

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

March 2008

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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.

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* sp. is frequently present in UK sweet pepper crops and can cause limited lesions at stem nodes, spreading lesions on stems and an internal fruit rot. Internal fruit rot caused significant losses on several nurseries in 2007.

Background and expected deliverables

Fusarium stem and *Fusarium* fruit rot of sweet pepper have 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

Identification of Fusarium species associated with pepper

An internal fruit rot of pepper caused by *Fusarium* sp. is reported to be a new and increasing problem in Belgium. As with *Fusarium* fruit rot observed in this project, external necrotic lesions can also develop on mature fruit with internal *Fusarium* rot. *Fusarium* sp. were isolated from affected fruit on 15 nurseries in Belgium and identified by DNA analysis. Four isolates were identified as belonging to the *F. oxysporum* complex, two were identified as *F. proliferatum* and five as an unknown species related to *F. nygamai* and *F. lactis* (both in the *Gibberella fujikuroi* species complex). All isolates were pathogenic when inoculated into fruit.

A set of six isolates of *Fusarium* sp. isolated from pepper plants and seeds in the UK during this project were sent to Belgium in December 2007 for comparison by DNA testing with those isolated in Belgium. Until the results of these tests are available, and because of the diversity of colony forms of *Fusarium* isolated from pepper, we shall refer to the cause of the current *Fusarium* stem rot and *Fusarium* internal fruit rot of pepper in the UK as *Fusarium* sp.

Pathogenicity of Fusarium sp. to pepper

The ability of *Fusarium* sp. from pepper and *F. oxysporum* from other hosts 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 and tomato and with *Fusarium* sp. from pepper. Isolates obtained from pepper and cucumber stems appeared more aggressive than other isolates.

When young pepper plants, cv. Special, were inoculated at fresh de-leafing scars and over the roots with spores of *Fusarium* sp., using isolates obtained from sweet pepper, no stem lesions or permanent wilting had developed 8 weeks later. *Fusarium* sp. was re-isolated from the roots and leaf scars of 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 *Fusarium* sp., 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.

Seven out of nine strains of *Fusarium* sp. collected from pepper plants caused small, dark brown lesions when inoculated as mycelium onto fresh de-leafing scars of pepper plants, cv. Fiesta. Although some strains resulted in lesions at up to 70% of inoculation sites, none had girdled the stem or caused any plant wilting after 4 weeks.

Fruit rot of pepper caused by *Fusarium* sp. generally develops from the inside. One possible infection route is via flowers. It is suggested that possible outcomes of flower infection are abortion of small fruit, or an internal rot that develops slowly as fruits ripen. An experiment was designed to test the pathogenicity of *Fusarium* sp. to pepper by inoculation of flowers with a spore suspension. At 8 weeks after spray-inoculation of flowers with either water or a suspension of *Fusarium* sp. (using a strain obtained from a fruit rot), only a small number of aborted and mature fruit were found to be infected. No conclusions can be drawn as to the importance of flower infection due to the low incidence of infected fruit.

In conclusion, *Fusarium* sp. is commonly found in pepper crops and occasionally, under certain conditions, is able to cause a stem rot and an internal fruit rot. The precise conditions under which these develop have not been identified.

Effect of temperature and humidity of Fusarium stem rot

The growth rate of three isolates of *Fusarium* sp. 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 *Fusarium* sp. is a relatively weak pathogen of sweet pepper. The conditions under which limited stem node lesions develop into spreading lesions have not been identified.

Effect of stem bindings on Fusarium stem rot

Experimental work in another project using a sap flow sensor on pepper stems in a commercial crop resulted in plant wilting and death after 2-3 weeks; *Fusarium* sp. was consistently associated with rotting tissue beneath the sensor. An experiment was devised to determine the effect of a stem binding designed to mimic a sap flow sensor on occurrence of stem rot. Lengths of stem were covered in silicone grease and wrapped in silver foil. A soft rot developed along the length covered in grease and several plants wilted and died. *Fusarium* sp. was readily recovered. Extensive stem rot only occurred on stems inoculated with *Fusarium* sp. prior to grease treatment. Silicone grease treatment resulted in linear bubbling along stems. It is suggested that silicone grease causes physical or physiological damage to stem cortical tissue sufficient to allow *Fusarium* sp., a weak pathogen of pepper stem, to develop.

Occurrence of Fusarium sp. 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 affecting 1.6% and 12% of seeds respectively. No *Fusarium* was recovered after hypochlorite treatment of seed (1% hypochlorite for 5 mins). 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%. An experiment to determine effect of hypochlorite seed treatment on pathogen transmission was inconclusive due to low levels of seed infection.

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 using agar plates. When examined by a DNA sequence test, three isolates (from pepper seed, rotting fruit stalk and roots) showed a 100% similarity to *F. oxysporum*; a fourth isolate (from a pepper fruit with internal *Fusarium* rot) showed a 99% similarity. One isolate obtained from air was identified by a DNA test as *F. graminearum*.

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 *Fusarium* sp. The incidence of internal stem base infection was no greater in plants that had shown *Fusarium* 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. The 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. A small black hole was often present at the flower end of fruit with *Fusarium* internal fruit rot. This observation is consistent with Belgium studies which indicate that internal fruit rot originates from infection of flowers.

Susceptibility of some protected vegetable crops to Fusarium sp. isolated from pepper

Inoculation of roots and fresh leaf scars of young cucumber, lettuce, pepper and tomato plants with a conidial suspension of *Fusarium* sp. 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 *Fusarium* sp. associated with fruit and stem rot of pepper is not strongly pathogenic to any of these crops.

Occurrence of Fusarium fruit rot and stem rot on pepper crops in the UK

In 2006, six nurseries in the Lee Valley (Essex and Herts) growing glasshouse crops of sweet pepper were examined for evidence of *Fusarium* fruit and/or stem rot in April and September. 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.

In June 2006, a sample of cv. Fiesta fruit that had developed pale brown spots and dimples during storage in a packhouse was received. A *Fusarium* sp. consistent with *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 *Fusarium* sp. can cause a post-harvest fruit rot.

In May 2007, two nurseries in Humberside and Yorkshire were examined for *Fusarium* fruit and/or stem rot. *Fusarium* sp. was found associated with rotting fruit on both nurseries, affecting both aborted and mature fruit. Stem node browning was found on cv. Ferrari on one nursery and *Fusarium* sp. was recovered from 5 out of 16 lesions tested.

Samples of visibly healthy class 2 fruit were taken from three nurseries in Essex and Hertfordshire in mid-July 2007 and examined for growth of *Fusarium* sp. within them. *Fusarium* sp. was found sporulating in 1, 4 and 15% of the fruit examined.

These results indicate that *Fusarium* sp. is relatively common in pepper crops in England but causes stem rot only very rarely. *Fusarium* internal fruit rot was present at low levels in a number of crops and can become a significant problem, especially if fruit is stored before marketing.

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

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* sp., 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 lesions 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. No occurrences of multiple lesions on a stem were observed (as is found with cucumber *Fusarium* stem rot), indicating that infection is probably not systemic.

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.

Control of Fusarium stem rot

Pepper seeds and crop debris (fallen flowers, aborted fruit, dead leaves) have been identified as potential sources of *Fusarium* sp. that may lead to *Fusarium* stem rot. Experiments were done to determine the effect of extra crop-hygiene and sodium hypochlorite seed treatment on the incidence of *Fusarium* stem lesions in a commercial crop. Extra crop-hygiene consisted of picking-up all fallen fruit, flowers and debris, and picking-off aborted fruit, every 2 weeks from February until June. Seed treatment consisted of treating known infested seed with sodium hypochlorite at 1% for 5 mins prior to sowing the seed. In both experiments, *Fusarium* stem rot occurred at a very low level (<2% of stems affected) and no spreading lesions or dead stems had developed by the end of the season. No conclusions can be drawn as to the effectiveness of these treatments due to the low level of *Fusarium* stem rot.

Summary

Current knowledge about Fusarium stem rot and internal fruit rot of sweet pepper in the UK is summarised below.

Factor	Knowledge
Cause:	<i>Fusarium</i> sp., probably <i>F. oxysporum</i> , causing both stem and internal fruit rot.
Stem rot symptoms:	A limited dry, brown rot at nodes, occasionally extending to girdle the stem and cause plant wilting; sporulation of <i>Fusarium</i> sp. is sometimes found on affected tissue.
Fruit rot symptoms:	An internal rot, occasionally developing into an external rot. Rarely seen until at, or after, harvest.
Importance:	Stem rot is currently uncommon, usually at a low level, though occasionally damaging. Fruit rot is increasingly common and can cause significant harvest and post-harvest losses.
Pathogenicity:	A relatively weak pathogen of the growing crop.
Specificity:	No evidence of a host-specific strain of <i>F. oxysporum</i> adapted to pepper.
Risk to other crops:	No damage caused to cucumber, lettuce or tomato plants.
Seed-borne:	Yes, but transmission not proven.
Systemic vascular disease:	Probably not.
Possible sources:	Seed, crop debris (especially aborted fruit). Spread in air and irrigation water.
Infection points:	Stem infection at nodes, probably direct infection of wounded tissue, or via senescent leaves or sideshoots. Fruit infection probably via flower parts, also through wounds (e.g. pest damage); possibly also via the calyx.
Conditions for infection:	Probably surface wetness on, or prolonged high humidity around, flowers or stem wounds; temperatures in range 20-25°C are favourable.

Financial benefits

Stem and fruit rot of sweet pepper caused by *Fusarium oxysporum* or a closely related species 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 had caused stem lesions on two nurseries and a post-harvest fruit rot on another nursery, the latter resulting in the loss of 10-15% of packaged fruit during storage. In 2007, where visible healthy fruit on one nursery were examined at harvest between May and September, 8.3% were found to be infected internally. 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.

Action points for growers

Symptoms

- Note the symptoms of sweet pepper *Fusarium* stem rot and *Fusarium* internal fruit rot caused by *Fusarium* sp. (see the photographs in HDC report PC 232a, February 2006).
- *Fusarium* stem lesions appear to develop most commonly from March to May. Spreading stem lesions caused by *Fusarium* sp. can appear similar to those caused by grey mould (*Botrytis cinerea*) and Sclerotinia (*S. sclerotiorum*). If in doubt about the cause of a stem rot, seek expert advice.

Crop hygiene

- Prompt removal of attached and fallen aborted fruit and of dead flowers may reduce the risk of *Fusarium* infection in a crop. Such fruit and flowers are frequently colonised by *Fusarium* sp. and could act as sources of infection.
- Remove any dead stems or plants promptly and carefully; bag them *in situ* before carrying them out of the house.
- There is limited evidence that *Fusarium* stem rot is favoured by warm temperatures (20-25°C) and a high humidity. Use of ventilation to reduce humidity and condensation on plant parts (e.g. flowers) may reduce risk of the disease.

Management of *Fusarium* internal fruit rot

- Fusarium fruit rot generally develops from the inside outwards and although fruit may appear perfectly healthy (from the outside) at harvest, Fusarium rot can subsequently develop post-harvest. If Fusarium internal fruit rot is known to be present in a crop, harvest fruit as soon as they are ready and do not store them before sale.
- Physical damage to fruit caused for example by handling or pest attack (e.g. tortrix caterpillar), may increase the risk of Fusarium internal fruit rot. Minimise fruit damage.

Management of Fusarium stem rot

- Further research work is required to devise improved methods for control of Fusarium stem rot.
- Grower experience indicates that when Fusarium stem node lesions are cut out, the plant generally grows satisfactorily for the remainder of the season. However, this can involve considerable staff time and there is no experimental evidence that removal of these limited stem lesions is necessary to ensure plant survival.
- Avoid stem damage as this can facilitate infection by *Fusarium* sp.

SCIENCE SECTION

In year 1 of this project, experiments were done to investigate the pathogenicity to pepper of a *Fusarium* sp. found associated with stem lesions and rotting fruit and the occurrence of this disease in the UK. A *Fusarium* sp. isolated from pepper stem lesions was shown to be capable of rotting detached pepper fruit and fruit stalks. However, isolates of *F. oxysporum* from cucumber, *Hebe*, *Lisianthus*, stock and tomato caused similar damage. Inoculation of the roots of young pepper plants with strains of *Fusarium* sp. obtained from pepper did not result in any wilting after 8 weeks. Inoculation of fresh de-leafing scars with mycelium of *Fusarium* sp. obtained from pepper resulted in small (10-20 mm long) dark brown lesions that failed to develop further. It was concluded that the *Fusarium* sp. is a relatively weak pathogen of pepper.

A survey of pepper crops in the Lee Valley (Essex and Herts) confirmed *Fusarium* sp. on attached or fallen fruit on all of six nurseries examined. *Fusarium* stem node lesions were found on just one nursery. A sample of pepper fruit, cv. Fiesta, rejected at a packhouse due to development of pale brown spots and dimples that developed during storage, was found to be infected internally by *Fusarium* sp.

In a commercial crop affected by stem node and fruit stalk lesions, *Fusarium* sp. was recovered from 52% of 108 stem node lesions and 11% of 30 fruit stalk lesions; no other pathogens were recovered. These results suggest that *Fusarium* sp. may be invading stem node and fruit stalk lesions initially caused by some other factor, rather than causing the lesions *per se*.

A *Fusarium* sp. was recovered at a low level from seed of cvs. Fiesta and Kelly. *Fusarium* sp. was also recovered from irrigation run-off water and passive air-sampling of glasshouse containing pepper crops.

In year 2 of this project (2007), the objectives were to:

- Investigate the effect of early-season crop hygiene on occurrence of *Fusarium* stem rot;
- Investigate the effect of hypochlorite seed treatment on *Fusarium* stem rot;
- Conduct further pathogenicity tests using mycelial (fungal threads) and conidial (spore) inocula;
- Determine the occurrence of *Fusarium* stem and fruit rot in some pepper crops outside the Lee Valley.

Effect of early season hygiene on Fusarium stem rot

Introduction

In the first year of this project, a *Fusarium* sp. was commonly found on fallen aborted fruit on the glasshouse floor and occasionally on fallen flowers and leaf petioles. *Fusarium* sp was also detected on attached aborted fruit and during passive sampling of glasshouse air. An experiment was devised to determine if regular removal of fallen crop debris during the early part of the season reduced or delayed the occurrence of Fusarium stem rot.

Materials and methods

Site and crop details

The experiment was located in a commercial crop of sweet pepper cv. Fiesta in the Lee Valley, Essex, in a glasshouse with a history of Fusarium stem rot. The crop was grown in rockwool slabs on hanging gutters.

Treatments

1. Nursery standard crop maintenance
2. All attached and fallen aborted fruit and necrotic tissue removed by hand every 2 weeks from mid-February until 29 June 2007 (additional to standard crop maintenance)

Experiment design and statistical analysis

There were 10 replicates of the two treatments arranged alternately along one pathway. Each plot consisted of 20 pepper stems, 10 on either side of a pathway, directly opposite each other. A 5 m guard was left at the ends of the pathway. There was no overhead air circulation fan in the chosen pathway.

Assessments

The central 16 stems in each plot in the experiment were examined every 2 weeks from 6 March to 2 July and the numbers of stem node lesions recorded. Lesions were removed as they occurred and a sample of them was plated onto potato dextrose agar (PDA) in a laboratory to determine the identity of associated fungi. Rotting fruit were also checked for Fusarium visible symptoms and removed. A further assessment was done on 30 October, checking for stem lesions and dead stems.

Results and discussion

Fusarium stem rot and Fusarium fruit rot occurred at a very low level (Table 1) irrespective of treatment and no conclusions could be drawn as to the effect of early season hygiene on the diseases.

There were no spreading lesions or dead stems in the experimental area at the end of cropping in October. The very low level of Fusarium stem rot throughout the nursery in 2007 was in marked contrast to 2006 when a total of 9% of stems was found to be affected between April and July. The reason for the differing levels of the disease between two successive seasons is unknown; a similar range of varieties was grown each season, and growing practices and environmental conditions were broadly similar.

Table 1: Effect of debris removal on occurrence of Fusarium stem rot in pepper – 2007

Assessment date (2007)	Total number of Fusarium lesions on 160 stems:		Total number fruit with Fusarium	
	Clean area	Dirty area	Clean area	Dirty area
6 March	0	0	0	0
20 March	0	0	0	0
3 April	0	0	0	0
17 April	1	2	1	0
1 May	0	0	0	1
15 May	0	0	S	S
2 July	0	0	0	0

S – several

Effect of hypochlorite seed treatment on Fusarium stem rot

Introduction

In the first year of this project, a *Fusarium* sp. was recovered from some batches of pepper seed. The spore type and colony appearance of strains on seed were very similar to that of *Fusarium* strains recovered from stem lesions. Our hypothesis was that infected seed may introduce *Fusarium* sp. into a crop. An experiment was done to determine if sodium hypochlorite treatment of pepper seed infected with *Fusarium* sp. reduced the occurrence of Fusarium stem rot.

Materials and methods

Site and crop details

An experiment was located in a commercial crop of sweet pepper grown in the Lee Valley, Essex. A batch of pepper seed, cv. Fiesta, shown to be infected with *Fusarium* sp. was used. The level of infection by *Fusarium* sp., as determined by plating 300 non-sterilised seeds onto PDA, was 1.5%. Young plants were raised from these seeds in rockwool cubes by a commercial plant propagator.

Treatments

1. Seed untreated.
2. Seed soaked in sodium hypochlorite at 1% for 5 mins.

In previous work (PC 232a), treatment of pepper seed with 1% sodium hypochlorite for 5 minutes reduced infection from 3.4% to zero.

Experiment design and statistical analysis

There were 10 replicates of the two treatments arranged alternately along a row. Each plot contained 15 plants and each plant had two main stems.

Assessments

All stems were examined every 2 weeks from 6 March to 2 July 2007 and the numbers of stem lesions assessed. A further assessment was done just before crop pull-out in late October.

Results and discussion

No *Fusarium* stem lesions and only a very low incidence of fruit affected by *Fusarium* sp. were found in the experimental area (Table 2). Assuming plant infection arises from the seed, this low level of disease is not surprising given the low incidence of seed infection; unfortunately, no batches of seed with a higher level of seed infection were available at the time when the propagator required the seed for sowing. No conclusions can be drawn as to the efficacy of sodium hypochlorite seed treatment for control of *Fusarium* stem or fruit rot due to the low levels of disease.

Table 2: Effect of hypochlorite seed treatment on occurrence of *Fusarium* stem rot in pepper – 2007

Assessment date (2007)	Total N° <i>Fusarium</i> stem lesions on 150 plants (300 stems)		Total number fruit with <i>Fusarium</i>	
	Treated seed	Untreated seed	Treated seed	Untreated seed
6 March	0	0	0	0
20 March	0	0	0	0
3 April	0	0	0	1
17 April	0	0	2	0
1 May	0	0	0	0
15 May	0	0	S	S
2 July	0	0	0	0

S - several

Pathogenicity of *Fusarium* sp. to pepper stems from mycelial inocula

Introduction

In previous experiments, inoculation of pepper plants with *Fusarium* sp. from pepper only rarely resulted in stem lesions. An experiment was devised to compare the pathogenicity to pepper stems of 10 isolates of *Fusarium* sp. obtained from pepper seed, pepper plants, and the air from a glasshouse growing a pepper crop using plates of a selective agar (Komada's medium). Mycelial inocula were used to mimic the occurrence of *Fusarium* sp. in necrotic petioles of leaves attached to the stem. An isolate of *F. oxysporum* obtained from wilting stock (*Matthiola incana*) plants was also examined.

Materials and methods

Crop details

Pepper plants cv. Fiesta were grown in 1 L rockwool cubes in a gravel trays in a heated glasshouse (20°C) in January – February 2007. Plants were irrigated and fed by drenching nutrient solution onto the cubes.

Treatments

Details of isolates are given in Table 3. Isolates were identified by conventional microscopic examination.

Table 3: Origin and presumptive identification of isolates of *Fusarium* spp. used to inoculate pepper stems

Origin of <i>Fusarium</i> sp.		ADAS Code	Presumptive identification
1.	Uninoculated control	-	-
2.	Pepper seed cv. Britney	M	<i>F. oxysporum</i> *
3.	Pepper seed cv. Special	D	<i>Fusarium</i> sp.
4.	Pepper seed ex rotting fruit	W	<i>F. oxysporum</i> *
5.	Pepper stem	H	<i>F. oxysporum</i> *
6.	Pepper stem node	I	<i>F. oxysporum</i> *
7.	Pepper fruit rot	F	<i>Fusarium</i> sp.
8.	Pepper fruit, under calyx	U	<i>Fusarium oxysporum</i> *
9.	Aborted pepper fruit	Q	<i>Fusarium oxysporum</i> *
10.	Aborted pepper fruit	R	<i>Fusarium oxysporum</i> *
11.	Stock (<i>Matthiola incana</i>)	T	<i>Fusarium oxysporum</i> *
12.	Passive air sample in greenhouse	B	<i>F. graminearum</i> *

*Identification confirmed by CSL. Isolates were grouped into six groups based on broad morphological features and a representative isolate from each group was sequenced.

Inoculation

Mycelial inocula (c. 10 mm diameter) taken from 10-14 day old cultures on PDA were applied to fresh de-leafing scars on the stem, near the plant head, and held in place with masking tape. Plant heads were then covered with a polythene bag for 48 h, secured loosely around the stem to allow air exchange.

Assessments

Stems were examined at 2, 3 and 4 weeks after inoculation to determine the number of lesions and their size; lesion length extending up and down the stem was measured.

Experimental design and statistical analysis

Each treatment was applied to two de-leafing wounds (at least 5 cm apart) on a single plant. There were five replicate plants per treatment, arranged in a randomised block design. Results were examined by calculating standard deviations and confidence limits (these are not shown in the table to maintain clarity of data).

Results and discussion

Small lesions were visible at some inoculation sites after 2 weeks. The number and size of lesions had increased slightly by 4 weeks (Table 4). None of the lesions girdled the stem. None of the isolates were strongly aggressive to pepper. Most strains caused some lesions, including the strain of *F. oxysporum* from stock. The uninoculated control, two strains from pepper fruit and a strain of *F. graminearum* from air caused no damage. All of the non-zero figures in the column of total lesion numbers are significantly different ($P < 0.005$) from the uninoculated control.

Table 4: Pathogenicity of *Fusarium* spp. to pepper from mycelial inocula after 4 weeks – 2007

Treatment (source of <i>Fusarium</i> sp.)	Total number of lesions (of 10)	Mean lesion length (mm)
1. Uninoculated	0	0.0
2. Seed cv. Britney	7	1.1
3. Seed cv. Special	7	0.8
4. See ex fruit rot	6	1.1
5. Pepper stem	5	0.8
6. Stem node	7	0.9
7. Fruit rot	0	0.0
8. Fruit calyx	0	0.0
9. Aborted fruit	5	0.7
10. Aborted fruit	3	0.8
11. Stock plant	4	0.4
12. Air	0	0.0

These results confirm those obtained in year 1, that *Fusarium* sp. ex pepper is not strongly pathogenic to pepper stems.

Pathogenicity of *Fusarium* sp. to pepper flowers and fruit from conidial inocula

Introduction

Fusarium fruit rot of sweet pepper appears to develop from the inside; visibly healthy fruit can be found with an internal tissue rot and sporulation of *Fusarium* sp. on the inner wall, especially around the fruit tip (flower end). Our hypothesis was that fruit infection arises from infection of flowers. An experiment was designed to determine the pathogenicity of *Fusarium* sp. to pepper fruit by inoculation of flowers and young fruit from a spore suspension.

Materials and methods

Crop details

Treatments were applied to plants of cv. Fiesta grown in 1 L Rockwool cubes. Plants were irrigated and fed with a nutrient solution and grown for 3 months in a heated glasshouse in 2007.

Treatments

1. Flowers (at least 2 fully open per plant), developing fruit and fresh stem wounds sprayed with a spore suspension of *Fusarium* sp. in water
2. Flowers, developing fruit and fresh stem wounds sprayed with water

Inoculation

A spore suspension of *Fusarium* sp. (originally ex rotting pepper fruit) in sterile distilled water (SDW) was prepared and adjusted to 5×10^5 spores/mL. Two leaves in the mid-layer were removed from each plant, leaving a small stub (c. 10 mm long). Two shoots in the plant head were also removed leaving a stub (c. 25 mm). All flowers, developing fruit and leaf and shoot stubs on a plant were spray-inoculated using a hand-held pressurised sprayer late in the afternoon on an overcast day. The floor was wetted to increase humidity around the experiment. The area of inoculated plants was surrounded by a polythene curtain, and the glasshouse vents were kept shut for 48 h after inoculation to help maintain a high humidity and warm temperature. Prior to inoculation, all aborted fruit were picked from plants, and all fallen fruit and flowers were picked up and removed from the glasshouse.

Experiment design and analysis

There were 10 replicates of each treatment arranged in a randomised block design. Each plot consisted of two plants, with a single untreated guard plant between adjacent plots. Results were examined by calculation of standard deviations and confidence limits for a binomial distribution.

Assessments

Plants were examined weekly for 8 weeks and the numbers of aborted fruit (attached or fallen) and the numbers of stem lesions were recorded. Aborted fruit were examined microscopically for internal or external growth of *Fusarium* sp. after incubation in a damp chamber for 7 days.

As they matured, fruit were removed at the normal picking stage and examined for internal and external *Fusarium* fruit rot.

Results and discussion

The plants grew slowly due to an aphid infestation which was not well controlled using biological control (*Aphidius*). At 8 weeks after inoculation, *Fusarium* sp. was found in one aborted fruit from inoculated and uninoculated plants, and in one mature fruit from uninoculated plants. None of the differences between inoculated and uninoculated plants were statistically significant at the 95% confidence level.

No conclusions can be drawn from this experiment due to the small numbers of affected fruit that developed and the lack of any *Fusarium* stem lesions.

Table 5: Effect of spray-inoculation with *F. oxysporum* on occurrence of *Fusarium* fruit rot

Treatment	Proportion of fruit affected by <i>Fusarium</i> sp.			
	Aborted fruit after:		Mature fruit after:	
	2 weeks	8 weeks	6 weeks	8 week
1. Inoculated	1/4	-	0/9	0/14
2. Uninoculated	1/6	-	1/5	0/13

Effect of inoculation with *F. oxysporum* and stem bindings on development of *Fusarium* stem rot

Introduction

Experimental work in another project (PC 269) using a sap flow sensor on pepper stems resulted in plant wilting and death after 2-3 weeks. Stem tissue directly beneath the sap flow sensor had become soft and decayed. Laboratory examination of affected tissue revealed consistent association of *Fusarium* sp. with the rotting tissue. Our hypothesis was that the sap flow sensor had resulted in initial stem damage and this had become colonised by *Fusarium* sp. naturally present on or within stems resulting in a soft rot of stem cortical tissue. An experiment was devised to determine the effect of various stem bindings on *Fusarium* rot of pepper stems.

Materials and methods

Crop details

Pepper plants cv. Kelly were grown in a heated glasshouse in 1 L pots in M3 compost until around 0.3-0.4 m tall. Stem bindings c. 70 mm long were then applied to two closely-spaced de-leafing scars, created by breaking off the lowest two leaves and to one internode length mid-way up the plant (i.e two stem bindings per plant). Stem bindings were applied 2 hours after inoculation with a spore suspension of *Fusarium* sp. (isolate H, ex stem lesion) at 1 x

10⁶ conidia /mL in SDW. Isolate H was previously demonstrated to be weakly pathogenic to pepper (see Table 3).

Treatments

Treatment details are given in Table 6. The paper towel was folded to produce a 4-sheet thickness and dipped in SDW before binding to the stem. The silicone grease (DC4 ex Intek Adhesives) was applied around the complete circumference of the stem. The insulation foam binding was secured at the top and bottom with electrical tape.

The stem binding treatments were designed to exclude air (silicone grease) and to maintain a section of stem wet (wrapped in moist paper towel). Silicone grease is not used commercially on peppers but this treatment was previously observed to facilitate development of *Fusarium* stem rot. Surface wetness may occur naturally on stems due to condensation.

Table 6: Details of stem bindings

Treatment	Inoculated with <i>Fusarium</i> sp.	Binding
1.	No	None
2.	No	Silicone grease to exclude air; re-applied weekly
3.	No	Silicone grease wrapped in silver foil and insulation foam (as used in a sap flow sensor)
4.	No	Moist paper towel enclosed in clingfilm; re-wetted weekly
5.	Yes	None
6.	Yes	Silicone grease to exclude air; re-applied weekly
7.	Yes	Silicone grease wrapped in silver foil and insulation foam (as used in a sap flow sensor)
8.	Yes	Moist paper towel enclosed in clingfilm; re-wetted weekly

Assessments

All inoculation sites were examined after 2 and 4 weeks for occurrence of lesions and the presence of *Fusarium* sp. After the final assessment, both node and internode sections of bound areas of stem were examined for occurrence of *Fusarium* sp. by isolation onto PDA.

Experiment design and statistical analysis

The experiment was a randomised block design with five replicates. Each plot consisted of one plant. Results were examined by calculating standard deviations and confidence limits (these are not shown in the tables in order to maintain clarity of data).

Results and discussion

At 2 weeks after inoculation, slight bubbling of internode surface tissue in a linear pattern was visible on a few stems, there were no distinct lesions. At 4 weeks after inoculation, these symptoms were visible on some stems in all treatments except treatments 1 and 5 (unbound stems). The bubbling developed in lines running up and down the stem, and covered almost all of the bound sections and not beyond. This symptom appeared to be some form of physiological response to binding, possibly due to exclusion of air or prevention of moisture exchange. Both inoculated and uninoculated stems developed this symptom when covered in silicone grease or moist paper towel. All stems bound with foil or paper towel (T3, T4, T7, T8) turned pale green in colour.

Firm black and/or soft brown nodal and internodal lesions had developed by 4 weeks after inoculation with *Fusarium* sp. on several stems in treatments 3 (silicone grease wrapped in foil; uninoculated), 6 (silicone grease; inoculated) and 7 (silicone grease wrapped in foil; inoculated) (Table 7). Inoculated stems treated with silicone grease (T6 and T7) had a significantly greater number of black lesions than the inoculated unbound control (T5) ($p < 0.05$). One plant in treatment 6 and two in treatment 7 wilted severely (Table 7). Growth of *Fusarium* sp. was visible on one stem in treatment 7. *Fusarium* sp. was recovered from almost all node and internode pieces of inoculated and bound stems, and from around half of the inoculated unbound stems. *Fusarium* sp. was also recovered from some uninoculated stems, especially those covered in silicone grease and wrapped in foil (T3) (Table 8).

Table 7: Effect of stem bindings and inoculation with *Fusarium* sp. on occurrence of Fusarium stem rot of pepper after 4 weeks

Treatment	N° black lesions (of 5)		N° brown lesions (of 5)		N° wilting plants (of 5)
	Node	Internode	Node	Internode	
<u>Not inoculated</u>					
1. None	0	0	0	0	0
2. Silicone grease	0	0	0	0	0
3. Silicone grease + foil	2	0	3	4	0
4. Moist towel + film	0	0	0	0	0
<u>Inoculated</u>					
5. None	0	0	0	0	0
6. Silicone grease	4	1	0	0	1
7. Silicone grease + foil	5	3	0	2	2
8. Moist towel + film	0	0	0	0	0

Table 8: Recovery of *Fusarium* sp. from pepper stems four weeks after various inoculation and binding treatments

Treatment	Number of stem pieces (of 25) from which <i>Fusarium</i> sp. isolated
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	Node	Internode
<u>Not inoculated</u>		
1. None	7	7
2. Silicone grease	6	7
3. Silicone grease + foil	14	12
4. Moist towel + film	7	11
<u>Inoculated</u>		
5. None	14	12
6. Silicone grease	25	24
7. Silicone grease + foil	25	25
8. Moist towel + film	25	24

Isolation of *Fusarium* sp. from node tissue was significantly greater in T6, T7 and T8 (all inoculated) than in T3 (uninoculated) and T5 (inoculated) ($p < 0.05$); isolation from T3 and T5 was significantly greater than from T1, T2 and T4. Isolation of *Fusarium* sp. from internode tissue was significantly greater in T6, T7 and T8 (all inoculated) than in all other treatments ($p < 0.05$).

These results show that application of silicone grease to pepper stems resulted in damage to the stem tissue. Lesions subsequently developed at node and internode areas, and on stems inoculated and uninoculated with *Fusarium* sp. The incidence of lesions, especially black lesions, was significantly greater on inoculated than uninoculated plants. In the 4 week period of the experiment, lesions girdled the stem on 3 of 10 inoculated plants that were treated with silicone grease and resulted in irreversible plant wilting. No wilting occurred on any uninoculated plants (Table 7).

The application of a stem binding to inoculated plants (T6, T7, T8) increased recovery of *Fusarium* sp. compared with unbound plants; possibly this was due to reduced survival of the fungus (e.g. from drying out) on unbound stems, or conversely, a proliferation of *Fusarium* sp. on bound stems due to more conducive environmental conditions. Interestingly, *Fusarium* sp. was also recovered from uninoculated stem pieces tested, albeit at a lower incidence than from inoculated stems (37% and 85% respectively).

In conclusion, it seems probable that application of silicone grease to pepper stems results in a physiological and/or physical damage to cortical tissue, and that this damage is sufficient to facilitate *Fusarium* sp. to infect and cause a stem rot. No stem rot was observed where stems were inoculated and left unbound, or where they were inoculated and bound with moist paper towel and clingfilm.

In previous work on young ash trees it was observed that silicone grease applied weekly to a length of the tree trunk beneath a sap flow sensor resulted in trunk constriction over the

length of the greased area (Wiltshire *et al.*, 1995); trunk constriction was also observed when the trunk was greased and no sap flow sensor or other physical restriction to radial growth was used.

It was concluded that damage may have resulted from a limitation to respiration of the cambium because of restricted gas exchange. In our experiment where stems were bound with moist paper towel and clingfilm, it is possible that gas exchange was less restricted than where stems were covered in silicone grease and bound with silver foil, the physical damage to stem tissue was less, and may have been insufficient to allow *Fusarium* sp. to cause a rot within the duration of the experiment.

On both the inoculated experimental plants treated with silicone grease and the plants on a commercial nursery treated with a sap flow sensor, the lesions bearing *Fusarium* sp. did not extend beyond the silicone treated area. This supports the suggestion that *Fusarium* sp. is generally not an aggressive pathogen of Fusarium stems.

Occurrence of Fusarium fruit and stem rot in commercial pepper crops

Introduction

In 2006, a *Fusarium* sp. was found associated with attached or fallen rotting fruit in all of six glasshouse crops examined in the Lee Valley; Fusarium stem rot was found on just one of these nurseries. In order to determine how widespread Fusarium fruit and stem rot is over a wider area of the UK, commercial crops in Humberside and Yorkshire were also examined in 2007.

Materials and methods

Crop and fruit assessments

Crops of sweet pepper were examined on one nursery in Humberside and one in Yorkshire in May 2007. A minimum of 10 rows in each crop were examined. All stem lesions and a sample of fallen fruit were collected for laboratory examination. Samples of intact packed Class II (misshapen) fruit from three nurseries in Essex and Hertfordshire were examined for internal Fusarium. In addition, a sample of reject fruit (cv. Plenty) was collected from the Hertfordshire nursery.

Laboratory examination

Stem lesions and fruit were examined directly again after incubation in a damp chamber for around 7 days. Fungi were identified by microscope examination.

Results and discussion

Stem node browning was found in the crop of cv. Ferrari in Yorkshire and *Fusarium* sp. was recovered from 5/16 lesions. *Fusarium* sp. was found within aborted and mature fruit in all three crops examines in Humberside and Yorkshire (Table 9).

Class II fruit examined immediately after harvest in three crops in the Lee Valley (Essex and Herts) were all found to contain *Fusarium* sp. at levels ranging from 1 to 15%. A sample of cv. Plenty rejected at harvest due to dimples on the fruit had a high level of infection (37%).

Table 9: Occurrence of *Fusarium* fruit and stem rot in UK crops – 2007

Nursery and county	Variety (colour)	Date assessed	Fusarium fruit rot (n° positive/n° examined)	Fusarium stem rot (n° positive/n° examined)
<u>Crop assessments</u>				
1. Humberside	Ferrari (red)	4 May	6/10	0/16
	Boogie (orange)		5/7	0/16
2. Yorkshire	Ferrari (red)	4 May	8/19	5/16
<u>Fruit assessments</u>				
3. Essex	Prego (green)	12 July	1/75 (1%)	-
4. Essex	Boogie (orange)	12 July	2/52 (4%)	-
5. Herts	Plenty (red)	12 July	29/78 (37%)	-
	Fiesta (Yellow)		11/75 (15%)	-

In a related project (PC 227a), the occurrence of *Fusarium* within visibly healthy pepper fruit was monitored at intervals from 7 May to 10 September during 2007. Overall, *Fusarium* sp. was found in 8.3% of fruit. Infection was present both on seeds and on the internal wall, especially at the flower end of fruit. Although the infection in many fruit would probably have gone unnoticed by a consumer, in some fruit there was obvious fungal growth on seeds and in others there was a pale brown rot on the internal fruit wall. Many of the fruit affected by *Fusarium* internal rot were observed to have a small black or dark brown hole at the flower end. Rejection of pepper fruit at packhouses, due to *Fusarium* internal rot, and returns from stores due to the same disease, were reported by several growers to be a significant problem in 2007.

In most instances *Fusarium* sp. did not cause an external fruit rot until harvest, if at all. It is suggested that development of *Fusarium* sp. within pepper fruit to cause tissue rotting may remain quiescent until the fruit ripens. This would explain the general lack of external *Fusarium* fruit rot in the growing crop and the increased occurrence of *Fusarium* rot in fruit stored after harvest.

Studies on Fusarium diseases of pepper in Belgium and Canada

Belgium

An internal fruit rot of pepper caused by *Fusarium* spp. is reported to be a new and increasing problem in Belgium (Aerts *et al.*, 2007). As with the *Fusarium* fruit rot observed in this project, external necrotic lesions can also develop on mature fruit with internal *Fusarium* rot. *Fusarium* spp. were isolated from affected fruit on 15 nurseries in Belgium and identified by DNA analysis. Four isolates were identified as belonging to the *F. oxysporum* complex, two were identified as *F. proliferatum* and five as an unknown species related to *F. nygami* and *F. lactis* (both in the *Giberella fujikaroi* species complex). All isolates were pathogenic when inoculated into fruit.

A survey showed that *Fusarium* spp. could only be isolated from within fruit when a small hole or necrosis was visible where the dried pistil is attached to the fruit, and occasionally when external damage was visible. This indicates that fruit infection is most probably via the pistil. The survey also showed that varieties producing large flowers were more susceptible than other varieties. Infra-red monitoring of flowers showed that dew point was reached for several hours during the night/early morning. It was suggested that condensation of water on flowers makes them more susceptible to infection by *Fusarium* spp. It is also suspected that varieties that retain their flowers for a long time may be more susceptible to *Fusarium* internal fruit rot (R. Aerts, pers.comm.) In Belgium, only *F. solani* has been found associated with stem lesions (R. Aerts, pers. comm.)

In artificial inoculation experiments on mature fruit, there was no infection on unwounded fruits and a high infection rate on wounded fruit; isolates from the *G. fujikuroi* complex appeared more aggressive than those from the *F. oxysporum* complex.

In an artificial inoculation experiment on flowers, spray inoculation with *F. nygami* resulted in infections on mature fruit (41.5%) and green fruit (31%). Levels of fruit infection following sprays of water were 4% and 0% respectively.

Canada

An internal fruit rot of sweet pepper was first described in Alberta, Canada, in 2003 (Yang *et al.*, 2005). The disease became a serious marketing issue as growers were unable to screen out affected fruit. In research undertaken by Alberta Research Council, more than 40 *Fusarium* sp. isolates collected from fruit were examined by DNA analysis. These isolates were identified as *Fusarium lactis* (previously identified as *F. proliferatum*) and *Fusarium*

solani. *Fusarium solani* caused rapid infection and external fruit rot developed within 14 days. *F. lactis* caused internal infection of fruit and developed slowly, showing symptoms around 40 days after inoculation. In a survey of eight commercial greenhouses, *F. lactis* was found to be the main causal agent of Fusarium internal fruit rot.

A similar disease caused by *F. subglutinans* was reported in British Columbia in 2002.

Overall Conclusions

Disease occurrence

1. A commercial nursery that suffered a relatively high incidence (c.10%) of Fusarium stem lesions on a range of pepper varieties in 2006 had very low levels of the disease on a similar range of varieties in 2007. There was no clear explanation for this difference.
2. A *Fusarium* sp. was found associated with rotting pepper fruit on two nurseries examined in Humberside and Yorkshire as well as four crops in the Lee Valley. A low incidence of stem node lesions was found in the crop in Yorkshire. These results, when combined with the 2006 survey in this project, indicate that the occurrence of *Fusarium* sp. in pepper crops in the UK is relatively common.
3. In 2007, *Fusarium* sp. was found within visibly healthy marketable pepper fruit at levels up to 15%. Post-harvest Fusarium fruit rot was a cause of fruit rejection by packhouses and stores. It is suggested that *Fusarium* infection of pepper fruit may remain latent until ripening.

Pathogenicity of *Fusarium* sp.

4. Seven out of nine strains of *Fusarium* sp. obtained from pepper plants caused limited lesions when applied to fresh de-leafing scars of pepper cv. Fiesta. Two strains from pepper and one from glasshouse air did not cause lesions, while a strain of *F. oxysporum* from stock also caused limited lesions. These results indicate *Fusarium* sp. is a weak pathogen of pepper stems.
5. Application of a silicone grease to pepper stems resulted in a breakdown of cortical tissue leading to plant wilting and death. *Fusarium* sp. was consistently recovered from rotting tissue whether or not plants had been inoculated with the fungus. This observation suggests that significant damage to pepper stems can facilitate rotting by *Fusarium* sp.

Disease control

6. A study on seed disinfection with hypochlorite and pathogen transmission from seed was inconclusive due to the low levels of *Fusarium* sp. on available seed batches.
7. A study on enhanced crop hygiene as a possible measure to reduce *Fusarium* stem rot was inconclusive due to the low level of *Fusarium* stem rot that developed in the crop.

Technology transfer

Getting to the heart of pepper rots. *HDC News* **142**, 11-12.

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