Contract report for the Horticultural Development Council

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

PC 232a

February 2006

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The results and conclusions in this report are based on a series of experiments conducted over one year. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

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

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GROWER SUMMARY

Headline

- The fungus associated with this fruit and stem rot of pepper that has been increasing in occurrence was identified as *Fusarium oxysporum*; this is the first record of this problem in the UK.
- The disease cycle remains unclear and examination of young plant/ symptomless infection is on-going.

Background and expected deliverables

A *Fusarium* rot of fruit has been observed at one glasshouse on several varieties of rockwool-grown sweet peppers and has increased for several years in succession in spite of thorough hygiene measures. In spring 2005, there were substantial losses. The disease also caused brown lesions on fruit stalks and stem nodes and occasional plant death.

Fruit and stem rot caused by *Fusarium solani* is a recognised, relatively uncommon, disease of sweet pepper in the UK (Fletcher, 1994). However, in the recent case of *Fusarium* fruit and stem rot, the fungus was identified as *Fusarium oxysporum*. This is the first report of *F. oxysporum* associated with a disease in sweet pepper in the UK, although fruit and stem rot of sweet pepper caused by *F. oxysporum* is reported to have occurred recently, and to be increasing, in the Netherlands (Aad Vijberg and J. Barker, pers. comms.). Worldwide, there is no published report of pepper fruit and stem rot caused by *F. oxysporum* and so information that could help towards control of the disease is lacking.

Seed transmission of *F. oxysporum* has been reported in several crop species where *Fusarium* diseases occur. This is a potential route by which *F. oxysporum* may have arisen on the UK nursery. The disease symptoms in pepper are unusual in that no wilting, or root or stem base rot has been observed, symptoms that are commonly seen in other crop diseases caused by *F. oxysporum.*

The overall aim of the current work is to develop a better understanding of the biology of *Fusarium* fruit and stem rot of pepper caused by *F. oxysporum* and to assess the risk to other major glasshouse vegetable crops. Ultimately, the commercial objective of this work is to devise a rational control strategy based on a sound understanding of disease sources, methods of spread, and factors influencing disease development.

Summary of results and conclusions

Symptoms

Symptoms of pepper fruit and stem rot associated with *F. oxysporum* are shown in Figures 1-10. *F. oxysporum* was consistently isolated in pure culture from rotting fruit and from fruit stalk and stem lesions. Fruit rots originated at the blossom end and at the shoulder beneath the calyx. Affected tissue was soft, water-soaked and often bore sporulating *Fusarium*. Within fruit, white or pink growth of *Fusarium* was often visible on and around seeds. Apparently healthy fruit were sometimes affected by *Fusarium* internally. Fruit rot occurred more commonly on mature than immature fruit, especially those maturing early in the year; it was more common on coloured than green fruit.

A brown rot occurred on fruit stalks, especially on the soft tissue close to the stem where fruit are cut. An internal brown rot affected tissue within the fruit stalk, and longitudinal streaks were sometimes visible externally. No sporulation was observed on these tissues although *F. oxysporum* was readily recovered.

On stems, *Fusarium* was associated with pale brown lesions at nodes. Occasionally lesions extended up and down the stem for 5 – 100 cm, penetrated internally and caused plant death. *Fusarium* growth was sometimes visible, especially at nodes. No vascular staining at the stem base or wilting was observed in plants with nodal lesions.

Fusarium oxysporum was also found on attached blackened, pea-sized or slightly larger aborted fruit and occasionally on fallen petals and leaf petiole bases. It was commonly found as a white or pale-pink growth around the calyx of mature fruit.

Pathogenicity tests in a pepper crop

In August 2005, an area of a red pepper crop cv. Special, with no symptoms of *Fusarium*, was inoculated with *F. oxysporum* at freshly-cut stem wounds, unwounded fruit stalks or via the flowers. After nine weeks, no rot had occurred at the stem or stalk inoculation points even though the fungus was consistently recovered from these sites.

However, *Fusarium* fruit rot occurred in all treatments including the uninoculated control but the incidence of fruit rot was not significantly increased by inoculation.

External fruit rot was greatest on plants inoculated on the fruit stalk (18%), and also occurred at a high level on plants inoculated on the roots (15%) or at fresh stem wounds (17%); it affected 10% of fruit on uninoculated plants.

Internal fruit rot occurred at levels ranging from 3% (following flower inoculation) to 17% (following stem wound inoculation). Growth of *Fusarium* around the edge of the calyx was most common (75% of fruit) on fruit inoculated at this position nine weeks previously but also affected 57% of fruit on uninoculated plants.

Fruit rot was considerably greater on red than green fruit.

F. oxysporum was recovered from within the stem of plants inoculated on the roots (6/15 plants), and also from uninoculated plants (3/15); it was commonly recovered from the roots of both inoculated and uninoculated plants. *F. oxysporum* was also recovered from inside fruit with no visible symptoms, and from discoloured specks and dimples on the fruit shoulder.

However, given the level of fungal infection/ isolation from uninoculated fruit and that inoculation of a range of plant/fruit parts failed to significantly increase the disease expression no conclusions can be drawn on possible infection routes at this stage.

Occurrence of Fusarium in the air in a glasshouse pepper crop

An agar selective for *F. oxysporum* was used to investigate the occurrence of the fungus in the air within pepper crops. Agar plates were exposed for 24 hours. In August 2005, *Fusarium* was commonly trapped throughout two glasshouses containing affected pepper crops. Five colony types were identified, three of which resembled isolates obtained from pepper fruit and stem rots. In December 2005, *Fusarium* was again recovered, though less frequently than in August.

Pathogenicity tests on detached pepper fruit

Detached pepper fruit, cv. Boogie, were inoculated with 21 isolates of *Fusarium* using mycelial inocula. All isolates caused fruit rot following wound inoculation of the fruit side, including an isolate from another host (column stock).

Twelve of the 21 caused rot following inoculation of unwounded fruit. Isolates from pepper seed and glasshouse air caused lesions at more than 15% of inoculation sites. Other isolates from a pepper crop, and an isolate from column stock, failed to rot unwounded fruit. Rot was more common following inoculation at the blossom end of fruit than at the calyx end.

During the 3-week incubation period, many fruit, including uninoculated fruit, developed growth of *Fusarium* and of *B. cinerea* internally.

Isolation of Fusarium from packeted seed

Tests on pepper seeds of four varieties showed three of them were infected by *Fusarium*, with levels of 0.8%, 0.8% and 3.4%. The infection was removed by surface sterilising. From a glasshouse experiment in this project it was seen that pepper fruit without any external rotting could contain fully developed seed affected by *F. oxysporum* and it would be possible for these seeds to be harvested.

Symptoms of *Fusarium* fruit and stem rot associated with *F. oxysporum*

1. White growth of *Fusarium* around the calyx at the stalk end.

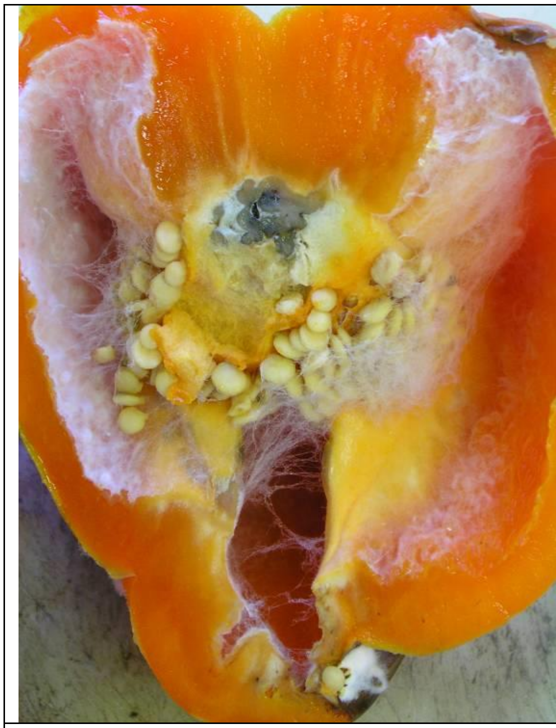
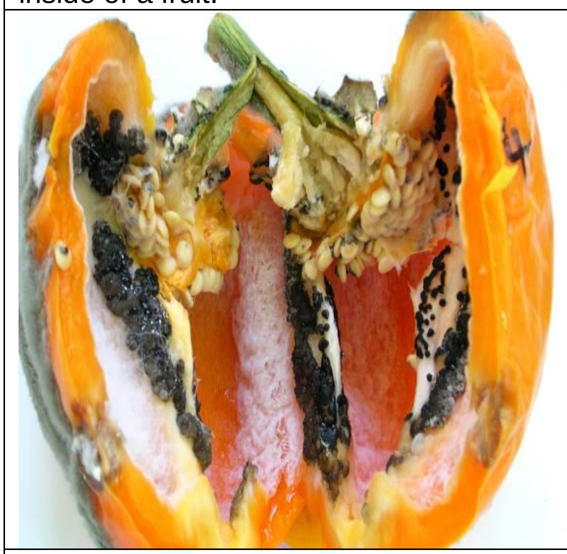
2. Dark brown stem lesion extending from a node.

3. *Fusarium* associated with cracks and dimples on the fruit shoulder.

4. Brown rot of vascular tissue at a fruit picking site on the main stem.

5. *Fusarium* rot of attached fruit.

6. Fruit stalk rot.

	
7. White growth of *Fusarium* on the inside of a fruit.	8. *Fusarium* and some browning of seed.
	
9. Black sclerotia of botrytis developing around pepper seed (*Fusarium* also present).	10. *Fusarium* rot of fruit stalk close to the main stem.

Financial benefits

Fruit and stem rot of sweet pepper caused by *Fusarium oxysporum* is known to have resulted in annual losses on one English production site in excess of £20,000, through a combination of unmarketable fruit and the cost of staff time in cutting out lesions to limit disease development. The source and means of spread of the disease is unknown and without an effective control strategy the disease could occur in other areas of the country and cause further economic loss in the pepper industry.

Action points for growers

- Note the symptoms of *Fusarium* fruit and stem rot in sweet pepper. Ensure that the cause of any fruit or stem rotting is correctly diagnosed.
- Be aware that after touching a sporulating *Fusarium* stem lesion, hands will be contaminated with spores of the fungus and they could spread the disease (e.g. via fruit harvest scars) to healthy plants.
- Sporulating material which can include aborted fruit should be bagged up where it is removed and disposed of in a sealed bag to reduce the risk of aerial spread by spores.
- Until the disease cycle is determined, growers should maintain a strict nursery hygiene regime.

SCIENCE SECTION

Introduction

During spring 2005, a glasshouse crop of rockwool-grown sweet peppers in the Lea Valley was monitored for disease as part of a project on energy saving (PC 227). A *Fusarium* rot of fruit was found by ADAS in early March and increased to cause substantial losses (c. 20% of a day's pick) by mid-April. The disease also affected fruit stalks and stem nodes, causing brown lesions. Varieties affected were Boogie, Fiesta and Special. *Fusarium* was also observed sporulating on attached aborted fruit in the crop. Observations suggested that infection might have arisen at both the calyx and opposite (flower) ends on fruit. No root death or wilting was observed.

Following culture in the laboratory, the fungus was identified by ADAS as *Fusarium oxysporum;* this identification was confirmed by CSL (ref: 20505388). Previously, fruit and stem rot of sweet pepper in the UK has been ascribed to *Fusarium solani* (Fletcher, 1994). This was the first report of *F. oxysporum* associated with a disease of sweet pepper in the UK. Stem and fruit rot of sweet pepper caused by *F. oxysporum* is reported to have occurred recently in the Netherlands and is seen as an increasing problem there (Aad Vijberg, pers. comm.). *Fusarium solani*, *F. oxysporum* and *F. circinatum* have all been associated with pepper fruit rot in Holland (J Barker, pers. comm.).

The disease has occurred on the UK nursery for several years in succession, increasing each year. Thorough cleaning and disinfection at the end of each season, and maintenance of a clean nursery during the growing season, have not resolved the problem. Losses are estimated to be around £20,000 per annum, a combination of the value of unmarketable fruit and the cost of staff time in cutting out stem lesions.

HDC report PC 232 reviewed *Fusarium* diseases on sweet and chilli peppers. Several different *Fusarium* species and types are reported to cause disease in pepper in other countries. In addition to *F. solani* (Cerkauskas, 2001; Lamb *et al.*, 2001; Tyson, 2001), these include an internal fruit rot caused by *F. circinatum* (syn. *F. subglutinans*) in Canada (Utkhede and Mathur, 2003, 2004), and a wilt caused by *F. oxysporum* f. sp. *vasinfectum* in North America (Miller *et al*., 1996). However, there appears to be no published report of a fruit and stem rot caused by *F. oxysporum*. This means that no details on the source, multiplication and spread of the disease can be examined to look for parallels in the present UK situation.

Seed transmission of *F. oxysporum* has been reported in several crop species affected by *Fusarium* wilt, or *Fusarium* crown and root rot diseases (e.g. basil, cucumber, tomato, column stock, cyclamen). This is a potential route by which *F. oxysporum* may have arisen on the Lea Valley nursery. A high proportion of seed from sweet pepper fruit affected by an internal fruit rot in Canada yielded *F. circinatum*. If seed is the source of *F. oxysporum*, the disease symptoms in pepper are unusual in that no wilting, or root or stem base rot has been observed. The path by which the fungus spreads from infected seed or young plants to cause fruit and stem node infection would need to be determined (e.g. is there symptomless systemic infection?).

The objectives of the current work are:

1. To conduct Koch's postulates on detached fruit and growing sweet pepper plants and determine if *F. oxysporum* can cause disease.

2. To investigate aspects of disease epidemiology including occurrence on seed, infection sites on the plant, and aerial spread.

Symptoms of *Fusarium* fruit and stem rot

Fruit symptoms

On mature fruit, an internal brown rot was seen in the pith and vascular tissue of the fruit stalk, especially at the end close to the stem where fruit are cut for harvest. Brown longitudinal streaks were sometimes visible externally if the internal fruit stalk rot penetrated close to the epidermis. Fruit rot was visible externally at the blossom-end (where the style remains attached to its expanded ovary, the fruit), or at the shoulder below the calyx. Rotted tissue was soft, appeared water-soaked and often was covered in white sporulating growth of *Fusarium*. Within the fruit, white *Fusarium* sporulation was visible across the seed-bearing region and some seeds and associated flesh were brown. Fruit symptoms were seen most commonly on fruit maturing early in the year (March-April), and more on coloured than green fruit. Occasionally an external rot developed on immature (2-3 week old) green fruit.

Stem symptoms

On stems, *Fusarium* produced dry, pale-brown lesions with a dark-brown border centred on leaf and fruit nodes. Usually the lesions extended about 50 mm either side of a node. Occasionally a dry, dark-brown streak extended up one side of the stem across several nodes, for one or two metres, with the darkened tissue penetrating 2-3 mm internally across the cortex and vascular strands. Pale pink mycelium or spores of *Fusarium* were sometimes visible externally, particularly on cut nodes. Several stem lesions had both botrytis and *Fusarium* present, with stems quickly becoming dominated by botrytis following damp incubation in the laboratory. Neither wilting nor stem base rot was observed in the crop, nor root necrosis. Botrytis stem infection tended to spread further away from the initial infection site and to have a bleached or pale brown colour.

Fusarium on crop debris

Blackened, pea-sized and slightly larger aborted fruit were common in the crop, some with an externally visible white fungal bloom. This fungal growth was frequently seen on the calyx next to the fruit stalk. *Fusarium* microconidia were confirmed following laboratory examination. Aborted fruit sometimes had rot within the cleft at the base of the fruit. Often, aborted blackened fruit with no external *Fusarium* were found to contain white growth of *Fusarium* within them. Botrytis and *Fusarium* were each found on old petals, sometimes the petals had dropped down to rest on the calyx of fruit below. Leaves were sometimes dropped and held in plants, and many had white spores on them. *Fusarium* was isolated from a petiole base.

Effect of inoculating different plant parts with conidia of *F. oxysporum*.

Introduction

The parts of sweet pepper that are susceptible to infection by *F. oxysporum* are unknown. Information on potential infection sites that lead to *Fusarium* fruit and stem rot is required in order to help develop a rational control strategy. An experiment was therefore devised with the following objectives:

1. To determine the susceptibility of sweet pepper flowers, fruit, fruit stalks, stem wounds and roots to infection by conidial inocula of *F. oxysporum* ex. pepper.

2. To conduct Koch's postulates on a rockwool crop of sweet pepper plants, cv. Special, and confirm that *F. oxysporum* can cause disease.

Method and materials

The experiment was conducted on a nursery that had a history of *F. oxysporum* fruit and stem rot, but in an area of red peppers cv. Special which had not had any *Fusarium* problems in the current season. Growing conditions were the same in the trial area as the main crop area. Each treatment plot comprised one head from each of the five plants, on a single rockwool slab. There were seven treatments with three replicates, arranged in two adjacent rows. Treatments were various inoculation points on the plants, selected on the basis of information on common sites of rotting recorded following natural infection elsewhere on the nursery, and on likely entry points based on information from other *Fusarium* diseases.

Cultures of *F. oxysporum* isolate AR05/32 (salmon coloured) and two colour types from isolate AR05/67, previously isolated from aborted fruit at the host nursery, were grown on agar for fourteen days. Conidial suspensions were prepared from the three isolates and filtered through muslin to remove mycelial strands. *Fusarium* micro- and macro-conidia were counted and suspensions diluted to 1×10^6 spores/ml, before producing a mixture of equal volumes of each isolate. Plants were inoculated with a 20 μl drop of this concentration dispensed with a micropipette. The droplet size was small enough to remain in place without running off the tissue. For the root inoculations, a 20 μl drop of 1×10^6 spores / ml was added to 100 ml of sterile distilled water and applied across the top of each rockwool block with a syringe. Each inoculation site thus received 2×10^4 spores. The inoculation treatments were:

1. Roots (unwounded)
2. Stem wound soon after fruit removal
3. Fruit stalk near junction with stem (unwounded)
4. Fully open flower, inoculated inside the petals (unwounded)
5. Senescent flower petals on young fruit (unwounded)
6. Calyx end of semi-mature (half size) fruit (unwounded)
7. Uninoculated

Stem nodes were re-cut with a sterile scalpel prior to inoculation. For fruit stalk inoculation, one full-sized green fruit per plant was selected, and the dark green band close to the stem inoculated. For senescent flower inoculation, a flower attached to a 10-20 mm diameter fruit was selected. In a few plants where neither flowers nor fruit of the required stage were present, it was necessary to apply the treatment at more than one position on an adjacent plant within the same slab. All the inoculation points were labelled. Commercial harvesting was stopped from above the lowest inoculation points.

The plants were inoculated on the 23 August 2005 when the crop had already been picked to about 2 m above the ground. The crop was assessed after two and six weeks for visible rotting on each inoculation point and whether flowers or fruit had fallen off. At the first assessment, aborted fruits were collected and examined in the laboratory to confirm the presence of *Fusarium*. From seven weeks, mature (unpicked) rotted fruit were collected from recorded plants (in case they dropped off), examined for *Fusarium*, and the location on the fruit of the rot recorded.

The final assessments were done on 27 October 2005, just before commercial harvesting was completed. The inoculation points were again scored for visible external fruit rotting and fruit was also cut open longitudinally to look for rotting or sporulation on the internal flesh and seeds. Fruit maturity was recorded. In the first replicate all fruit was assessed internally. In the second two replicates only two fruit per inoculation point were cut open, this was the fruit at the inoculation height and the node above. If the fruit at the inoculation height was absent then fruit from the two nodes above were sampled. The location of rotting and/or *Fusarium* growth was recorded on each fruit. Samples of a range of fruit symptoms were photographed. Isolations were made on PDA after surface sterilisation with sodium hypochlorite. A sample of healthy looking fruit from uninoculated plants was also taken.

Root rot was assessed and % browning scored after slitting open the rockwool blocks and the slabs from all the root-inoculated (T1) and uninoculated (T7) plants. Samples of roots were collected and examined for *Fusarium*.

Whole stems from all the plots of the root-inoculated (T1) and uninoculated (T7) plants were sampled intact. In the laboratory each stem was checked visually for vascular browning by slicing around the stem at 50 cm intervals, taking the first sample at rockwool block level. The highest samples were from 250 cm up the plant. Once browning was found, samples were surface sterilised with sodium hypochlorite, and isolations made from the cortex and vascular tissue from 0 cm, 25 cm and 50 cm above it, and any extension of browning visible at these heights was recorded. If no browning was visible, samples were taken for isolation from the stem base and 50 cm above.

Main stem sections were cut to 15 cm above and 15 cm below the inoculation points of the stem wound (T2) and fruit stalk (T3) inoculated plants, taking a random sample of two plants per treatment from each of the 3 replicates. In the laboratory, tissue samples from the node and 8 cm above and below were plated out. Samples were also taken from near the stem at the picking point (softer tissue) of the fruit stalk, and at the end of the stalk where it joined the fruit calyx. Some samples of fruit and seed tissue were also plated on PDA.

Using the number of fruit affected and the number examined at each inoculation site it was possible to calculate the percentage of fruit affected and the standard error of that figure. The 95% confidence intervals were calculated for each inoculation site. Two inoculation sites were said to be significantly different if the mean of one was not contained in the confidence interval of the other.

Results

<u>Symptoms</u>

No plant wilting, stem rot or fruit stalk rot were observed on inoculated plants. *Fusarium* fruit rot developed in all treatments, with both external and internal tissue affected. Some apparently healthy fruit showed an internal white/pale pink fungal growth when they were cut open (most often covering the upper half of the fruit) which did not usually cause necrosis or obvious rotting. Other fruit showed a white or pale pink powdery growth of *Fusarium* around the rim of the calyx, but no tissue rot, either externally or internally. The majority of the fruit affected by *Fusarium* had not been specifically inoculated with *F. oxysporum* either when it was a flower, or directly on to their flesh or fruit stalks.

Inoculation with *F. oxysporum* had no statistically significant effect on the occurrence of fruit rot. The proportion of fruit that developed a visible *Fusarium* rot (external rot) appeared to be slightly greater on plants inoculated by a root drench (15%), at a stem wound (17%) or a fruit stalk (18%) than plants not inoculated (11%), or inoculated on flowers or the calyx (3-10%) (Table 1). Between 14 to 17% of fruit on plants had internal rotting following root, stem wound, calyx and senescent flower inoculation. Eight percent of fruit had internal rotting when plants were inoculated via an unwounded fruit stalk, similar to that for uninoculated plants. The presence of *Fusarium* growth around the rim of the calyx was greatest on plants inoculated at the back of the calyx (75%), but this was not significantly different from the incidence on uninoculated plants (58% of fruit affected) (Table 2).

When the occurrence of fruit rot was examined by stage of fruit ripening (Table 3), it was evident that there was very low incidence on green fruit (1/86) and a considerably greater incidence on firm-ripe red fruit (19/185) and over-ripe red fruit (13/17). *Fusarium* growth was commonly present around the calyx of green fruit (48/86 fruit) even though no rot was present, at a similar level to its occurrence on red fruit (115/185).

<u>Isolation of *Fusarium* from stems</u>

Browning of the vascular tissue was only found in one plant, at the base. This was an uninoculated plant, and *Fusarium* was isolated from 25 cm above the base. *Fusarium* was also isolated, from 0 and 50 cm, in two other uninoculated plants. *Fusarium* was isolated from six out of the fifteen root-inoculated plants, with *Fusarium* at 50 cm in three of the plants (no isolations were made above 50 cm). *Fusarium* was only isolated twice at both 0 cm and 50 cm on the same plant.

<u>Isolation of *Fusarium* from inoculation sites</u>

At the stem wound and fruit stalk inoculation sites, although no rot or vascular browning developed, *F. oxysporum* was nearly always recovered from inoculation points. In the stem-wounded plants, *Fusarium* was also isolated above or below the inoculation point in half of the stems. Four out of six plants inoculated at the fruit stalk had *Fusarium* in the stem at both 8 cm above and below the inoculation point; *Fusarium* was also consistently isolated from near the calyx on the fruit stalk.

Root necrosis and discolouration was observed in all treatments. *Fusarium* was isolated from both the root-inoculated and the uninoculated plants, more consistently (7/15) from the latter. A *Pythium* sp. was also isolated, and may have caused the darker browning.

Isolation of *Fusarium* from fruit

When fruit was cut open, including many with no external symptoms, often the seeds were covered in pale pink fungal growth. The seeds were not discoloured or shrivelled by the fungus growing on them.

Fusarium oxysporum was recovered from the flesh of fruit with no symptoms, and from seeds and seed-bed pith inside externally affected fruit. The fungus was isolated from discoloured specks and dimples in the fruit flesh on the shoulder, side and blossom-end. There was no visible skin damage here.

Fusarium growth on crop tissue

The pale pink growth of *Fusarium* was detected on dropped flowers (some of which had lodged on fruit hanging below), aborted fruit and in shoulder cracks on ripe fruit. Within the inoculated area of crop, *Fusarium* was frequently visible as a pale pink fungal growth around the calyx on visibly healthy fruit of all ages.

Table 1. Effect of inoculating different parts of pepper plants with *F. oxysporum* on the occurrence of *Fusarium* fruit rot – 2005.

Treatment (inoculation site)	**Total no. fruit examined**	**% fruit with: Any rot *Fusarium***	**External rot**	**Internal rot**
1. Roots	65	20.0 (5.0) b	15.4 (4.5) b	15.4 (4.5) b
2. Stem wound	52	21.2 (5.7) b	17.3 (5.2) b	17.3 (5.2) b
3. Fruit stalk	49	20.4 (5.8) b	18.4 (5.5) b	8.2 (3.9) ab
4. Flower	31	3.2 (3.2) a	3.2 (3.2) a	3.2 (3.2) a
5. Senesced flower	35	14.3 (5.9) ab	2.9 (2.8) a	14.3 (5.9) ab
6. Calyx	40	20.0 (6.3) b	10.0 (4.7) ab	15.0 (5.6) ab
7. Uninoculated	28	10.7 (5.8) ab	10.7 (5.8) ab	7.1 (4.9) ab

( ) = standard error

Means within a column sharing a common letter are not significantly different at *P* = 0.05.

Table 2. Effect of inoculating different parts of pepper plants with *F. oxysporum* on growth of *Fusarium* around the fruit calyx.

Treatment (inoculation site)	**Total no. fruit examined**	**% fruit with growth of *Fusarium* at the calyx**
1. Roots	65	56.9 (6.1) ab
2. Stem wound	52	65.4 (6.6) bc
3. Fruit stalk	49	44.9 (7.1) a
4. Flower	31	67.7 (8.4) bc
5. Senesced flower	35	40.0 (8.3) a
6. Calyx	40	75.0 (6.8) c
7. Uninoculated	28	57.1 (9.4) abc

( ) = standard error
Means within a column sharing a common letter are not significantly different at P = 0.05.

Table 3. Occurrence of *Fusarium* fruit rot (%) according to fruit colour.

Treatment (inoculation site)	**Green fruit**		**Firm red fruit**		**Over-ripe red fruit**	
	External rot	**Internal rot**	**External rot**	**Internal rot**	**External rot**	**Internal rot**
1. Roots	0	0	5	7	100	100
2. Stem wound	0	0	10	16	50	33
3. Fruit stalk	0	0	12	4	83	50
4. Flower	0	0	5	5	0	0
5. Senesced flower	0	8	0	14	0	0
6. Calyx	0	0	12	19	100	100
7. Uninoculated	0	0	12	6	0	0
Total proportion	0/86	1/86	14/185	19/195	13/17	10/17

Discussion

Inoculation of pepper plants with a mixture of *F. oxysporum* isolates obtained from pepper failed to cause rot at any of the inoculation sites. Inoculation also failed to cause an increase in the incidence of fruit that subsequently developed *Fusarium* fruit rot. It is therefore not possible from this experiment to draw conclusions on possible routes by which *Fusarium* infects pepper to cause fruit and/or stem rot. Possible reasons for the lack of tissue rotting include a) insufficient inoculum (2 x 10^4 spores/site), b) insufficient time between inoculation and the final assessment (nine weeks) for rot to develop. There was a clear effect of fruit age on occurrence of *Fusarium* fruit rot, with the incidence of rot significantly greater on red than green fruit. Green fruit were not immune to rot, occasional affected green fruit being observed in the surrounding crop.

Trapping *Fusarium* from the air within a glasshouse pepper crop

Introduction

Spore trapping was done to determine the occurrence of *Fusarium oxysporum* in the air in two glasshouses containing pepper cv. Special. Sampling was first done in the main picking period and then repeated at the start of subsequent crops in the same two houses.

Methods

An agar selective for *F. oxysporum* (Komada's medium) was used. Plates were set out at ten locations (two plates per location) within two glasshouse blocks in both of which *Fusarium* fruit and stem rot had occurred in early 2005. Plates were laid out on 23 August and 13 December. Locations were selected across the houses to include between plants on the plastic ground covering, on the slabs and on the concrete pathway, with plates also at knee and head height up the plants on the first date. The lids were replaced after 24 hours exposure, and then the plates were returned to the laboratory and incubated for 14 days before describing colony types, checking for sporulation and counting the number of colonies on each plate. Subcultures were taken of the main colony types, and representative isolates of *Fusarium* were retained for testing for pathogenicity to pepper fruit, and stored for any subsequent work. Suspect isolates of *F. oxysporum* were grown-on to confirm species identification.

Results and discussion

When *Fusarium* colonies were sub-cultured and morphology and colour described on PDA it was seen that many of the cultures developed stronger colouration with age, in particular on the underside. This made comparison more difficult.

<u>August</u>
Five different colony types of pink *Fusarium* were identified and counted (Table 4). Three of the colonies (2, 4 and 5) resembled isolates taken from fruit and stem tissue in October 04. These were respectively pink aerial mycelium with a cherry underside, pale pink with a creamy yellow underside, and peach with a peach underside. The peach isolate (type 5) resembled the most common form isolated from pepper fruit and stems, but it had the least number of colonies on the air sample plates. This was isolate E and later caused one pepper to rot at the blossom end and lesions following wounding when used to inoculate detached fruit. The most common colony type (type 2), with yellow salmon aerial growth and a red underside, was isolate B in the detached pepper inoculations and produced a large lesion, but only following wounding.

<u>December</u>
Five colony types were counted after being categorised by aerial mycelium and underside colour. There were significantly fewer colonies on the plates than in August, most having zero *Fusarium* (Table 4). Most of the colonies were pale or darker peach, but there were no certain type matches against the August samples.
Table 4. Occurrence of *Fusarium* on selective agar plates after 24 h exposure in a pepper crop.

Date and glasshouse	**Mean number of colonies per plate for *Fusarium* types:**	**Range/**

						plate
	Type 1	**Type 2**	**Type 3**	**Type 4**	**Type 5**	
23 August						
Glasshouse 3	8.0	8.1	4.4	1.7	1.3	3 - 42
Glasshouse 4	10.6	14.4	8.3	3.1	0.0	24 - 50
	Type 6	Type 7	Type 8	Type 9	Type 10	
13 December						
Glasshouse 3	0.05	0.05	0.1	0.05	0.1	0 - 1
Glasshouse 4	0.1	1.4	0.0	0.95	0.0	0 - 12

Inoculation experiments on mature harvested fruit

Introduction

Pepper fruits were used to compare the pathogenicity of *F. oxysporum* and other *Fusarium* isolates obtained from pepper, pepper glasshouse air, and from another crop species. The objectives were:

1. To compare the ability of various isolates of *F. oxysporum* to cause rotting of ripe pepper fruit, with and without wounding.
2. To determine the relative susceptibility of different positions on pepper fruit to infection and rotting.

Methods

<u>Sources of *Fusarium* isolates</u>
Fusarium isolates were selected to give a mixture of sources. Some sources provided more than one isolate if there were differences in colony colour. They included the original *F. oxysporum* isolates (AR05/32 and AR05/67) used to inoculate the pepper crop in August 2005, plus isolations from various symptoms on pepper. In addition, cultures were grown from spores trapped from the air at a pepper nursery with the problem. An isolate of *F. oxysporum* from stock (*Matthiola incana)* was included for comparison. There were 21 *Fusarium* isolates, and an uninoculated control (Table 5).

<u>Inoculation and incubation</u>
Isolates were grown on potato dextrose agar (PDA) plus streptomycin for 14 days and applied to the fruit as a 6 mm diameter agar plug taken from near the edge of the colony. The mycelium side was placed against the fruit. Orange, commercially-harvested marketable peppers (cv. Boogie) were used. The fruit was washed under running tap water and blotted dry before use. Fruit of a similar ripeness were used within each replicate.

Two experiments were run concurrently on 10 November 2005, one with wounded, the other with unwounded fruit. For each experiment, the inoculated fruit were randomised in produce trays and held from rolling over by laying in a cardboard fruit-tray liner. The produce trays were stacked over trays of water to raise humidity and left wrapped in clear polythene in a room at 18°C.

Seventeen healthy fruit were not inoculated and were left out of the humid trays to monitor any natural fungal infection on the peppers.

Table 5. Origin and description of *Fusarium* isolates used to inoculate pepper fruit.

Code	Colony colour & texture of aerial/ underside colour	Isolate source	Isolate location
Control			
A	Agar	N/A	N/A
Isolates from glasshouse air			
B	Salmon & yellow weft / gold & red	N/A Air trap	Block 4 crop
C	Gold woolly / cherry red	N/A Air trap	Block 4 crop
E	Pale pink sparse / peach	N/A Air trap	Block 3 crop
G	White sparse / red veined peach	N/A Air trap	Block 3 crop
Isolates from pepper			
H	Pale pink sparse / peach	Inside stem	General crop
I	White sparse velvet / violet veins	Stem node	General crop
J	Pale pink / red veined peach	Calyx fruitlet	General crop
K	Pale pink sparse / peach	Petiole base	General crop
Isolates from seed			
L	White velvety / white, purple core	Seed	Britney
M	Pale pink woolly / creamy yellow	Seed	Britney
N	Pale pink velvety / creamy yellow	Seed	Britney
O	Peach velvety / peach	Seed	Special
Isolates from aborted fruit			
Q	Pale pink velvety / pale peach	Aborted fruit	AR05/32
R	Peach / peach with some cherry red	Aborted fruit	AR05/67 (1)
S	Pale pink velvet / white, red core	Aborted fruit	AR05/67 (2)
Isolate from column stock			
T	Cherry velvety / violet red, white	Stock plant	AR03/76
Isolates from pepper fruit			
D	Violet velvety / violet veined white	Fruit dimple	Trial plot 6
F	Pale peach velvety / red vein peach	Blossom-end	Trial plot 19
U	Peach velvety / salmon	Under calyx	Trial plot 12
V	Peach / violet veined peach	Rotted fruit	Trial plot 7
Isolate from pepper seed			
W	Pale pink /peach	Seed ex. fruit	Trial plot 4

Inoculation of wounded fruit

Inoculation of the side of the fruit was carried out at two positions after making a 7-mm cut in the skin surface just before adding the agar plug. Wounding was used because preliminary tests with some isolates had not caused any rotting of the side of the fruit when the skin was left intact. The 22 treatments were randomised in four replicate blocks, with each of the two inoculation positions per fruit being a separate replicate. Two different isolates were placed on the two wound sites per fruit.

Inoculation of unwounded fruit

Each isolate or control agar plug was placed at the calyx margin (touching the fruit flesh shoulder), and at the blossom-end (within the cleft at the base of the fruit). The 22 isolates were randomised as a replicate block within each fruit tray. There were three replicate blocks for each end of the fruit.

Assessments

Fruit were examined externally at weekly intervals for 21 days and then internally after 28 days. The presence of mycelium growing on the fruit surface from the plugs was recorded and the diameter of rotting was measured on lesions with and without the staining attributed to *Fusarium*. Records were made of any external fungal sporulation, including that of botrytis. When the fruit was cut open longitudinally to check for internal rotting and fungal growth, an index was used for *Fusarium* where 1 = mycelial growth around the inoculation position, 2 = up to half the internal area covered, 3 = over half of the inside covered by *Fusarium* mycelium.

Results

Wound inoculation

Rot developed around most of the agar plugs on the wounded sides of fruit (Table 6) and also on some of the unwounded fruit ends, often producing a brown-tinged depression which penetrated the flesh and often had local *Fusarium* growth internally. Rot developed at one of the four wounded but uninoculated sites. By the final assessment, many isolates had started to produce fungal growth within the fruit (Table 6).

The fungal inoculations resulted in lesions ranging in diameter from 10 mm (peach isolate O from seed) to 30 mm (yellow salmon isolate B from the air) while there was very little rot beneath the control agar plugs (Table 6). The stocks flower isolate also produced lesions after wounding, but not on intact tissue (Table 7). Staining was not so apparent on wounded tissue and it is possible that symptom development was different from that which would occur on normally unwounded tissue in the glasshouse crop.

Unwounded inoculation

Tissue rot was evident after 21 days, variously associated with *Fusarium* and botrytis. Stained lesions that appeared to be associated with *Fusarium* were counted (Table 7). These appeared as a brown-edged depression and were most common at the blossom-end of fruit. Botrytis was most common at the calyx end. Infection success after 21 days was greater with isolates from air and seed (Table 8).

There was no significant difference between isolates in the number of inoculation sites developing lesions after 21 days, or the incidence of fruit with internal *Fusarium* or botrytis after 28 days.

Fusarium fruit rot development was more common at the blossom-end (22% of pepper tissue or air source inoculum plugs) compared with the calyx end (10%). The isolate from stock did not produce any rotting. Over 23% of the rots on unwounded fruit were caused by isolates from seed, 21% from isolates collected from the air, and 8% to 13% from pepper fruit, stem and leaf isolates (Table 8).

During the 28 day incubation period the majority of the fruit became affected by internal pale pink *Fusarium* growth, which frequently covered at least half of the inside of the fruit and also grew over the seeds (Tables 7). There was seldom any staining of the flesh or seeds covered by the spreading mycelium. Botrytis commonly developed at the calyx (stalk) end, covering the calyx and the outside of the fruit shoulder with dense sporulation, and also formed sclerotia that were particularly abundant inside the fruit, alongside the internal ovary ridges and amongst the seed. Some fruit had a 'V' shaped waxy marking spreading externally from the calyx, and this may have been caused by the growth of the botrytis on the internal ridges.

Only two of the 17 uninoculated fruit remained healthy after 28 days on the bench. Externally there was a small amount of botrytis sporulation around the calyx and some flesh waxiness. There was no *Fusarium* mycelium, only a brown staining of the orange flesh visible under the epidermis at differing locations on each fruit. Internally, botrytis sclerotia were present in 15 fruit. Pale pink *Fusarium* grew within fruit on stained areas and across the seeds. Three fruit had internal *Fusarium* without any botrytis. The high number of fruit showing internal *Fusarium* without inoculation may reduce the value of the inoculated scores for internal *Fusarium*; however, the number and extent of external lesions following inoculation will show true differences in isolate pathogenicity.

Discussion

Twenty-one isolates of *Fusarium* from pepper, glasshouses air and column stock had all caused rot of wounded, detaching pepper fruit, and at most inoculation sites, after 21 days. By contrast, only 12 of the isolates caused rot on unwounded fruit in this time, and none caused rot at more than 33% of inoculation sites (Table 9). Isolates from pepper seed and glasshouses air were most likely to cause rot. These results indicated that wounded pepper fruit are more susceptible to *Fusarium* that unwounded fruit.

*Table 6***.** Symptom development following inoculation of wounded pepper fruit with various isolates of *Fusarium* (four replicates for each isolate).

Isolate	**Proportion of inoculation sites (of 4) developing lesions**		**Mean external lesion diameter (mm) after:**		**Fungal growth inside fruit at 28 days**	
	7 days	**14 and 21 days**	**14 days**	**21 days**	***Fusarium* (0-3) mean of 4 values**	**Botrytis present (out of 4)**
Control						
A	0	1	3.5	4.0	1.8	2
Isolates from glasshouse air						
B	1	4	23.3	30.5	3.0	4
C	0	4	15.8	18.8	2.0	2
E	2	3	13.8	17.8	3.0	3
G	2	3	17.8	20.3	2.8	3
Isolates from pepper						
H	2	4	21.0	28.5	2.5	3
I	4	4	19.8	20.0	3.0	4
J	1	4	19.0	27.8	2.0	1
K	1	4	17.3	23.0	3.0	4
Isolates from seed						
L	2	4	14.3	16.8	2.5	3
M	2	4	22.3	24.3	2.3	4
N	3	4	20.0	24.3	3.0	4
O	1	3	10.0	10.5	3.0	4
Isolates from aborted fruit						
Q	3	3	14.3	19.0	3.0	4
R	1	3	13.5	14.3	1.5	2
S	4	4	19.8	23.5	2.5	2
Isolate from column stock						
T	4	4	20.3	24.3	3.0	4
Isolates from pepper fruit						
D	0	4	13.8	16.8	3.0	3
F	2	4	17.3	20.8	2.0	2
U	1	3	10.8	18.8	3.0	4
V	2	4	13.5	16.3	1.5	3
Isolate from pepper seed						
W	1	4	18.5	24.5	3.0	3

Table 7. Symptom development following inoculation of unwounded pepper fruit with isolates of *Fusarium.* Calyx and blossom-end results combined (3 replicates of each).

Isolate	**No. of inoculation sites (of 6) developing *Fusarium* lesions after 21 day**	**Fungal growth inside fruit at 28 days:**	
		***Fusarium* index (0-3) mean of 6 inoculation points**	**Botrytis (out of 3 fruit)**
Control			
A	0	2.0	2
Isolates from glasshouse air			
B	0	2.2	2
C	2	2.0	2
E	1	2.0	1
G	2	3.0	3
Isolates from pepper			
H	0	2.0	2
I	0	2.0	1
J	2	2.7	2
K	0	2.0	2
Isolates from seed			
L	1	2.0	2
M	2	3.0	3
N	0	3.0	3
O	2	2.0	2
Isolates from aborted fruit			
Q	2	3.0	3
R	0	3.0	3
S	0	2.7	3
Isolate from column stock			
T	0	2.0	2
Isolates from pepper fruit			
D	0	3.0	3
F	1	2.7	3
U	1	3.0	3
V	1	2.0	2
Isolate from pepper seed			
W	2	3.0	3

There was no significant difference between isolates in the incidence of external *Fusarium* lesions after 21 days, or internal botrytis or *Fusarium* after 28 days.

Table 8. Occurrence of *Fusarium* growth and fruit rot at stained lesions 21 days following inoculation of unwounded fruit by isolates grouped by source. Calyx and blossom-end results combined to give 6 inoculation points per isolate.

Isolate source	**% inoculation points producing**		
	No. isolates in class	**Mycelial growth over fruit**	**Stained lesions associated with *Fusarium***
Air trap	4	66.7	20.8
Stem / petiole	4	50.0	8.3
Seed	5	83.3	23.3
Aborted fruit	3	55.6	11.1
Rotted fruit	4	58.3	12.5
Stock plant	1	83.3	0.0

Table 9. Occurrence of *Fusarium* rot after 21 days following inoculation of unwounded fruit according to colour of isolate in culture. 3 points per isolate.

Colony colour	**Isolates in each class shown by code**	**% of inoculation points producing stained lesions at the calyx end**	**% of inoculation points producing stained lesions of the blossom-end**
Yellow salmon, red below	B	0.0	0.0
Gold red, red below	C	33.3	33.3
White, peach below	E G H J KOUW	16.7	25.0
White, purple below	D I L S	0.0	8.3
Pale pink, yellow below	M N	0.0	33.3
Pale pink	Q	33.3	33.3
Peach, peachy red below	F R	0.0	16.7
Purple red, white below	T	0.0	0.0
Peach, violet veined peach below	V	0.0	33.3

Isolation of *Fusarium* from packeted seed

Introduction

The occurrence of *Fusarium* on pepper seed was investigated. Commercial batches of four varieties of sweet pepper seed were examined for *F. oxysporum* and other *Fusarium* species.

Methods

500 sweet pepper seeds of four sweet pepper varieties, Britney, Fiesta, Ferrari and Special were taken from unopened packets and plated on Komada's medium. Half of the seeds were plated directly and half after surface sterilisation. After incubation for 14 days, any fungal growth was recorded for each of the seeds. Any growth from seeds resembling *Fusarium* was sub-sampled onto separate agar plates, grown on and the species identified. Representative isolates were retained for testing their pathogenicity to detached pepper fruit and stored for any subsequent work.

Results and discussion

Seed tests showed that in packeted seeds a proportion of seeds from three out of four varieties were infected, with levels of 0.8%, 0.8% and 3.4% producing *Fusarium* (Table 10). The infection was however removed by surface sterilising.

Table 10. Recovery of *Fusarium* from pepper seed

Variety	**Surface sterilisation**	**Number of seed developing *Fusarium***	**Total number of seeds tested**
Britney	Yes	0	500
	No	17	500
Ferrari	Yes	0	250
	No	0	260
Fiesta	Yes	0	260
	No	2	250
Special	Yes	0	250
	No	2	250

Inoculation of young plants of cucumber, lettuce, pepper and tomato.

Introduction

In order to devise effective control strategies for *Fusarium* fruit and stem rot of pepper it is necessary to determine whether or not pepper plants can become infected as young plants, prior to setting out in the cropping house, possibly as symptomless infections. Additionally, if the fungus has alternative hosts these could provide a source of infection during propagation. The objectives of the work here were:

1. To determine if *F. oxysporum* can infect sweet pepper plants using isolates retrieved from seed, stems and fruit of an affected pepper crop.

2. To determine the susceptibility of lettuce, cucumber and tomato to infection by *F. oxysporum* from pepper, using conidial inocula applied to roots and to stem wounds.

Methods

Two experiments are in progress:

Inoculation of rockwool pepper plants
Young rockwool grown pepper plants at the 8-10 true leaf stage were inoculated with a conidial suspension of a mixture of isolates retrieved from seed, stems and fruit of the 2005 crop. The plants were placed on capillary matting in trays and grown on in a warm glasshouse. Plants were inoculated at fresh leaf scar (2/plant) and by drenching conidia over the roots.

Inoculation of potted seedlings
Young plug plants of cucumber, lettuce, pepper and tomato (at the 2-4 true leaf stage) were inoculated by drenching of conidia over the roots, using a mixture of *F. oxysporum* isolates obtained from pepper. Control plants were left uninoculated. Plants were grown on in compost in 9 cm pots for six weeks and examined for *Fusarium* disease. Re-isolation for fungi was made.

Results and discussion

This work is continuing and will be reported in the next annual report.

Conclusions

Symptoms

1. *Fusarium oxysporum* is associated with fruit, fruit stalk and stem rots on pepper. It also occurs on crop debris (aborted fruit, flower petals, leaf petiole bases).
2. Visible fruit rot is more common on red than green fruit, and especially on over-ripe fruit at the calyx end.
3. *Fusarium* is commonly found inside fruit (sometimes of apparently healthy fruit), growing over seed and sometimes causing a tissue rot.
4. *F. oxysporum* occurs within the stems of apparently healthy plants, often without vascular staining, and as growth around the calyx of fruit without causing rot.

Pathogenicity

1. Isolates of *F. oxysporum* from a pepper crop, pepper seed and the glasshouse air of a pepper crop caused rot of unwounded detached pepper fruit following mycelial inoculation. Rot was more common following inoculation at the blossom end than the calyx end. Other isolates from pepper, and an isolate from stock, failed to rot unwounded fruit.

2. A mixture of three isolates of *F. oxysporum* from pepper applied as spores (2×10^4 conidia per inoculation site) failed to cause rot at inoculation sites (root, stem wound, fruit stalk, fruit) or plant wilting, in a growing pepper crop. *F. oxysporum* was frequently recovered from inoculated tissues nine weeks after inoculation.

3. Inoculation of plants with *F. oxysporum* had no significant effect on the incidence of fruit developing *Fusarium* rot.

Biology of the disease

1. *Fusarium oxysporum* can occur in glasshouse air and on pepper seed and crop debris, as well as on the growing crop and harvested fruit.

2. Several different colour types of *F. oxysporum* are associated with the crop.

3. *Fusarium oxysporum* was found at a low level (up to 3.4%) on Britney, Fiesta and Special pepper seed. No *Fusarium* was recovered after surface sterilisation in sodium hypochlorite.

4. *F. oxysporum* was detected in the air of a glasshouse growing pepper in August 2005 (old crop) and in December 2005 (new crop).

5. *F. oxysporum* was recovered from unstained tissue within the stem of plants, both following root–inoculation (6/15 plants) and from uninoculated plants (3/15 plants).

Technology transfer

Protected vegetables: *Fusarium* risks assessed. *HDC News* (in preparation) (Tim O'Neill).

Acknowledgements

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