasshouse. A first experiment used leaf nodes 1 and 2 from the stem base; a second experiment used nodes 2 and 3. Results were examined by analysis of variance with the uninoculated treatments excluded.

Treatments were:

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1. Uninoculated, low humidity, 20°C
2. Inoculated, low humidity, 20°C
3. Uninoculated, high humidity, 20°C
4. Inoculated, high humidity, 20°C
5. Uninoculated, low humidity, 25°C
6. Inoculated, low humidity, 25°C
7. Uninoculated high humidity, 25°C
8. Inoculated, high humidity, 25°C

Results and discussion

At eight days after inoculation, pale brown lesions with a dark brown border had developed at most of the sites inoculated with *F. oxysporum* and at none of the uninoculated control sites in both experiments. Lesion spread was greater up and down the stem than around it, extending to around 10 mm after 8 days. In experiment 1, there was a greater proportion of lesions at stem node 2 ($^{20}/_{20}$) than at the more woody stem node 1 ($^{11}/_{20}$). Consequently stem node 1, the lowest on the stem, was not used in subsequent experiments. More consistent lesion development occurred in experiment 2 when nodes 2 and 3 were used.

In the first experiment, lesion length after 8 days was greatest at 25ºC and 100% RH. The effect of humidity was statistically significant (Table 1.4). In the second experiment, neither temperature nor humidity affected lesion size (Table 1.5). After 6 weeks, lesion length did not exceed 20 mm and none had completely girdled the plant stem. No wilting occurred. Isolations were made from a sample of stem lesions at the end of the experiment and *F. oxysporum* was recovered consistently.

This experiment confirms that *F. oxysporum* obtained from pepper is able to cause stem node lesions on pepper from a mycelial inoculum, in contrast to earlier experiments using conidial inocula, which failed to cause lesions. A high inoculum level such as that found in mycelium on agar could occur in a crop in the form of senescent tissue (e.g. trimming stubs, dead side shoots) around stem nodes, particularly towards the end of a cropping season. It is interesting to note that in experiments investigating stem rot of glasshouse sweet pepper by *Fusarium solani* (Fletcher, 1994), mycelial inoculation of fresh de-leafing scars resulted in lesions after 10 days and stem girdling after 20 days, where soft stem tissue was inoculated. This result is similar to ours in that the development of stem lesions was less on the more woody tissue at the stem base. In commercial crops affected by *F. solani* stem rot, significant crop loss did not occur until October, the final month of cropping, a time that coincided with wet weather and a large amount of senescent lateral tissue.

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Table 1.4: Effect of temperature and humidity on infection of pepper from mycelial inocula of *F. oxysporum* – Experiment 1

Treatment	Temp (ºC)	RH	Number of stem lesions (out of 10 wound sites)	Mean length of stem lesion (mm) after 8 days
1. Uninoculated	20	Low	0	0.0
2. Inoculated	20	Low	6	2.7
3. Uninoculated	20	High	0	0.0
4. Inoculated	20	High	8	3.6
5. Uninoculated	25	Low	0	0.0
6. Inoculated	25	Low	8	2.3
7. Uninoculated	25	High	0	0.0
8. Inoculated	25	High	9	6.7
Significance: humidity (H)				<0.001
Significance: temperature (T)				NS
Significance: T x H				NS

Results examined excluding the uninoculated treatments; NS - not significant.

Table 1.5: Effect of temperature and humidity on infection of pepper from mycelial inocula of *F. oxysporum* – Experiment 2

Treatment	Temp	RH	Number of stem lesions (out of 10 sites)	Mean length of stem lesion (mm) after 8 days
1. Uninoculated	20	Low	0	0.0
2. Inoculated	20	Low	10	8.2
3. Uninoculated	20	High	0	0.0
4. Inoculated	20	High	10	7.8
5. Uninoculated	25	Low	0	0.0
6. Inoculated	25	Low	10	7.6
7. Uninoculated	25	High	0	0.0
8. Inoculated	25	High	10	9.3
Significance: humidity (H)				NS
Significance: temperature (T)				NS
Significance: T x H				NS

Note: results examined excluding the uninoculated treatments; NS not significant.

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1.4 Effect of temperature on growth of *F. oxysporum*

Objectives

To determine the effect of temperature on mycelial growth of *F. oxysporum* isolates obtained from pepper.

Methods

The effect of temperature on the growth rate of three isolates of *F. oxysporum* (isolates H, I and V) obtained from pepper stem, node and fruit, was determined. Mycelial plugs (6 mm diameter) of *F. oxysporum* taken from the leading edge of a culture were placed in the centre of PDA plates. Plates were incubated at 5, 10, 15, 20, 25, and 30°C. The diameter of mycelial growth was measured and a growth rate (mm/day) calculated. There were three replicate plates per isolate and two diameters at 90° to each other were measured.

Results and discussion

Mycelial growth was greatest at 25°C for all three isolates and there was little growth below 10°C or at 30°C (Fig 1.1).

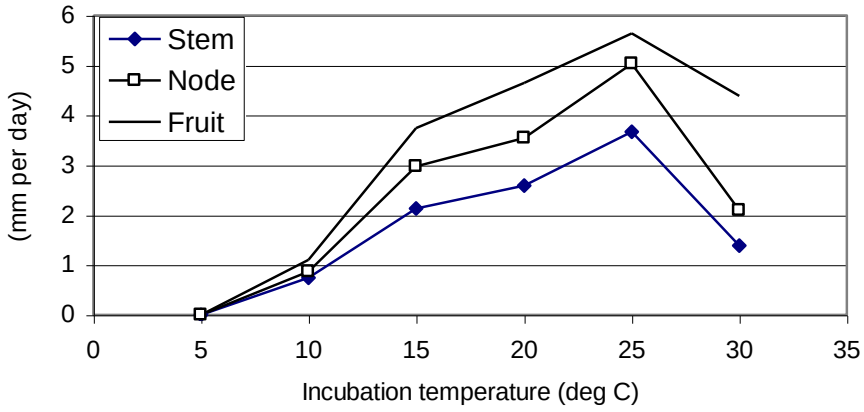

Fig. 1.1: Effect of temperature on mycelial growth rate (mm/day) of three isolates *of F. oxysporum* obtained from different tissues of pepper plants.

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2. Occurrence and identification of *Fusarium* species on pepper seed, in glasshouse irrigation run-off water and in glasshouse air

Introduction

In initial studies undertaken in PC 232a, isolates of *Fusarium* species were recovered at a low level from some pepper seed (0.8-3.4%), and on passive spore traps placed in a pepper crop to sample the glasshouse air. A number of colony types were recovered from the glasshouse air, some of which resembled isolates obtained from pepper fruit and stem rots.

In this study, two further lots of seed were examined for occurrence of *Fusarium* species. Work was undertaken to confirm the identity of the *Fusarium* colony types commonly isolated from pepper crops and glasshouse air. Sampling of glasshouse air and irrigation run-off water was undertaken in spring 2006 to determine if air and water can be sources of *F. oxysporum*.

2.1 Occurrence of *Fusarium* species on pepper seed

Method

Two lots of packeted pepper seeds, cvs Fiesta and Kelly, were examined for occurrence of *Fusarium* by plating 250 seed per lot onto PDA+S. For cv. Fiesta, seed was plated both directly and after surface disinfection in sodium hypochlorite (1% for 5 minutes). For cv. Kelly, seed was plated directly. Plates were examined after 14-21 days for colonies typical of *Fusarium* species. Representative isolates were examined microscopically for macro- and micro-conidia typical of *F. oxysporum*.

Results and discussion

Fusarium sp., probably *F. oxysporum*, was recovered from both seed lots, from 1.6% of cv. Fiesta and from 12% of cv. Kelly. No *Fusarium* sp. was recovered from hypochlorite-treated seed. Further work is required to confirm species identification of these isolates (e.g. by a DNA test) and to confirm the pathogenicity to pepper plants of isolates recovered from seed.

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2.2 Occurrence of *Fusarium* species in glasshouse air and water

Objective

To determine in a pepper crop if irrigation water applied to rockwool slabs or run-off water draining from slabs contains *F. oxysporum.*

Methods

A 500 ml sample of nutrient solution was collected from one dripper and 500 ml of waste solution from the main drain tank (emptied daily) in each of four glasshouse blocks at a commercial pepper nursery in April 2006. Samples were transported to the laboratory in a coolbox and spores allowed to settle to the bottom for 24 h at 4°C. The upper 450 ml was then discarded and the remaining 50 ml was spun down for 15 mins at 2,000 rpm. The residue was re-suspended in 1 ml of sterile distilled water (SDW) and 50 µL aliquots were spread onto five Petri plates of Komada's medium. Plates were incubated at 21°C in the light. The number of *Fusarium* colonies was recorded after 5 and 10 days. As a positive control, a known suspension of *F. oxysporum* in 500 ml water was prepared and processed with the test samples. SDW was used as a negative control.

The air was sampled passively in two glasshouses by exposing 20 plates of Komada's medium for 24 h. Plates were distributed throughout the pepper crops.

Fusarium stem rot symptoms were present in all houses at the time of water and air sampling. *Fusarium* sp. had been confirmed on aborted fruit in the crop prior to sampling.

Results and discussion

Fusarium sp. was confirmed in water and air samples (Table 2.1). There were large numbers of colony forming units (cfu) in the waste solution and only rarely was *Fusarium* found in the irrigation water. Most colonies were peach or white coloured. Large numbers of colonies developed on the plates exposed to glasshouse air for 24 hours. Colony types were predominantly white/purple, white/cherry and salmon. Examination of a sample of isolates by CSL confirmed that some but not all isolates were *F. oxysporum* (see Section 2.3).

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Table 2.1: Detection of *Fusarium* in irrigation water, run-off water and the air of a glasshouse pepper crop affected by Fusarium stem rot – April 2006

Sample	No. agar plates with *Fusarium* after 10 days	Mean No. cfu /100 ml	Main colony type
Water			
Positive control in water	6/6	240	Peach
Negative control	0/6	0	-
Irrigation water			
House 1	1/6	0	White
House 2	0/6	0	-
House 3	3/6	20	Peach
House 4	0/6	0	-
Run-off water			
House 1	-	6	White
House 2	-	34	White
House 3	-	116	White
House 4	-	170	White
Air			
House 1	20/20[a]	-	Various
House 2	18/18[a]	-	Various

[a] >50 colonies/plate

2.3 Identification of *Fusarium* isolates

Objective

To determine the identity of selected isolates of *Fusarium* sp. obtained from pepper seed, pepper crops and glasshouse air and irrigation water.

Method

Twenty isolates, selected to provide a range of colony types and sources, were cultured on PDA and sent to CSL for identification based on morphological characteristics. At CSL the 20 isolates were grouped according to morphological features into six groups. The DNA from a representative isolate was then sequenced to determine species identity.

Results and discussion

Seventeen of the isolates were confirmed as *F. oxysporum* (Table 2.2). The other isolates were identified as *F. graminearum* (one) and *Acremonium* sp. (two).

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These results confirm that the fungus associated with pepper fruit and stem rot is *F. oxysporum,* and that isolates of *F. oxysporum* may occur on seed, aborted fruit, in glasshouse air and irrigation run-off water and that isolates show great variation of colony type.

Table 2.2: Identification of *Fusarium* isolates associated with pepper seed, pepper fruit and stem rot and found in pepper glasshouses

Source of isolate	ADAS code	Colony colour (top/bottom)	Group (CSL)	Identification
Seed (from isolations made in 2005)	L	White/dark cherry	1	*F. oxysporum*
	M	White/pale Peach	2	*F. oxysporum*
	O	Peach	1	*F. oxysporum*
	W	Peach	1	*F. oxysporum*
Stem node rot	H	Peach	1	*F. oxysporum*
	I	White	2	*F. oxysporum*
	28/4/06	Peach	1	*F. oxysporum*
	26/5/06	White/cream	5	*Acremonium sp.*
Fruit stalk lesion	28/4/06	White/dark Cherry	2	*F. oxysporum*
	26/5/06	White/cream	2	*F. oxysporum*
Rotted fruit	U	Peach	1	*F. oxysporum*
	V	Peach	1	*F. oxysporum*
Aborted fruit	Q	White/dark Cherry	2	*F. oxysporum*
	R	Peach	2	*F. oxysporum*
Roots	GH4 row 1	White/pale Peach	1	*F. oxysporum*
	GH4 row 4	White/cherry	3	*F. oxysporum*
Water	GH4 run-off	White/dark Cherry	3	*F. oxysporum*
	GH2 run-off	White	6	*Acremonium sp.*
Air	B	Pink/cherry	4	*F. graminearum*
	E	Peach	1	*F. oxysporum*

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3. Investigation of systemic infection

3.1 Occurrence of *Fusarium* within stems

Introduction

The recovery of *F. oxysporum* from pepper seed (see section 2.1), and the acknowledgment that *F. oxysporum* occurs as a vascular wilt pathogen in many crops (e.g. cucumber, cyclamen, *Hebe*, stock, tomato), raises the question as to whether stem lesions on pepper arise from vascular infection originating from the use of infected seed. In order to test this hypothesis, the stem bases of plants that had, and had not, developed stem node lesions were collected and examined for *F. oxysporum*.

Method

In a crop of cv. Special, all stems that developed Fusarium stem node lesions during March – July 2006 in one area of the crop were tagged. There were very few new Fusarium stem node lesions that developed after July. In November 2006, 49 tagged and 50 untagged stems (i.e. stems which had not developed Fusarium stem node lesions) were cut down and a 30 cm length of the stem removed from just above where the stem splits to form two heads. The stem sections were examined in the laboratory for vascular staining and transverse sections were plated onto PDA + streptomycin. Isolation plates were examined for *Fusarium* after 14 days.

Results and discussion

There was no vascular browning in the base of any of the stems. On the PDA plates, there was growth of *Fusarium* consistent with *F. oxysporum*, from just two stems; one each from the sets of tagged and untagged stems. The result of this experiment suggests that systemic vascular infection is not the source of Fusarium stem node lesions.

3.2 Location of *Fusarium* within pepper fruit

Introduction

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Within fruit, sporulating *F. oxysporum* is sometimes found on developing seeds in the central cavity, and associated with pale brown lesions on the internal wall of intact, symptomless fruit. It is unclear how this internal infection arises. The occasional recovery of *F. oxysporum* from fruit stalk lesions gives some support to the hypothesis that fruit infection arises from systemic infection through the fruit stalk.

Fusarium sporulation can also sometimes be found as a white powdery growth, underneath the calyx margin at the proximal end of fruit. It is possible that occurrence of *Fusarium* here may lead to infection within fruit of the white tissue suspended from the calyx that supports seed development. Samples of fruit with and without external symptoms of *Fusarium* were examined to provide quantitative information of the location of *Fusarium* in pepper fruit.

Materials and methods

Isolations onto PDA were made from 50 apparently healthy red fruit (cv. Special), taking three pieces of tissue (c 2 × 2 mm) from within the fruit stalk and from the white tissue supporting developing seed. Isolations were also made from within the fruit stalk of four fruit with visible Fusarium rot at the distal end and internally.

Results and discussion

Of 50 fruit with no symptoms of Fusarium rot, *Fusarium* was recovered from tissue supporting seed in six fruit, from within the fruit stalk of five fruit, and from both locations in just one fruit (Table 3.1). Of four fruit with visible Fusarium rot, *Fusarium* was recovered within the fruit stalk and from firm white tissue above the seed of all fruit.

The lack of a consistent association of fruit stalk infection with internal fruit infection in visibly healthy fruit does not support the hypothesis that *Fusarium* is entering fruit from the fruit stalk.

Possible alternative entry points leading to internal fruit infection are the flowers, tissue affected by blossom end rot and around the calyx. On semi-mature and mature pepper fruit showing visible symptoms of Fusarium rot, infection often occurs around the distal end, where remnants of flower parts are occasionally visible. This suggests that infection may originate from flower infection. Fusarium rot at the distal end is also sometimes associated with blossom end rot, which usually appears in this position on fruit. Infection via the flower, or via tissue showing blossom end rot, both infer that fruit infection arises from outside the plant (e.g. as spores carried in the air or on bees).

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Table 3.1: Recovery of *F. oxysporum* from different locations within apparently healthy fruit

Type of fruit	No of fruit (of 50) from which *Fusarium* recovered at:				
	On seed	Internal tissue	Fruit wall	Fruit stalk	Fruit and stalk
Healthy	2	5	1	6	1

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4. Susceptibility of some protected vegetables to *F. oxysporum* isolated from pepper

Introduction

In order to devise effective control strategies, it is necessary to understand the biology of *F. oxysporum* associated with pepper fruit and stem rot. This includes the host range. An experiment was devised to test the susceptibility of cucumber, lettuce and tomato to *F. oxysporum* from pepper using conidial inocula applied to roots and stem wounds.

Methods

Plug plants of cucumber, lettuce, pepper and tomato at the 2-4 true leaf stage were potted into Levington M3 compost in 9 cm diameter pots (M2 compost for lettuce). They were inoculated 1 week later by drenching conidia (10^6/plant in 30 ml water) over the roots using a mixture of three isolates (Q, I and V, from aborted fruit, nodal lesion and rotted mature fruit) obtained from pepper. Stem wounds were created by removal of a lower leaf and a 50 µL drop of spore suspension (10^7 spores/ml) was immediately applied. Control plants were left uninoculated. Plants were grown for 9 weeks in a heated glasshouse. Plants were fed weekly with potassium nitrate (150 g/L). Plants were placed on capillary matting in gravel trays to prevent cross-infection through drainage water. There were 10 plants per plot and four replicate blocks. Results were examined by analysis of variance (ANOVA).

Plants were examined at 3, 6, and 8 weeks after inoculation for evidence of stem lesions, wilting plants or other symptoms. Pepper fruits were left on the plant and examined for Fusarium rot. At 9 weeks after inoculation, all the plants in two blocks were examined for *Fusarium* within the stem and on roots. Transverse sections around 1 cm thick were taken at intervals throughout the stem (every 5 cm for lettuce, 10 cm for pepper, 20 cm for cucumber and tomato up to 1 m), surface sterilised in sodium hypochlorite and plated onto PDA. Five root pieces per plant (1 cm long) were tested. Stem tissue was also examined for vascular browning, in the stem base, mid-stem and upper stem. Inoculated leaf scar tissue was excised, surface sterilised, and plated onto agar to check for viable *F. oxysporum*. Aborted pepper fruit were collected from plot trays and examined for *Fusarium* sporulation immediately and after incubation.

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Results and discussion

Neither inoculation of roots nor inoculation of fresh leaf scars with conidia of *F. oxysporum* from pepper resulted in symptoms on any of the four crops. None of the pepper fruit developed Fusarium rot. Stem lesions developed on two pepper plants adjacent to rotting fruit; *Botrytis cinerea* was isolated from both rotting stem tissue and the fruit. At 9 weeks after inoculation, *Fusarium* was isolated from the stems of many plants, both inoculated and uninoculated (Table 4.1); only a few plants showed vascular staining and this was not consistently associated with inoculation.

Fusarium was recovered at a high incidence from the roots of all plants (Table 4.2) and from most leaf scar inoculation sites. *Fusarium* was confirmed on 13/23 aborted fallen pepper fruit collected from the floor, occurring on fruit from inoculated and uninoculated plants at a similar incidence.

These results indicate that the *F. oxysporum* associated with Fusarium fruit and stem rot of pepper is not strongly pathogenic to cucumber, lettuce, pepper or tomato. The recovery of *F. oxysporum* at a high incidence from within roots and stem tissue of both inoculated and uninoculated plants suggests a high level of symptomless infection. This may have occurred due to transmission between plots, or may occur naturally (e.g. from seed).

Table 4.1: Recovery of *Fusarium* from surface-sterilised stem tissue 9 weeks after inoculation with *F. oxysporum*

Treatment				Proportion stem sections with *Fusarium* (%)	Maximum height recovered (cm)	No. plants with stem browning (of 30)
	Crop	Site	Inoc			
1	Cue	-	-	29/60 (48)	100	0
2	Cue	Leaf	4	24/60 (40)	100	1
3	Cue	Root	4	33/60 (55)	100	2
4	Let	-	-	21/52 (40)	30	4*
5	Let	Leaf	4	26/58 (45)	30	1
6	Let	Root	4	49/73 (67)	40	0
7	Pep	-	-	30/45 (67)	30	1
8	Pep	Leaf	4	16/40 (40)	20	0
9	Pep	Root	4	9/52 (17)	40	1
10	Tom	-	-	40/60 (67)	100	0
11	Tom	Leaf	4	9/53 (17)	100	0
12	Tom	Root	4	8/59 (14)	100	0

* Infected by *Rhizoctonia;* Inoc - inoculated

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Table 4.2: Recovery of *Fusarium* from surface-sterilised roots and inoculated leaf scars 9 weeks after inoculation with *F. oxysporum*.

	Treatment			No plants (of 10) from which *Fusarium* recovered		
	Crop	Site	Inoc	Stem	Roots	Leaf scar
1	Cue	-	-	9	9	-
2	Cue	Leaf	4	8	8	10
3	Cue	Root	4	10	10	-
4	Let	-	-	5	5	-
5	Let	Leaf	4	7	9	5
6	Let	Root	4	10	2	-
7	Pep	-	-	10	10	-
8	Pep	Leaf	4	10	9	8
9	Pep	Root	4	5	5	-
10	Tom	-	-	10	10	-
11	Tom	Leaf	4	8	10	8
12	Tom	Root	4	4	10	-

Inoc - inoculated

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5. Occurrence of Fusarium fruit and stem rot on pepper crops in the Lee Valley - 2006

Introduction

F. oxysporum associated with a fruit and stem rot of pepper has been confirmed on one nursery in the UK where it has occurred each year since at least 2004. Defra Plant Health were consulted when the problem was first shown to be different from the fruit and stem rot of pepper caused by *Fusarium solani*; they determined that no statutory action was required. It is possible that the disease may also be present on other nurseries in the UK and has not been diagnosed. It was therefore decided to examine pepper crops on six nurseries in the Lee Valley for symptoms of Fusarium fruit and stem rot. On the original nursery, the disease was monitored at regular intervals to provide information on how the disease progresses with time.

5.1 Occurrence of *Fusarium* fruit and stem rot in commercial pepper crops

Objective

To determine if, and to what extent, Fusarium fruit and stem rot occurs in pepper crops in the Lee Valley in 2006.

Methods

Crops were examined on six nurseries in April and on three nurseries in September 2006. On each nursery several rows were walked in one or more houses and the crops examined for fruit and stem rot symptoms. Samples were collected for laboratory examination to confirm the identity of fungi associated with symptoms.

Results and discussion

In April 2006, sporulating *Fusarium* was found on fallen fruit (usually mid-size to mature) on all nurseries (Table 5.1). When examined in the laboratory the appearance of macro and micro-conidia of most samples was consistent with *F. oxysporum*. *Fusarium* was also found occasionally on attached aborted fruit. Stem node lesions and fruit stalk browning were found on only one nursery in addition to the nursery where the problem has occurred for several years. Varieties with *Fusarium* on stem lesions were Boogie, Fiesta, Ferrari and Special. Varieties with *Fusarium* on fallen fruit were Boogie, Fiesta, Ferrari, Plenty, Prego, Special and Tahiti. No *Fusarium* was found on Forward or Romero. All of the crops were grown on rockwool slabs. These results indicate that *F. oxysporum* was commonly present

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in pepper crops and was associated with fruit rot, but only rarely (2 out of 6 nurseries) did it cause stem rot.

In June 2006, samples of fruit cv. Fiesta were received from another nursery in the Lee Valley. Fruit had appeared marketable when graded but developed brown spots on the outside and mould on the inside after storage in the packhouse for up to 6 days. *Fusarium oxysporum* was confirmed within affected fruit. The problem was reported to have caused post-harvest rot in 5-10% of packed fruit during cool storage.

In September 2006, *Fusarium* was again confirmed on three of the nurseries re-visited in the Lee Valley. Plant losses due to *Fusarium* were very low.

In conclusion, *F. oxysporum* appears to be relatively common in pepper crops in the Lee Valley but damage by the fungus is relatively rare.

Table 5.1: Occurrence of Fusarium stem lesions, and sporulating *Fusarium* on fallen fruit, in pepper crops on six nurseries in the Lee Valley – April 2006

Nursery and block	Variety	Fusarium stem node rot and fruit stalk browning	*Fusarium* on fallen fruit	Other symptoms
1. New block	Ferrari	4	4	
F block	Ferrari		4	
2. Main	Plenty		4	
	Fiesta		4	
	Boogie		4	
	Ferrari		4	
	Palermo			
3. Various	Prego		4	
	Spirit			Wilting plant
	Special			
4. Main	Tahiti		4	*Fusarium* on attached fruit
5. Various	Fiesta		4	
	Boogie			
6. Various	Romero			
	Fiesta		4	
	Boogie		4	
	Plenty			
	Forward			

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5.2 Occurrence of Fusarium stem rot and internal fruit infection in one crop - 2006

Objective

To monitor the occurrence of stem node rot, fruit stalk browning and mature fruit rot in a pepper crop between April and July and to determine the association of *Fusarium* with each symptom.

Method

On a nursery with a history of Fusarium fruit and stem rot, all plants in two pathways in each of two crops of cv. Special were carefully examined at 7-28 day intervals between 29 March and 11 July. A total of 578 plants were examined in a glasshouse containing plants grown to the nursery standard conditions and 675 plants in an adjacent house containing plants grown under an energy-saving regime. The position along the row of each plant that developed a stem node rot or fruit stalk browning was noted. Lesions were cut out wherever they occurred. All lesions were examined for *Fusarium* by plating onto PDA. Additionally, samples of roots were collected twice, and samples of 50 mature class II fruit on four occasions. The incidence of fruit that showed internal *Fusarium* sporulation or seed discolouration was assessed immediately in the laboratory.

Results and discussion

The most common symptom was stem node lesions, with 20-24 lesions found at each inspection between 29 March and 24 May (Table 5.2). There was generally only one lesion per stem so this represents 1.7-2.0% of plants affected each time. The number of stem node lesions declined during June and none was found in July. By 5 July, the cumulative number of stems that had developed stem node lesions over the 3 month monitoring period was 108, representing 8.9% of stems assuming one lesion per stem. Fruit stalk lesions were found at a relatively high incidence in late March and late May, but few were found at other times. The incidence of stem node and fruit stalk lesions was reported to have remained very low during July, August and September. *Fusarium* was confirmed within 13, 6, 2, 8 and 6% of fruit in March, May, June, July and October respectively.

Fusarium was isolated at a high incidence (> 50% of samples) from stem node lesions and from aborted flowers and fruit (Table 5.3). It was recovered at a low incidence from fruit stalk lesions (11.1%), and roots selected at random (8.6%). The level of recovery from stem node lesions varied with time.

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F. oxysporum was also isolated from occasional extensive stem lesions (1m long) found in the crop.

An examination of the location of affected plants (Appendix 1) revealed around four times as many single affected stems as clusters of two or more affected stems.

There was a significantly higher incidence of Fusarium stem node lesions in the energy saving than in the control compartment at each assessment date (Tables 5.4–5.5). However, there was no obvious difference in mean daily temperature or humidity deficit before and during the development of stem node lesions (weeks 1-19) that might explain this difference (see HDC report PC 227a, March 2007).

These results indicate that *Fusarium* is commonly associated with flower petals, aborted fruit and stem node lesions. It was rarely associated with fruit stalk browning, suggesting that this symptom may have a non-pathogenic cause. There was a rapid increase in the occurrence of stem lesions during April and May. No explanation was found to account for differing levels of Fusarium stem rot in two compartments.

Table 5.2: Occurrence of symptoms associated with *Fusarium* fruit and stem rot of pepper in a crop of cv. Special, March – July 2006

Date assessed	Incidence of symptoms on 1203 stems			Incidence of fruit with internal *Fusarium*
	Stem node lesion	Fruit stalk lesion	Attached fruit rot	
March 29	24	15	-	7/54
April 28	20	2	0	-
May 10	24	0	1	-
May 24	23	10	0	3/50
June 7	14	1	0	-
June 21	3	2	1	1/50
July 5	0	0	0	-
July 11	-	-	-	4/50
Sep 26	-	-	-	3/50
Total	108	30	2	

- not sampled.

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Table 5.3: Incidence of *F. oxysporum* isolated from different tissues and symptoms in a commercial crop – 2006

Date sampled	No. samples developing *F. oxysporum* / No. tested				
	Stem node lesion	Fruit stalk lesion	Fallen flower	Aborted fruit	Roots
March 29	21/24	1/15	7/8	19/19	11/200
April 28	2/20	1/2	-	-	-
May 10	5/24	0/0	-	-	8/20
May 24	19/23	1/10	-	-	-
June 7	8/14	0/0	-	-	-
% affected	52.4	11.1	87.5	100	8.6

Table 5.4: Effect of energy saving on occurrence of Fusarium stem rot in pepper between 29 March and July 5, 2006

Date Assessed[a]	Wk No.	Occurrence of stem node lesions[b]					
		Compartment 3 (energy saving)			Compartment 4 (control)		
		No. lesions	Cumulative plants affected		No. lesions	Cumulative plants affected	
			Number	%		Number	%
April 28	17	19	19	2.8	5	5	0.9
May 10	19	15	34	5.0	4	9	1.7
May 24	21	13	45	6.7	5	14	2.7
June 7	23	5	50	7.4	3	17	3.2
June 21	25	3	52	7.7	0	17	3.2
July 5	27	0	52	7.7	0	17	3.2

[a] All stem node lesions were removed from both compartments on 29 March, so the figures below represent the occurrence of lesions after this date.
[b]Two pathways (four crop faces) were examined in each compartment, comprising a total of 675 stems in the energy-saving compartment and 528 in the control compartment.

Table 5.5: Pearson chi-square test comparing the proportion of stem node lesions in two compartments (energy saving and control) on five assessment dates – 2006

Date assessed	Chi-square (1 df)	Probability level
April 28	5.29	0.021
May 10	9.55	0.002
May 24	10.24	0.001
June 7	9.88	0.002
June 21	11.02	<0.001

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6. Research on *Fusarium* internal fruit rot of pepper in Canada

A poster on *Fusarium* internal rot of pepper fruit in Alberta, Canada was presented at the 8th European Federation of Plant Pathology Congress in August 2006 (Kharbanda *et al.,* 2006). The key points about the disease in Canada are:

- The disease is caused by *Fusarium lactis*, not *F. oxysporum* and has affected greenhouse crops of sweet peppers in Alberta and British Columbia.
- The inside of infected fruit is covered with white mycelium; fruit appeared healthy externally.
- The disease was first found in 2003 on cv. Symphony, an orange-fruited variety.
- *Fusarium lactis* is a weakly-virulent pathogen and previously has been recorded causing an internal fruit rot of figs.
- *Fusarium lactis* was found to be transmitted in air and on the legs and mandibles of bees.
- Occurrence of pepper fruit rot caused by *F. lactis* was believed to be related the temperature and humidity, though specific requirements are not known.

7. Conclusions

1. *Fusarium oxysporum* is associated with stem node lesions and a fruit rot of glasshouse sweet pepper crops in the UK. The fruit rot is usually internal and develops more commonly on mature fruit close to harvest, and post-harvest, than on developing fruit.

2. This disease is relatively new to the UK. It was present on several nurseries in the Lee Valley in 2006 affecting several different varieties.

3. Fifteen isolates of *Fusarium* obtained from pepper seed, crops or crop debris in 2005 and 2006 were all identified by a DNA test as *F. oxysporum*. No *Fusarium solani* (previously recorded as a cause of Fusarium fruit and stem rot on pepper in the UK) was found.

4. *F. oxysporum* is a relatively weak pathogen of sweet pepper, infecting fresh de-leafing wounds from mycelial but not conidial inocula. In experimental work, stem node lesions did not progress to girdle the stem, although this is reported to occur in commercial crops.

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3. The origin and conditions leading to both stem node lesions and fruit rot are unclear. No evidence was found to support the hypothesis that stem node lesions arise from infected seed.

4. *F. oxysporum* can be found in pepper crops on attached or fallen aborted fruit, fallen flowers, in the air and in irrigation drainage water.

5. Development of stem node lesions in one crop in the Lee Valley in 2006 occurred mainly in the period March – May, and not earlier (Dec – Feb) or later (June – November).

8. Technology transfer

1. Project review meeting, Lee Valley, 18 December 2006.
2. Presentation to growers, HDC protected Crops Energy Meeting, Cheshunt, 13 February 2007.

9. References

Kharbanda, P.D., Yiang, J., Lange, R., Howard, R.J. and Mirza, M. (2006). *Fusarium lactis:* cause of internal fruit rot of greenhouse sweet peppers. Proceedings 8th Conference of European Foundation for Plant Pathology, Copenhagen, 13-17 August 2006.

Fletcher, J.T. (1994). Fusarium stem and fruit rot of sweet peppers in the glasshouse. Plant Pathology 43:225-7.

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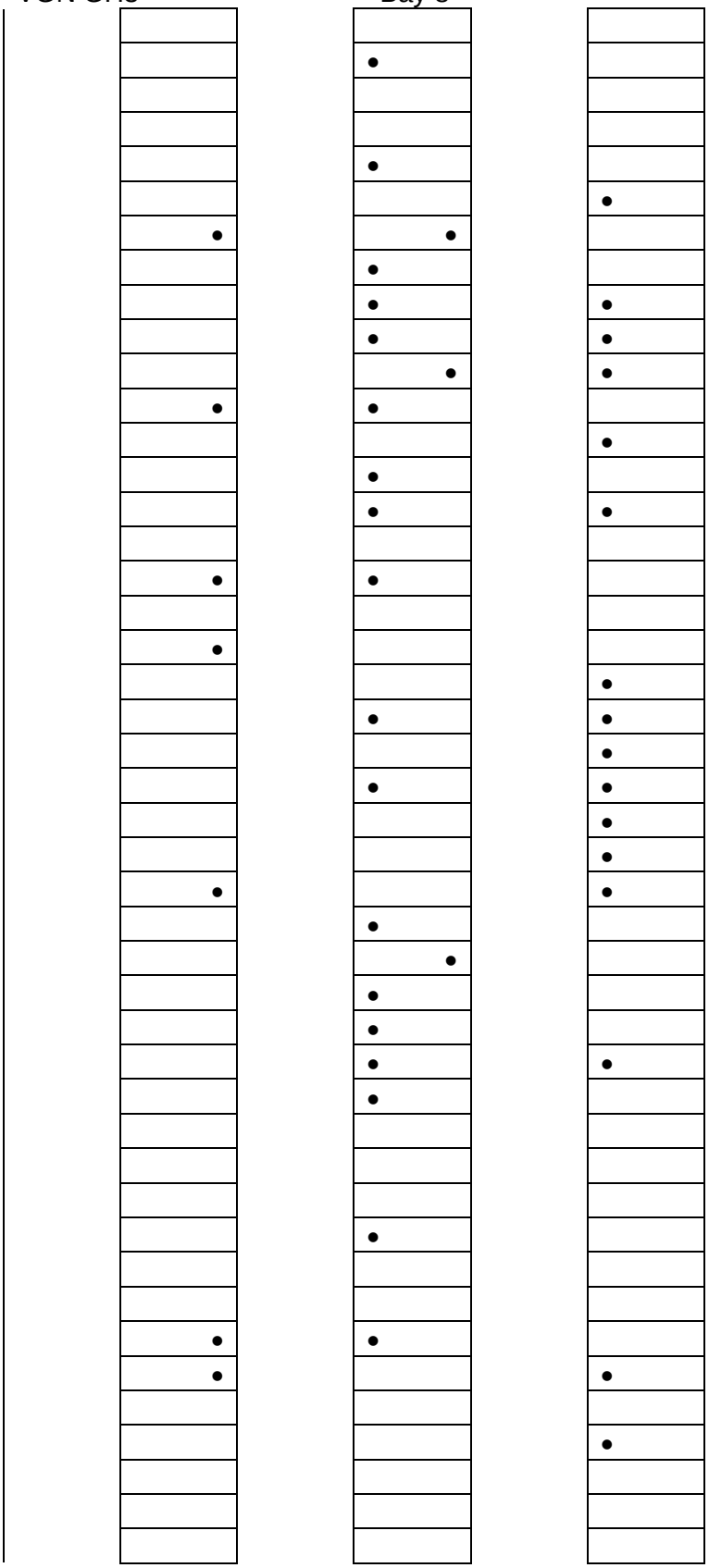
Appendix 1. Location of plants with stem node lesions in monitored crop: 29 March – 5 July 2006

Conventional (Control) block Glass House entrance →

VGN GH3 Bay 8



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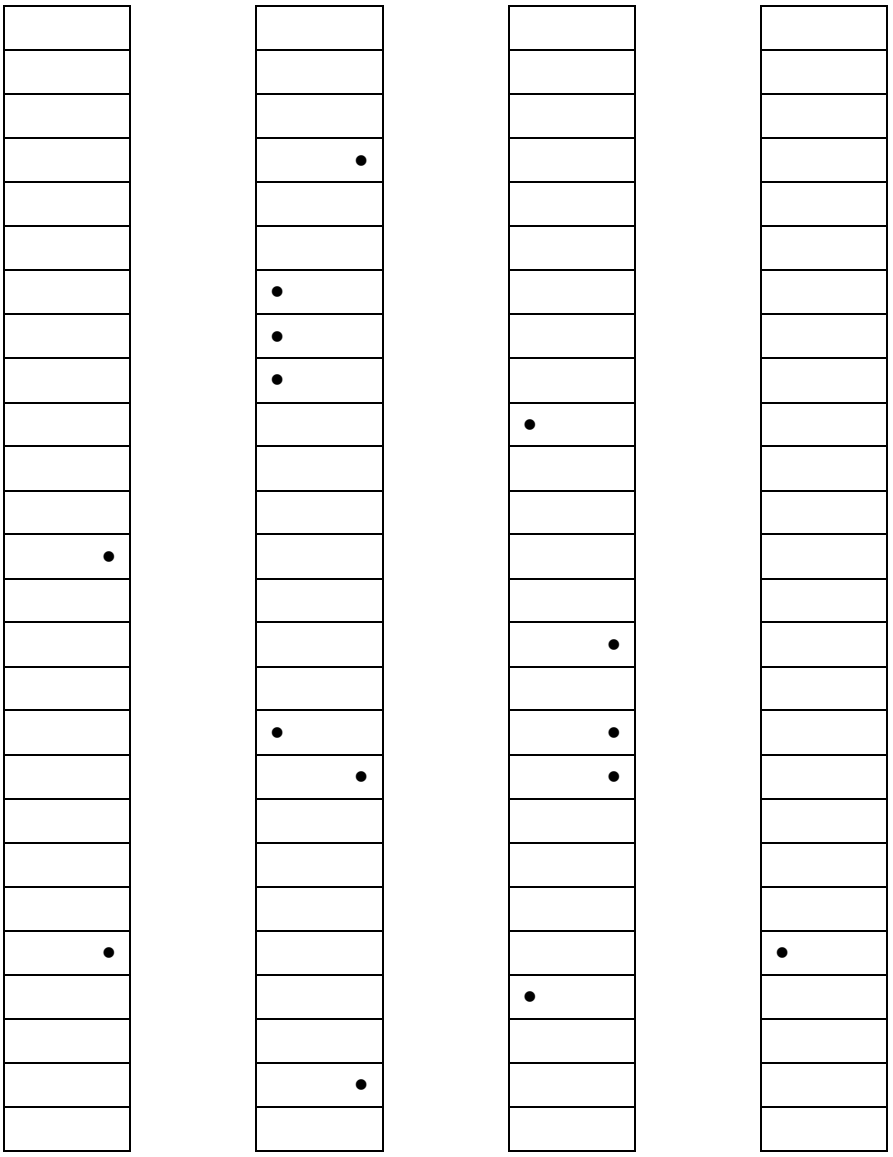
Energy saving block

VGN GH 4 Bay 3A

←Glass House entrance



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