Project title:	Sweet pepper: aspects of the biology and control of Fusarium fruit rot
Project number:	PE 007
Project leader:	Dr Tim O'Neill, ADAS
Report:	Annual Report, March 2012
Previous report	None
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Location of project:	Commercial nursery, Essex, ADAS Boxworth, University of Warwick
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Date project commenced:	1 April 2011
Date project completed:	31 March 2014

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

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AUTHENTICATION

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

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GROWER SUMMARY

Headlines

- Isolates of *Fusarium* species obtained from pepper fruit from various UK nurseries were identified as *F. lactis* (predominantly), *F. oxysporum* and *F. proliferatum*.
- Inoculation of sweet pepper flowers with spores of *Fusarium proliferatum* resulted in reduced fruit set and internal fruit rot.

Background and expected deliverables

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide since around 2000. In the UK a survey in 2007 showed infected fruits were present in many crops at levels from 1 to 37% (PC 260). The disease causes some losses on production nurseries but more importantly Fusarium continues to be a frequent cause of rejection by packers and complaints by supermarkets. Losses vary greatly between crops and seasons, and growers are generally unaware a problem may be present until harvest or postharvest. The fruit rot arises through infection of flowers by spores of *Fusarium*. Several *Fusarium* species have been associated with the disease, notably *F. lactis* and *F. oxysporum*. Observations in commercial crops indicate the disease is favoured by high humidity and fluctuating temperatures. At present there is no effective method of control. This project aims to reduce losses to Fusarium internal fruit rot through increased knowledge of factors associated with a high incidence of the disease and use of biofungicides and fungicides to control flower infection.

Summary of the project and main conclusions

Review of overseas research on pepper Fusarium internal fruit rot

Fusarium internal fruit rot of sweet peppers has also become a significant problem in recent years in Canada, Belgium and the Netherlands. The fungi associated with the disease are variously reported as *F. lactis*, *F. oxysporum. F. proliferatum* and *F. solani* in Alberta, Canada; as *F. subglutinans* in British Columbia, Canada; as *F. oxysporum, F. proliferatum, F. nygamai* and *F. lactis* in Belgium, and as *F. oxysporum*, *F. proliferatum, F. solani* and an unknown species related to *F. lactis* in the Netherlands.

Work in Belgium showed that pepper flowers may reach dew point for several hours during the night and early morning, and it was suggested that condensation of water on petals

makes them more susceptible to infection by *Fusarium* species. It was also reported that varieties with large petals, and varieties that retain petals for longer, were more susceptible.

Recent work in Canada confirmed the infection pathway of *F. lactis*. *Fusarium* spores (conidia) deposited on the stigma grew down the style and into the ovary within 5-6 days after inoculation. At 45 days after inoculation, typical internal fruit rot symptoms were observed and *F. lactis* was recovered from fruit tissue and seeds within externally symptomless fruit. It was suggested that *Fusarium* conidia were deposited on the stigma by insect pollinators or from the air.

Further work in Canada, by a different research group working with *F. subglutinans* (subsequently re-identified as *F. lactis*), identified some biological and chemical treatments that significantly reduced Fusarium internal fruit rot when applied to flowers 1 day before inoculation. Effective biological treatments included preparations of *Bacillus subtilis, Gliocladium catenulatum* and *Trichoderma harzianum*-T22. The fungicide Rovral (iprodione) also reduced Fusarium fruit rot.

Contact was made in 2011 with researchers in Belgium, Canada and the Netherlands. No new results were identified in Canada.

A research group in Belgium published results in early 2012 on the genetic diversity of *Fusarium lactis* species complex isolates obtained from sweet pepper. Out of 98 isolates obtained from Belgium (82), Canada (1), the Netherlands (9) and the UK (6), 74 were identified by molecular tests as *F. lactis* or *F. lactis*-like, 13 as *F. oxysporum*, nine as *F. proliferatum* and two as *F. solani*. Members of the *F. lactis* species complex showed large genetic and phenotypic diversity. It was suggested that the emergence of Fusarium internal fruit rot over the last 10 years in major sweet pepper growing regions was due to the introduction of new varieties with reduced resistance, and possibly to changes in greenhouse climate control that allowed higher relative humidity.

Work on the disease was done in the Netherlands in 2008-9 by Groen Agro Control, a private consultancy and research organisation, but their report is not in the public domain.

Molecular characterisation of Fusarium isolates associated with pepper Fusarium internal fruit rot in the UK

Out of six *Fusarium* isolates obtained from UK pepper crops and sent to Belgium for molecular characterisation, two were confirmed as *F. oxysporum,* three (peach-coloured isolates) were identified as *F. lactis*-like (but different from the type-strain), and one was identified as *F. proliferatum*.

Nineteen isolates of *Fusarium* sp. were obtained from fruit on four UK pepper nurseries and compared with reference DNA sequences of *F. andiyazi*, *F. lactis*, *F. oxysporum*, *F. proliferatum*, *F. nygamai* and *F. solani* by molecular tests at the University of Warwick. Eight isolates were found to be closely related to *F. lactis*, six to *F. oxysporum*, three to *F. proliferatum* and two to *F. solani.* An isolate obtained from a pepper stem base lesion on a fifth nursery was identified as *F. solani*.

Effect of Fusarium inoculum and flower age on infection

Two fully replicated experiments were done in commercial crops of sweet pepper in Essex. Flowers were individually inoculated and the stalks tagged so that fruits developing from them could be identified at harvest around 10 weeks later and examined for Fusarium internal fruit rot.

In May 2011, five methods of inoculation with an isolate of *F. proliferatum* were compared on fully open white flowers. Only 56 out of 280 inoculated flowers developed into mature fruit; 14 of these were infected with *Fusarium* internally. Inoculation of flowers with *Fusarium* spores by spraying in water, placing a small water droplet in the flower or dry spore transfer using a paintbrush, all resulted in infected fruit. Spray inoculation with a low *Fusarium* spore concentration was most successful (5 out of 8 fruit affected). None of 20 fruit developing from uninoculated flowers, or from flowers inoculated with water only, were affected by Fusarium.

In August 2011, a further set of flower inoculation treatments was examined using the same isolate of *F. proliferatum*. More fruit developed from flowers left untreated, inoculated with water only, or mist-inoculated with *Fusarium* spores (20-25 fruit from 60 flowers per treatment) than the other treatments (7-12 fruit from 60 flowers). Fusarium internal fruit rot only occurred in fruit that developed from inoculated flowers (Table 1). Inoculation of young white flowers and old brown flowers both resulted in Fusarium fruit rot, although at a significantly higher incidence from young white flowers (50%) than old brown flowers (19%). The level of infected fruit at harvest (35-56%) varied little with spore concentration or method of applying the spores. Mist inoculation, which resulted in a similar level of fruit set to that of uninoculated flowers, and a level of Fusarium fruit rot similar to that of inoculation with a high spore concentration, will most probably be used in future work to examine varietal susceptibility and some control options.

Eight isolates of *Fusarium* sp. recovered from affected fruit were characterised by molecular tests. Three isolates were identified as *F. proliferatum*, four as *F. lactis* and one as *F. lactis*-like. *F. lactis* may have occurred through natural dual infection; occurrence of *Fusarium*

within small aborted fruit from uninoculated flowers supports this explanation. Alternatively, as no internal rot occurred in fruit that developed from untreated flowers, it is possible that the original inoculum consisted of a mixture of *F. lactis* and *F. proliferatum*, rather than a pure culture of *F. proliferatum*.

Table 1. Effect of flower inoculation with *Fusarium proliferatum* and flower age on occurrence of Fusarium fruit rot in sweet pepper cv. Ferrari – November 2011 (Experiment 2)

Treatment	Total number fruit at harvest[a]	Occurrence of Fusarium (% of fruit harvested)			
		External rot	Internal rot	On seed	Any symptoms
1. Untreated	33	0	0	0	0
2. Water control	27	0	0	0	0
3. Mist	26	12	31	19	35
4. Dry spore transfer	18	11	17	33	33
5. Spray – low concentration (5 x 10^3/ml)	16	44	56	38	56
6. Spray – medium concentration (5 x 10^5/ml)	14	29	43	36	50
7. Spray – old flowers, medium concentration (5 x 10^5/ml)	21	14	19	5	19

[a] Fruit were harvested on 19 October and 2 November, 10 weeks after flower inoculation; data shown are for the combined harvests.

Financial benefits

Fusarium internal fruit rot of sweet pepper occurs in many UK sweet pepper crops, the severity varying with variety, nursery, glasshouse and time of year. The disease is more common in the spring and autumn when fruit take longer to ripen. Growers have reported that up to 20% of a day's pick may be affected. Assuming a farm-gate-value of 50p per fruit and a harvest of [1,000] fruit/ha on a single day, this represents a loss of £100/ha/day. Additional losses arise when infected fruits are not detected at harvest or in the packhouse, but the rot develops subsequently causing supermarket rejection or customer complaint to the supermarket, both of which incur a cost for the grower. The potential financial benefits of this work are an increased proportion of harvested fruit free from *Fusarium* internal infection and reduced risks of packhouse rejection, supermarket complaints and disruption to the supply chain.

Action points for growers

None at present.

SCIENCE SECTION

This project aims to build on knowledge of Fusarium internal fruit rot of pepper developed in HDC project PC 260 (completed in 2008), together with findings from overseas on similar or identical diseases, in order to identify crop and environment conditions favourable to infection. Biofungicides and fungicides registered or likely to be registered for use in the UK and with evidence of activity against *Fusarium* sp. will be evaluated for their efficacy as preventative treatments applied to pepper flowers.

The project objectives in Year 1 were:

1. To obtain and review the latest research results on this disease;
2. To confirm the identity of *Fusarium* species associated with internal fruit rot in the UK by molecular characterisation of isolates;
3. To determine the influence of inoculum type and flower age on infection.

1. Review of pepper Fusarium internal fruit rot

UK experience and research

In the UK a Fusarium internal fruit rot of sweet pepper was first identified in 2005 when it affected up to 20% of a day's pick on one nursery; the cause was identified as *F. oxysporum* (O'Neill, 2005). Subsequently the disease was shown to be present in many glasshouse crops, the severity varying with variety, nursery, glasshouse and time of year (O'Neill, 2008; Gill Wardell, pers comm.). The disease is more of a problem in the spring and autumn when fruit take longer to ripen (56-70 days), than in the summer (38-40 days). Isolates of *F. oxysporum* from sweet pepper caused a rot when wound-inoculated onto pepper fruit, but drench inoculation to roots did not cause a wilt in pepper, or in cucumber, tomato or lettuce indicating that isolates of the species from pepper fruit were not typical of a wilt pathogen. Many isolates from sweet pepper fruit showed a distinctive peach/orange colour in culture on potato dextrose agar (PDA), different from the more common purple or white colour of *F. oxysporum* isolates that caused wilt in tomato and other crops. The same peach/orange-coloured *Fusarium* was isolated from pepper seeds inside fruit affected with internal fruit rot.

Fusarium was found to be relatively common in UK pepper crops, especially on aborted fruit, crop debris on the floor and rotting mature fruit missed by pickers. On one nursery fruits damaged by tortrix moth caterpillar showed a high incidence of Fusarium rot with rot around the wound site; on other nurseries fruits incompletely fused at the flower end

showed a greater incidence of the disease. Increased crop hygiene to remove sources of inoculum is practised on some nurseries and this appears to give some control. Sodium hypochlorite seed treatment was shown to reduce occurrence of *Fusarium* sp. on packeted seed, but an experiment comparing the incidence of Fusarium fruit and stem rot on plants grown from treated and untreated seed was inconclusive due to a low incidence of the disease (PC 260). Occurrence of *Fusarium* sp. on commercial seed suggests that this is one possible means by which the disease is introduced to a nursery, although it is unknown how such seed infection might lead to fruit infection.

In 2007-2009, the incidence of Fusarium internal fruit rot on the same red fruit variety, cv. Special was assessed in three contiguous glasshouse blocks (Adams, 2010). An increased incidence of the disease was found to be associated with glasshouse block and not with de-leafing treatment. The host grower indicated that the block with the consistently greater incidence of Fusarium internal fruit rot tended to run at higher humidities than other blocks due to its location. Other growers have reported the disease is consistently worse in some blocks than others, suggesting an environmental influence on the disease.

Grower observations indicate the disease can be localised in a crop, possibly associated with environmental conditions favourable to infection, including high humidity or condensation. Flowers are produced near the top of pepper plants where the leaf canopy is relatively open; one might therefore expect the humidity around flowers to reflect that of the glasshouse or area of glasshouse.

Discussion with members of the Pepper Technology Group indicates that Fusarium internal fruit rot continues to be a frequent cause of rejection by packers and complaints by supermarkets for UK growers.

Overseas research

Occurrence and cause(s)

Fusarium internal fruit rot of pepper has been reported in Belgium, Canada and the Netherlands.

In Belgium the disease was first described in 2003. Isolates obtained from infected fruit on 15 nurseries were identified by molecular tests variously as *Fusarium proliferatum*, related to *F. nygamai* and *F. lactis* and belonging to the *F. oxysporum* complex (Aerts *et al.,* 2006). All isolates were pathogenic when inoculated into fruit.

In British Columbia, Canada, the disease was observed in 2001 when 40% of fruits in a commercial greenhouse were affected (Utkhede & Mathur, 2003). Only one fungal species

was consistently isolated and, based on morphology, this was identified as a *Fusarium subglutinans*-like species (subsequently re-identified as *F. lactis*). In Alberta, Canada, an internal fruit rot of sweet pepper was found in 2003 (Yang *et al*., 2009). Samples collected from nine greenhouses in 2004 resulted in 56 isolates of *Fusarium* that were identified by DNA analysis. Isolates were classified into *F. lactis* (32), *F. solani* (18), *F. proliferatum* (3) and *F. oxysporum* (3). In pathogenicity tests, isolates of *F. lactis* caused a slow internal fruit rot when inoculated onto flowers; *F. solani* caused a rapid external rot of fruit. *Fusarium lactis* was subsequently isolated and identified from infected sweet pepper fruit from greenhouses in Saskatchewan and Ontario. Based on molecular analysis, Kharbanda *et al*. (2006) reported that isolates from Alberta previously identified as *F. subglutinans* were highly similar to *F. lactis*.

Fusarium lactis has previously been reported as a cause of internal fruit rot on figs (*Ficus carica*) where it was reported to be weakly virulent (Nirenberg & O'Donnell, 1998).

In the Netherlands, an unknown *Fusarium* species related to *F. lactis*, *F. solani*, *F. oxysporum* and *F. proliferatum* were reported associated with pepper fruit rot (Aerts, pers. comm; Hubert *et al.,* 2003).

There is no report of Fusarium internal fruit rot of pepper in the APS Compendium of Pepper Diseases, a reference book primarily based on disease occurrence in the USA (Pernezny *et al*., 2003).

Recently, a comprehensive study was done by a Belgium research group to characterise European isolates of *Fusarium* from sweet pepper (van Poucke *et al.,* 2012). The study compared isolates by DNA sequence analysis of four genes, mating type and morphological features. The set of isolates examined comprised ones from Belgium (82), the Netherlands (9) and the UK (6); the latter were obtained from pepper glasshouses and seed by ADAS in 2006-2007. An isolate from Canada was also examined.

Of these 98 isolates, 74 were identified as *F. lactis* or *F. lactis*-like, 13 as *F. oxysporum*, nine as *F. proliferatum* and two as belonging to the *F. solani* species complex. There was an unusually high level of genetic diversity among isolates of the *F. lactis* species complex. Both mating types (*MAT1-1* and *MAT1-2*) were present in the *F. lactis* species complex, indicating the possibility of sexual compatibility and increased variation in the fungus.

Colony colour, colony morphology and the size of macroconidia of isolates belonging to the *F. lactis* species-complex differed, and these were sometimes variable even within a sequence type (*F. lactis* species complex was divided into 10 sequence types). Details of colony colour and morphology were not presented. When grown on banana leaf agar the

macroconidia produced by different isolates varied significantly in length and number of septa.

The authors noted that the large genetic and phenotypic diversity of *Fusarium* isolates that cause internal fruit rot of sweet pepper is striking, especially as the disease has only been observed in crops for less than 10 years. It was suggested that, given *F. lactis*, *F. proliferatum* and *F. oxysporum* can cause the disease, and that their pathogenicity to pepper is relatively low, the interaction between these Fusarium species and pepper fruit is not very specific. Instead, it was suggested that the recent occurrence of these fungi in pepper fruit is due to reduced resistance of new pepper varieties. This could explain why a number of Fusarium species can cause the disease and why it has emerged in many countries simultaneously. Inoculation experiments on older pepper cultivars are needed to test this hypothesis. Detection of *F. lactis* and *F. proliferatum* in the air of a tomato glasshouse in Belgium indicates that the fungi that cause Fusarium internal fruit rot of pepper may be generally present in the air, although at low concentrations.

It was also suggested that changes in climate control in greenhouses, adopted by growers as a consequence of higher energy prices, may allow higher relative humidity and increased condensation on flowers which could favour infection.

Biology

Microscopy studies have shown that internal fruit rot of sweet pepper caused by *F. lactis* is initiated through infection of the stigma and style during flowering (Yang *et al*., 2010). Flowers were artificially pollinated by gentle brushing and then inoculated by placing 10 µL of a conidial suspension (1-3 x 10^6 spores/mL in water + Tween 20) in the flowers. There was hyphal growth on the stigma (and anthers) at 1 day after inoculation and within the style and ovaries at 6 days after inoculation. At 45 days after inoculation, when externally symptomless fruit were sectioned, typical internal fruit rot symptoms were observed. Symptomless seeds from infected fruit also yielded colonies of *F. lactis.*

Tissues of the pepper style contain large intercellular spaces (Hu & Xu, 1985), which probably facilitate hyphal growth even for a weak pathogen such as *F. lactis.* Senescence of the flower tissues could also favour growth of *F. lactis*. Infection of the placenta and seeds occurred at a position close to the original location of the style (distal end of fruit, where remains of flower parts or a small black hole are sometimes found), supporting the hypothesis that infection occurs by hyphal growth through the style, rather than penetration through the fruit wall.

Mycelial growth on seed and strong discolouration of seed suggest that *F. lactis* probably utilises nutrients from the developing seeds to support its growth and cause infection of seed. In our assessments of the location of *Fusarium* sp. within pepper fruit in the UK, occurrence on seeds and placenta is more common than on the fruit internal wall; fruit which have an internal fruit rot almost invariably have obvious Fusarium infection on seeds also (O'Neill, unpublished).

The occurrence of *F. lactis* on symptomless seeds from infected fruit (Yang *et al*., 2010) suggests one way the fungus may be spread to different greenhouses. Hyphae were observed on the inside of the seed coat and in the endosperm (i.e. internal infection). In an examination of commercial pepper seed in the UK we also found *Fusarium* sp. on symptomless seeds, with growth occurring from both untreated and hypochlorite surface disinfected seeds (O'Neill, unpublished). Commercial pepper seed in Canada tested in 2003 was reported to be infected with *F. subglutinans* (= *F. lactis*) (Utkhede & Mathur, 2004).

Assuming seed is a pathway by which *F. lactis* is introduced into a glasshouse, it remains to be established how the fungus spreads from seeds to flowers.

Yang *et al*. (2010) also showed that pollen grains can become colonised by *F. lactis*. It is possible that pollinating insects may spread the fungus from flower to flower within a house by movement of pollen. Fungal hyphae and chlamydospores were observed on pollen grains on a bee collected in a greenhouse in Alberta, Canada. *Fusarium* sp. was isolated from dead bees collected from a pepper greenhouse in the UK (O'Neill, unpublished). In further work in Alberta, Canada, Fusarium spores were found in the air in infested greenhouses, and bees were found to carry fusarium spores on their mouthparts and legs (Anon., 2006).

In a study of flower infection in Belgium, flowers sprayed with a spore suspension of *F. nygamai* (probably a mis-identification, see Van Poucke et al., 2012) developed a high incidence (41%) of internal fruit rot (Aerts *et al.*, 2006). *Fusarium* was found sporulating on dried styles and petals of inoculated flowers, whereas *Penicillium expansum* was commonly found on uninoculated flowers. Using infrared photographs of pepper flowers taken over several days, it was reported that flowers partially closed during the night and that dew point temperature was reached for several hours. It was suggested that condensation on flowers probably makes them more susceptible to infection by *Fusarium* species.

Mycotoxin production

The identification of *F. lactis, F. oxysporum* and *F. proliferatum* as predominant causes of pepper Fusarium internal fruit rot raises the question of mycotoxin production as both species are members of the *Gibberella fujikuroi* species complex, a group known to produce mycotoxins. This species complex is known to produce fumonisins, beauvericin and other mycotoxins which can produce severe disease in humans (van Poucke *et al*., 2012). Isolates of *F. lactis* and *F. proliferatum* were examined by Belgium researchers for their ability to produce mycotoxins in red sweet pepper fruits. *F. proliferatum* produced limited amounts of beauvericin and traces of one fumonisin (van Poucke *et al.,* 2012). No mycotoxins were detected in pepper inoculated with *F. lactis sensu stricto*, while some isolates closely related to *F. lactis* produced moderate to high levels of beauvericin. Another study showed that there was no migration of beauvericin beyond the fungal affected fruit tissue, while fumonisins migrated to some extent (at least 15 mm) into the surrounding healthy tissue (Monbaliu *et al*., 2010). Mostly it was at a lower level in healthy tissue than in the lesions.

Control

Varieties are reported to differ in their susceptibility to Fusarium internal fruit rot, although none so far have been reported as resistant. In a variety susceptibility trial in Canada, white and orange peppers were reported to be more susceptible than yellow and brown pepper (Anon., 2006). Disease incidence ranged from 20% (cv. Marona) to 65% (cv. Sympathy); the same two varieties showed the greatest range in disease severity.

In a study in Belgium, it was reported that varieties that produce large flowers appear more susceptible (Aerts *et al.*, 2006).

Measures recommended to reduce occurrence of Fusarium internal fruit rot include good sanitation practice, careful disposal of infected fruits, maintaining good air circulation, keeping the relative humidity below 85%, and preventing wounding to the fruit (Anon., 2006).

Application of biological and chemical treatments to flowers for control of pepper fruit rot caused by *F. subglutinans* (= *F. lactis*) has been investigated in Canada (Utkhede & Mathur, 2005). A spore suspension of *F. subglutinans* (0.1 mL of 3 x 10^6 conidia/mL) in sterile distilled water was pipetted into the middle of flowers of the very susceptible cv. Sympathy one day after application of treatments. Fruit were assessed for internal fruit rot 60 days later. Details of the products examined and results of two experiments are summarised in Table 1.1 and 1.2. A significantly smaller proportion of pepper fruits from flowers treated

with Rovral (iprodione), Prestop (*Gliocladium catenulatum*) and Quadra 137 (*Bacillus subtilis*) were infected than those from inoculated control fruit at three or more of the five harvests in both years; Mycostop (*Streptomyces griseoviridis*) and PlantShield (*Trichoderma harzianum*) were less effective. Flowers treated with Rovral had significantly higher fruit weight at four out of five harvests in both years. These results suggest that Rovral, Prestop and some isolates of *Bacillus subtilis* have potential to prevent internal fruit rot of sweet pepper caused by *F. subglutinans* (= *F. lactis*).

Table 1.1. Detail of products evaluated for control of pepper internal fruit rot (adapted from Utkhede & Mathur, 2005)

Product	Rate (g/L)	Active ingredient
Rovral	1	iprodione
SoilGard	5	*Gliocladium virens* strain GL 21
Prestop	10	*Gliocladium catenulatum* strain J1446
Quadra 136	20	*Bacillus subtilis*
Quadra 137	10	*Bacillus subtilis*
Mycostop	1	*Streptomyces griseoviridis* strain K61
PlantShield	1	*Trichoderma harzianum* T22

Table 1.2. Effect of biological and chemical treatments on control of Fusarium internal fruit rot on cv. Sympathy (adapted from Utkhede & Mathur, 2005)

	Mean % fruit infected (over 5 harvests)		No. harvests (of 5) with significant control	
	2003	2004	2003	2004
Water (control)	46	50	-	-
Rovral	18	27	3	3
SoilGard	27	37	2	1
Prestop	25	30	4	3
Quadra 136	26	38	2	1
Quadra 137	25	30	3	3
Mycostop	23	36	2	2
PlantShield	24	30	2	2

Other fungicides with reported activity against *Fusarium* species and approved for use on protected pepper in the UK include fludioxonil (in Switch, SOLA 3172/2010) and azoxystrobin (Amistar). Up to three sprays of Switch are permitted on protected pepper, at

a maximum rate of 1 kg/ha and with a 7 day harvest interval; up to four sprays of Amistar are permitted at 1 L/ha with a 3 day harvest interval (SOLA 1295/2002).

During a visit to the Dutch Glasshouse Crops Research Station at Bleiswijk in August 2011, the subject of pepper Fusarium internal rot was discussed. Work on the disease was done for growers in 2008-09 by Groen Agro Control, a private research and consultancy company; results are not in the public domain. No work has been done recently or is in progress on this disease by the Glasshouse Crops Research Station; current effort on peppers is focused on bacterial fruit rot (*Pectobacterium carotovorum*), which caused losses during crop production in 2010.

2. Identification of Fusarium species associated with internal fruit rot

Introduction

Molecular characterisation by DNA sequence analysis of selected areas of the genome is the most definitive method possible to confirm the identity of *Fusarium* species associated with pepper internal fruit rot. DNA sequences for several taxonomically useful genes are available for most of the species associated with the disease overseas. The aim of this work was to collect *Fusarium* isolates from UK pepper crops and identify them to species level by sequencing PCR amplicons for three or four taxonomically useful genes.

Materials and methods

Details of Fusarium isolates

Isolates of *Fusarium* species from the ADAS culture collection, originally obtained from sweet pepper crops in the Lee Valley in 2006 and 2007 (Table 2.1), were sent to the Institute for Agricultural and Fisheries Research (IAFR) in Merelbeke, Belgium, for characterisation. Two of the isolates (F and H) examined in Belgium were also examined at Warwick University (WU5 and WU6).

Table 2.1. Details of UK Fusarium isolates obtained from pepper greenhouses examined by DNA tests in Belgium

Belgium reference	ADAS code	Obtained from:	Colony colour on Potato Dextrose Agar
Fus404	M	Pepper seed	White
Fus405	O	Pepper seed	Peach
Fus406	H	Pepper stem rot	Peach
Fus407	I	Pepper stem rot	Pink/purple
Fus408	Q	Aborted pepper fruit	White/pink
Fus409	F	Internal fruit rot	Peach
Fus410	B	Glasshouse air	Red/yellow, tufted

In 2011, isolates of *Fusarium* were obtained from fruit from three pepper nurseries (A-C) in the Lee Valley, Essex (Table 2.2), from bought packaged fruit where the production nursery was identified on the pack (nursery D), and from affected fruit that developed in an inoculation trial in this project (Experiment 2; see section 3) (Table 2.3). Isolates were grown on potato dextrose agar (PDA) and supplied to University of Warwick for identification by DNA characterisation.

Table 2.2. Details of Fusarium isolates obtained from sweet pepper from three UK nurseries examined by DNA sequence tests – first batch, 2011

WU ref	ADAS Ref	Source of sample			Colour in culture on PDA
		Nursery (code)	Variety	Tissue	
1	11/49a	C	Special	Fruit	Peach
2	11/49b	C	Special	Fruit	Peach
3	11/49c	C	Special	Fruit	Peach
4	11/49d	C	Special	Fruit	Peach
5	F	C	Fiesta	Fruit	Peach
6	H	C	Special	Stem	Peach
7	11/36a	A	Ferrari	Fruit	Peach
8	11/36b	A	Spider	Fruit	White
9	11/48a	B	Ferrari	Fruit	Purple
10	11/48b	B	Ferrari	Fruit	White/pink
11	11/48c	B	Ferrari	Fruit	White
12	11/48d	B	Ferrari	First	White
13	11/48e	B	Ferrari	Fruit	Purple
14	11/48f	B	Kelly	Fruit	White/pinky red
15	11/48g	B	Kelly	Fruit	White
16	11/48h	B	Kelly	Fruit	White/pinky red
17	11/48j	B	Kelly	Fruit	White/pinky red
18	11/48k	B	Kelly	Fruit	White/pinky red

Table 2.3. Details of Fusarium isolates obtained from sweet pepper from nursery A following flower inoculation and examined by DNA sequence tests – second batch, 2011

WU ref	Flower inoculation treatment (with *Fusarium proliferatum*)		Colour in culture on PDA
1-18	T7 – Spray to old flower	(Plot 22)	White/peach
3-20	T4 – Paint brush transfer	(Plot 1)	Pinky-red
7-24	T3 – Mist	(Plot 9)	Peach
8-25	T4 – Paint brush transfer	(Plot 24)	Yellow
10-27	T7 – Spray to old flowers	(Plot 2)	Peach
12-29	T5 – Spray to young plots	(Plot 23)	Light yellow
16-33	T4 – Paint brush transfer	(Plot 1)	Pinky-red
17-34	T7 – Spray to old flower	(Plot 22)	White/peach

Species identification by DNA sequencing

Isolates received at University of Warwick were grown in liquid medium (potato dextrose broth at room temperature with gentle agitation). After 7 days the mycelium was harvested, freeze dried and DNA extracted using a commercial kit (Qiagen DNeasy plant mini-kit following the manufacturer's instructions). Potential inhibitors were removed using a PVP mini-column and DNA stored at -20^{0}C. PCR followed standard conditions. Initial assessment was by agarose gel and all amplicons sequenced in-house.

Four genes were used during parts of this study (rRNA gene internal spacer [ITS]; elongation factor [TEF], calmodulin and rpb2). Primers used were either designed in-house (TEF PCR – Viki Vagany) or published (O'Donnell *et al*. 1998, 2000, 2007). The rRNA ITS is good for establishing identity to the genus level (i.e. as *Fusarium*) whilst the other three are appropriate to species and sub-species identification. The results presented here are based on the TEF gene. Other genes gave results consistent with TEF.

Results

Belgium tests

The set of seven UK isolates examined in Belgium comprised isolates obtained from packeted seed, fruit rots, stem rots and glasshouse air. The cultures varied in colour and appearance on PDA. Four different species were identified (Table 2.4). The three peach-coloured isolates, generally the most common type isolated from internal fruit rots in the UK, were all identified as *F. lactis*-like. This is consistent with the species identified as the

cause of internal fruit rot in Canada, and the most common cause in Belgium and the Netherlands. The two other species (*F. proliferatum* and *F. oxysporum*) identified from UK samples have also been previously associated with pepper internal fruit rot. The *F. graminearum* trapped from glasshouse air is probably not associated with disease in peppers; this species was found only once and has not been reported elsewhere associated with a pepper disease.

Table 2.4. Characterisation of *Fusarium* species obtained from UK pepper greenhouses by IAFR, Belgium

Belgium ref.	ADAS code	Source of isolate	Colony colour	Identification
Fus404	M	Seed	White	*F. proliferatum*
Fus405	O	Seed	Peach	*F. lactis*-like
Fus406	H	Stem	Peach	*F. lactis*-like
Fus407	I	Stem	Pink/purple	*F. oxysporum*
Fus408	Q	Fruit	White	*F. oxysporum*
Fus409	F	Fruit	Peach	*F. lactis*-like
Fus410	B	Air	Red/yellow, tufted	*F. graminearum*

UK tests – commercial crop samples

The initial set comprised 18 isolates of *Fusarium* obtained from five varieties over three nurseries, predominantly from fruit with internal Fusarium rot symptoms. The cultures varied in colour from white through to purple (Table 2.5).

A cluster analysis (Fig 2.1) based on DNA sequence results indicated four different *Fusarium* species. The six isolates from nursery C were identified as *F. lactis*-like; these were all peach-coloured. Two of these isolates were previously tested by a Belgium research group and also identified as *F. lactis*-like. One isolate from nursery B was identified as *F. lactis* while six isolates from this nursery were identified as *F. oxysporum*. These latter isolates were all white or white/pink in colour. One isolate each from nurseries A and B were identified as *F. proliferatum*; these were also white in colour. Two isolates from nursery B were identified as *F. solani*. None of the isolates was closely related to *F. andiyazi* or *F. nygamai*.

In a subsequent test on a Fusarium isolate obtained from pepper fruit from nursery D in Kent (WU 14-31), the fungus was identified as *F. lactis*.

Although there is some evidence here of colony colour on PDA being associated with species identification (Tables 2.4 and 2.5), later results (Table 2.6) showed no clear association. Elsewhere it has been reported that colony colour and colony morphology of

isolates belonging to different *F. lactis* species complex differed according to sequence type, and sometimes these were variable even within a sequence type (van Poucke *et al.,* 2012). Given that two species can sometimes develop the same colour, this character cannot be used to identify species.

Table 2.5. Characterisation of *Fusarium* isolates obtained from UK pepper greenhouses by Warwick University – first batch, 2011

WU ref	Nursery	Colony colour on PDA	Identification
1.	C	Peach	*F. lactis* - like
2.	C	Peach	*F. lactis* – like
3.	C	Peach	*F. lactis* – like
4.	C	Peach	*F. lactis* – like
5.	C	Peach	*F. lactis* – like
6.	C	Peach	*F. lactis* – like
7.	A	Peach	*F. proliferatum*
8.	A	White	*F. proliferatum*
9.	B	Purple	*F. solani*
10.	B	White/pink	*F. oxysporum*
11.	B	White	*F. solani*
12.	B	White	*F. oxysporum*
13.	B	Purple	*F. lactis*
14.	B	White/pinky red	*F. oxysporum*
15.	B	White	*F. proliferatum*
16.	B	White/pinky red	*F. oxysporum*
17.	B	White/pinky red	*F. oxysporum*
18.	B	White/pinky red	*F. oxysporum*

All isolates from fruit except isolate 6 (from a stem lesion).

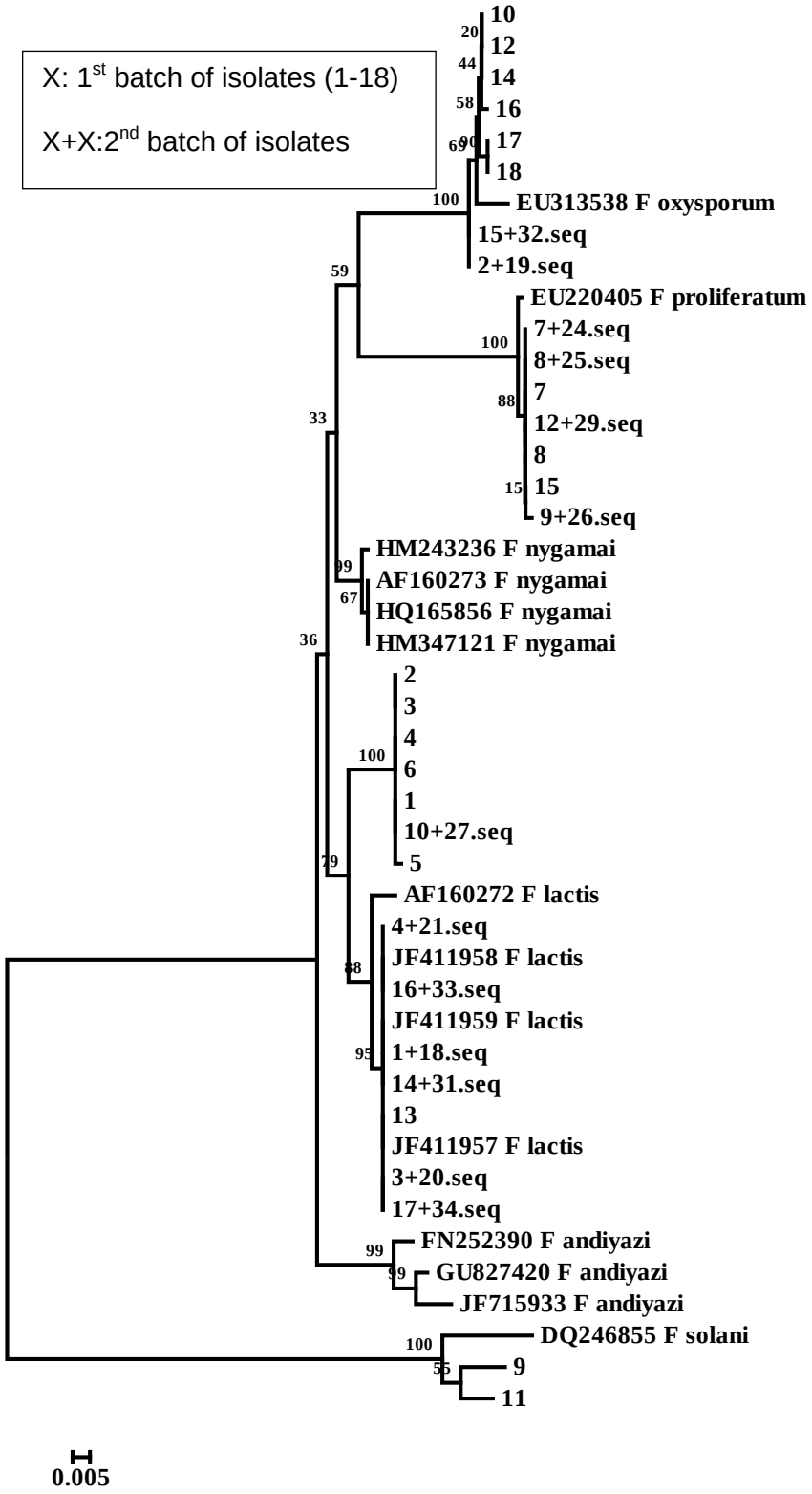

Fig 2.1. Relationship (based on cluster analysis) of isolates of *Fusarium* spp. from UK pepper crops to reference isolates of six *Fusarium* species (*F. andiyazi, F. lactis, F. oxysporum, F. proliferatum, F. nygamai* and *F. solani*). First batch labelled 1-18 (see Table 2.2); second batch labelled X+X (see Table 2.3). Numerical values at branch points represent bootstrap support for this topology.

UK tests on fruit developing from inoculated flowers

Eight isolates were obtained from fruit collected following inoculation of flowers with *F. proliferatum* (see section 3, Experiment 2) and characterised. Three of the isolates were identified as *F. proliferatum*, four as *F. lactis* (*sensu stricto*) and one as *F. lactis* (*sensu lato*) (Table 2.6). There are two possible explanations for identification of two species following inoculation with one species. One is that the original culture (11/36b) used to inoculate fruit was a mixture of two species, *F. proliferatum* and *F. lactis*. The second is that infected fruit in the glasshouse arose from a mixture of artificial and natural inoculation of flowers. The culture was obtained by transfer of mycelium from one affected fruit, and not by a single spore isolation method, so a mixed infection of these two species is possible. Considering the dual inoculation hypothesis, the *F. lactis* isolates recovered were obtained from treatment 4 (paint brush transfer inoculation) and treatment 7 (spray inoculation to old flowers). Possibly these treatments were less effective inoculation methods than spray inoculation of young flowers, allowing natural infection by *F. lactis* to swamp or jointly occur with any *F. proliferatum* infection. The lack of any rots in fruit developing from uninoculated flowers suggests this is unlikely; however, some commercial fruit outside the trial area were affected by Fusarium internal rot, indicating a level of natural infection in the house (e.g. from air-borne spores or transferred by pollinating insects). Moreover, tagged fallen aborted fruit from uninoculated flowers were found to be infected internally by *Fusarium* sp., again indicating natural infection. The identity of the species within aborted fruit from uninoculated flowers was not determined. A marked strain of *Fusarium* would need to be used in order to determine conclusively whether fruit rots in a particular experiment originate from artificial or natural inoculation.

Table 2.6. Characterisation of *Fusarium* isolates obtained from pepper fruit in inoculation experiment 1 by Warwick University – batch 2

WU ref	Treatment *	Colony colour	Identification
1-18	T7, plot 22	White/peach	*F. lactis* (ss)
3-20	T4, plot 1	Pinky red	*F. lactis* (ss)
7-24	T3, plot 9	Peach	*F. proliferatum*
8-25	T4, plot 24	Yellow	*F. proliferatum*
10-27	T7, plot 2	Peach	*F. lactis* (sl)
12-29	T5, plot 23	Light yellow	*F. proliferatum*
16-33	T4, plot 1	Pinky red	*F. lactis* (ss)
17-34	T7, plot 22	White/peach	*F. lactis* (ss)

* All fruit were inoculated at the flower stage with an isolate of *F. proliferatum*. T3 – spore drop inoculation; T4 – paint brush transfer; T5 – spray inoculation of flowers (low concentration); T7 – spray inoculation of flowers (high concentration); ss- *sensu stricto*; sl – *sensu lato*.

3. Effect of Fusarium inoculum and flower age on occurrence of internal fruit rot

Introduction

Pepper Fusarium internal fruit rot arises following infection of flowers (Yang *et al.*, 2010). Aaerts *et al.,* (2006) suggested that the disease was greater on varieties with larger, more persistent flower petals. Utkhede and Mathur (2005) demonstrated that certain fungicides and biofungicides applied to flowers can reduce pepper Fusarium internal fruit rot. The objective of our work was to devise a method for inoculating flowers with *Fusarium* sp. that results in development of internal fruit rot as a prelude to experiments on varietal susceptibility and control in Years 2 and 3.

Materials and methods

Site and crop details

Two experiments were done on a commercial nursery in Essex, in spring and autumn 2011. The work was done on plants of cvs Spider (Experiment 1) and Ferrari (Experiment 2) grown on rockwool slabs in a glasshouse block with a history of Fusarium internal fruit rot. The crop was grown and managed by nursery staff to normal commercial standards. No fungicides were applied to the crop, with the exception of sulphur for control of powdery mildew. Full crop diaries are given in Appendix 1. Temperature and humidity were recorded using EasyLog USB loggers suspended in the crop row at around 1.5 m height.

Treatments

Treatments are detailed in Table 3.1. All inoculations were done onto fresh white fully opened flowers except for treatment 7 in Experiment 2.

Table 3.1. Details of inoculation treatments to pepper flowers - 2011

Treatment	Method
Experiment 1 (5 May – 7 July 2011)	
1. Uninoculated	-
2. Water control	20 µl droplet of sterile distilled water (SDW) pipetted into flowers
3. Spore droplet	20 µl droplet of suspension of *Fusarium* sp., at 5 x 10^5 spores/ml, pipetted into flowers (around 10^4 spores/flower)
4. Dry spore transfer	Paint brush transfer of *Fusarium* sp. spores from a PDA plate onto flower petals, two wipes per flower
5. Spray inoculation (low concentration)	Spray of spores in water at 1 x 10^3/ml onto flowers (around 800 spores/flower)
6. Spray inoculation (medium concentration)	Spray of spores in water at 1 x 10^5/ml onto flowers (around 8 x 10^4 spores/flower)
7. Spray inoculation (high concentration)	Spray of spores in water at 1 x 10^7/ml onto flowers (around 8 x 10^6 spores/flower)
Experiment 2 (11 August – 2 November 2011)	
1. Uninoculated	-
2. Water control	20 µl droplet of SDW pipetted into flowers (around 10^4 spores/flower)
3. Mist inoculation	Spore suspension in water (5 x 10^5/ml) misted onto flowers (5 x 10^4 spores/flower)
4. Dry spore transfer	Paint brush transfer of spores onto petals
5. Spray inoculation (low concentration)	Spray of spores in water at 5 x 10^3/ml onto flowers (around 4 x 10^3 spores/flower)
6. Spray inoculation (medium concentration)	Spray of spores in water at 5 x 10^5/ml onto flowers (around 4 x 10^5 spores/flower)
7. Spray inoculation (old flowers)	Spray of spores in water at 5 x 10^5/ml onto old brown flowers (around 4 x 10^5 spores/flower)

Experiment design and data analysis

Each experiment was done as a randomised block design with four replicates (crop rows). Individual plots consisted of 15 marked stems each with one tagged flower. The 15 plants in a plot were a run of consecutive stems except where there was no flower at the correct stage of development; stems with no suitable flowers were left untagged and uninoculated. A gap of at least 5 stems was left between adjacent plots. For Experiment 2, a further set of

10 flowers was inoculated in each plot around one week after the first inoculation. The set of seven treatments in a block were arranged down one side of a pathway. All fruit that developed from tagged flowers were harvested at maturity for assessment of Fusarium fruit rot. Results were examined by Generalised linear modelling with logit transformation.

Inoculum production and inoculation

An isolate of *Fusarium* sp. obtained from fruit with Fusarium internal fruit rot from the same variety on the host nursery was used. A culture of this isolate (BX11/36a) was sent to University of Warwick and was subsequently identified as *F. proliferatum*.

Spores used for inoculation were obtained from 7-21 day old cultures on PDA. A spore suspension was made in sterile distilled water and filtered to remove mycelial strands. A few drops of Tween 80 wetter were added to reduce spore clumping and the concentration was determined and adjusted as required using a haemocytometer.

Flowers to be inoculated were tagged on the flower stalk with coloured wool in Experiment 1 and with elasticated jewelry labels in Experiment 2. Each stem with a tagged flower was marked with coloured tape to aid finding the correct fruit for harvest.

Spray inoculation was done using a hand-held plastic sprayer, using two squirts per flower. Mist inoculation was done using a scent mister, again with two squirts per flower. Flowers were sprayed at a distance of approximately 3 cm. This resulted in release of around 2 ml and 0.3 ml of spore suspension for the spray and mist treatments respectively; around 10% of the spray volume and 40% of the mist volume went onto or into the flowers. It is estimated this resulted in deposition on each flower of around 0.8 ml and 0.1 ml of spore suspension for spray and mist treatments respectively. Flowers were visibly wet after spray inoculation and damp after mist inoculation. Dry spore transfer was done using a fine artist's paintbrush.

In Experiment 1, both main stem and sideshoot flowers at the full open white stage were used. In Experiment 2, following guidance from the grower, only main stem flowers borne singly (not in clusters) were used, in an attempt to select flowers that were less likely to abort during fruit development due to fruit load or for other reasons.

Assessments

All fruit that developed from tagged flowers were harvested for disease assessment at fruit maturity (July 7 for Experiment 1; October 19 and November 2 for Experiment 2). Additionally in Experiment 1 only, inoculated flowers were examined at 2 weeks after

inoculation to determine the proportion of fallen flowers and set fruit. Fruit that had developed to less than half size were not assessed; these generally contained no seeds.

Fruit were stored in the laboratory for 4-5 days at ambient temperature in the dark and then assessed. Fusarium was recorded as external fruit rot, internal fruit rot and growth on the seed.

Isolates of *Fusarium* were cultured from a sample of affected fruit from the first inoculation of Experiment 2 for species identification at Warwick University, to determine if the species present was the same as that inoculated into flowers.

Results and discussion

The Fusarium isolate (BX11/36a; WU-8) used to inoculate these experiments was subsequently identified as *F. proliferatum* (see section 2). Both *F. proliferatum* and *F. lactis* were recovered from affected fruit. Results on re-isolation from affected fruit are presented in full and discussed in section 2.

Experiment 1

At 2 weeks after inoculation, many flowers in each treatment had fallen or set fruit and fallen (Table 3.2). The number of remaining attached fruit at this stage, out of 60 tagged flowers per treatment, ranged from just 14 to 42; spray inoculation with *Fusarium* sp. (T5-7) appeared to reduce fruit set at this stage.

Five fallen fruit with the coloured wool tags attached (i.e. fruit that had developed from inoculated flowers) were collected from each treatment, cut into four and placed on PDA to check for presence of *Fusarium*. *Fusarium* was confirmed from all fruit from all treatments, appearing as peach or white growth, occasionally as purple. There was no consistent difference in the colour of *Fusarium* cultures growing from aborted fruit in Treatments 1 and 2 (uninoculated) compared with those in other treatments.

The occurrence of *Fusarium* in aborted fallen fruit that developed from flowers not inoculated with the fungus (T1 and T2) is surprising. It suggests a background inoculum of *Fusarium* in the glasshouse with natural deposition on flowers (e.g. from air currents or via bee transfer) or less likely on aborted fruit.

Fruit were harvested at maturity on 7 July, 8 weeks after inoculation; the majority were fully red. Although 211 set fruit were present at 2 weeks after inoculation (65 of which were swelling), only 56 of these developed into full size fruit. Most of the others had fallen, while a few had developed to around golf-ball size.

Fifteen of the 56 fruit showed Fusarium rot at harvest, with the symptoms present both externally and internally on all of them (Table 3.3). None of the fruit visibly healthy at harvest was found to contain *Fusarium* as internal fruit rot or on the seeds. Fruit affected by Fusarium rot only occurred in Treatments 3-7, i.e. the five treatments where flowers had been inoculated with *F. proliferatum*. The low and medium concentration spray inoculations resulted in the greatest proportion of affected fruit (5/8 and 4/6 respectively). Data was not analysed statistically due to the low numbers.

All of the fruit affected by Fusarium rot had white mycelial growth of *Fusarium* visible on the seeds, and seeds were usually discoloured dark brown. Some fruit without Fusarium rot had seeds discoloured light brown, including the uninoculated treatments; no *Fusarium* growth was found on these light-coloured seeds.

The greatest number of set fruit (14 from 60 flowers) occurred in the uninoculated treatment (T1) and the least (3 from 60 flowers) in the high concentration spray inoculation (T7) (Table 3.3).

Table 3.2. Effect of inoculation treatment on fruit set after two weeks - Experiment 1

Treatment	Number of fruit developed from 60 flowers			Number of fallen flowers/fruit
	Set	Swelling	Total	
1. Uninoculated	25	13	38	22
2. Water control	31	11	42	18
3. Spore droplet	31	10	41	19
4. Dry spore transfer	27	8	35	25
5. Spray – low	11	10	21	39
6. Spray – medium	11	9	20	40
7. Spray – high	10	4	14	46

Table 3.3. Effect of flower inoculation with *Fusarium* sp. on occurrence of Fusarium fruit rot in sweet pepper cv. Spider – July 2011 (Experiment 1)

Treatment	Total number fruit at harvest[a]	Occurrence of Fusarium (% of fruit harvested)		
		External rot	Internal rot	On seed
1. Untreated	14	0	0	0
2. Water control	6	0	0	0
3. Spore droplet	12	17	17	8
4. Dry spore transfer	7	29	29	14
5. Spray – low (1 x 10^3/ml)	8	63	63	25
6. Spray – medium (1 x 10^5/ml)	6	67	67	67
7. Spray – high (1 x 10^7/ml)	3	33	33	33
Totals	56			

[a] Fruit were harvested on 7 July, 63 days after inoculation.

Experiment 2

Using the combined data from the two harvests, the number of fruit examined for each treatment ranged from 14 to 33 (Table 3.4). All of the inoculation treatments except for mist inoculation significantly reduced (P = 0.027) the number of flowers that developed into mature fruit. Although some fruit showed external Fusarium fruit rot, a greater proportion of fruit were affected internally (Table 3.4). All of the fruit affected by Fusarium were red or red/green in colour. None of 34 green fruit was affected by Fusarium.

The proportions of fruit with Fusarium external rot, internal rot or growth on the seed were significantly affected by treatment. No fruit developing from uninoculated flowers or flowers treated with water only (T1 and T2) were affected by Fusarium.

The lack of Fusarium in uninoculated fruit is consistent with Experiment 1 but contrasts with the occurrence of Fusarium inside fallen aborted fruit noted in Experiment 1. Possibly this may be explained in that fallen aborted fruit and mature fruit are self-selecting different samples i.e. flowers in any treatment where natural infection occurred tended to abort, and only flowers that did not receive a natural inoculum, or perhaps a lower level of inoculum, developed into mature fruit.

The low concentration spray inoculation (T5) resulted in the greatest incidence of Fusarium internal fruit rot (57%). This was significantly greater (P <0.001) than the dry spore transfer (18%) and spray inoculation of old flowers (19%), but not the mist inoculation (Table 3.4).

The low concentration spray inoculation with *Fusarium* spores also resulted in the greatest incidence of Fusarium external rot (45% of fruit). This was significantly greater (P = 0.003) than the mist inoculation (12%), dry spore transfer (11%) and spray inoculation of old flowers (15%) (Table 3.4).

There was evidence (Table 3.4) that old brown flowers were significantly less likely to develop Fusarium fruit rot or seed infection than fresh, fully-open white flowers inoculated with the same spore concentration. This difference was largely due to the lack of Fusarium fruit rot in any fruit developing from inoculated old brown flowers from the second inoculation (Table 3.5).

When the occurrence of any Fusarium symptoms was examined (i.e. fruit were classed as infected whether the rot was external, internal or just as Fusarium growth on seed), the proportion of affected fruit in Fusarium-inoculated treatments ranged from 40-56% (pick 1), 0-56% (pick 2) and 19-56% (combined).

Table 3.4. Effect of flower inoculation with *Fusarium* sp. and flower age on occurrence of Fusarium fruit rot in sweet pepper cv. Ferrari – November 2011 (Experiment 2)

Treatment	Total number fruit at harvest[a]	Occurrence of Fusarium (% of fruit harvested[b])			
		External rot	Internal rot	On seed	Any symptoms
1. Untreated	33	0	0	0	0
2. Water control	27	0	0	0	0
3. Mist	26	12 (6.2)	31 (9.8)	20 (8.6)	35 (10.5)
4. Dry spore transfer	18	11 (7.3)	18 (10.0)	33 (12.1)	35 (12.8)
5. Spray – low (5 x 10^3/ml)	16	45 (11.8)	57 (13.2)	36 (12.9)	56 (13.7)
6. Spray – medium (5 x 10^5/ml)	14	28 (11.2)	43 (14.1)	36 (13.9)	52 (14.8)
7. Spray – old flowers (5 x 10^5/ml)	21	15 (7.4)	19 (9.3)	5 (4.9)	19 (9.5)
Significance (27 df)	0.027	0.003	0.001	0.004	<0.001
LSD	2.8	-	-	-	-

( ) – standard error.
[a] Fruit were harvested on 19 October and 2 November, 69 and 70 days after inoculation; data shown are for the combined harvests.
[b] Logit transformed means; see Appendix 2 for raw data.

Crop environment

In Experiment 1, daily minimum temperature in the glasshouse was around 17ºC while the daily maximum temperature was usually over 30ºC and occasionally over 35ºC (Fig 3.1). Maximum humidity in the glasshouse was around 80% RH, with occasional days at around 83-85% (Fig 3.2). On the day of inoculation (5 May), the RH was around 72% on the afternoon of inoculation and rose to around 78% by early the following morning (Fig 3.3),

after which it fell rapidly to around 40%. Over the trial period, the mean day (9 am - 9 pm) and night humidities (9 pm - 9 am) were 64.8% and 79.7% respectively (Appendix 1).

In Experiment 2, daily minimum temperature was maintained around 17ºC and the maximum temperature was lower than in Experiment 1, around 25-30ºC (Fig 3.4). The relative humidity recorded in this experiment was generally higher than in the first experiment. Daily maximum humidity was in the region of 82-90%, occasionally above 90%, and tended to increase as the season progressed (Fig 3.5). On the days of the inoculation (11-12 August), the RH was 85% or above from around midnight until 9 am the following morning and remained at 70-80% during the day (Fig 3.6). On the day of the second inoculation (24 August) the RH was around 90% from midnight until 9 am the following day and was again high the following night (Fig 3.7). During the daytime, the RH fell to around 60%. Thus, the relative humidity around the crop in Experiment 2 was higher than that in Experiment 1 at and immediately following the time of flower inoculation. The mean day and night humidities during the trial period for Experiment 2 were 79% and 87% respectively (Appendix 1), higher than in Experiment 1.

However, there was no evidence that the overall proportion of flowers that developed into fruit with Fusarium internal rot was favoured by high humidity based on the humidity recorded in the crop row on the day of inoculation. The respective values were:

Experiment 1, 5 May: RH around 72-78%, Fusarium fruit rot 39% (14/36 fruit)

Experiment 2, 11-12 Aug: RH around 83-85%, Fusarium fruit rot 45% (27/60 fruit)

Experiment 2, 24 Aug: RH around 85-90%, Fusarium fruit rot 26% (9/35 fruit)

This may be because (1) different varieties were used in Experiments 1 and 2, which may differ in susceptibility; (2) the numbers of fruit examined in Experiment 1 and in Experiment 2 inoculation 2 were relatively few and so may not be a representative sample of the crop; (3) the RH around flowers may differ from that recorded in the crop canopy. More detailed monitoring of the crop environment in relation to development of Fusarium fruit rot will be done in 2012.

Table 3.5: Effect of inoculation time on occurrence of Fusarium rot in sweet pepper cv. Ferrari – Experiment 2, 2011

Treatment	Pick 1 – October					Pick 2 – November				
	No fruit[a]	% external	% internal	% seed	% any	No fruit[b]	% external	% internal	% seed	% any
1. Untreated	25	0	0	0	0	8	0	0	0	0
2. Water control	23	0	0	0	0	4	0	0	0	0
3. Mist	20	13	36	22	41	6	23	23	25	23
4. Dry spore transfer	12	15	25	39	42	6	0	0	17	19
5. Spray – low (5 x 10^3/ml)	11	32	54	33	54	5	50	50	29	49
6. Spray – medium (5 x 10^5/ml)	7	13	42	42	56	7	55	55	43	56
7. Spray – old flowers (5 x 10^5/ml)	10	31	40	10	40	11	0	0	0	0
Significance (27 df)	-	0.029	0.002	0.009	0.001	-	0.005	0.005	0.111	0.015

[a] 60 flowers per treatment inoculated; [b] 40 flowers per treatment inoculated.
All treatments were applied to fresh, fully open white flowers except for T7.

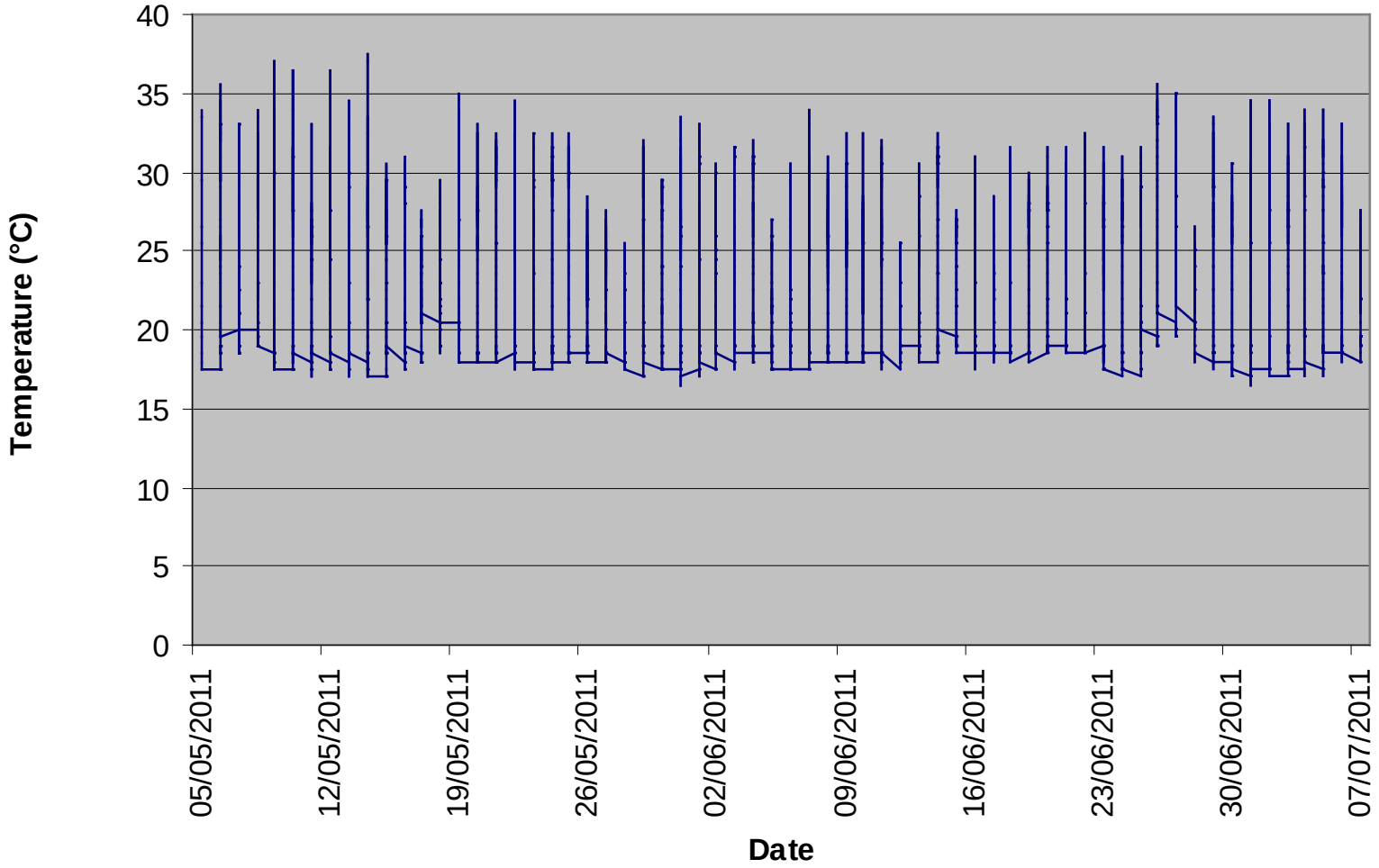

Figure 3.1. Experiment 1: Temperature recordings from a logger placed in the pepper crop in the greenhouse from 5 May to 7 July 2011 (flowers inoculated 5 May).

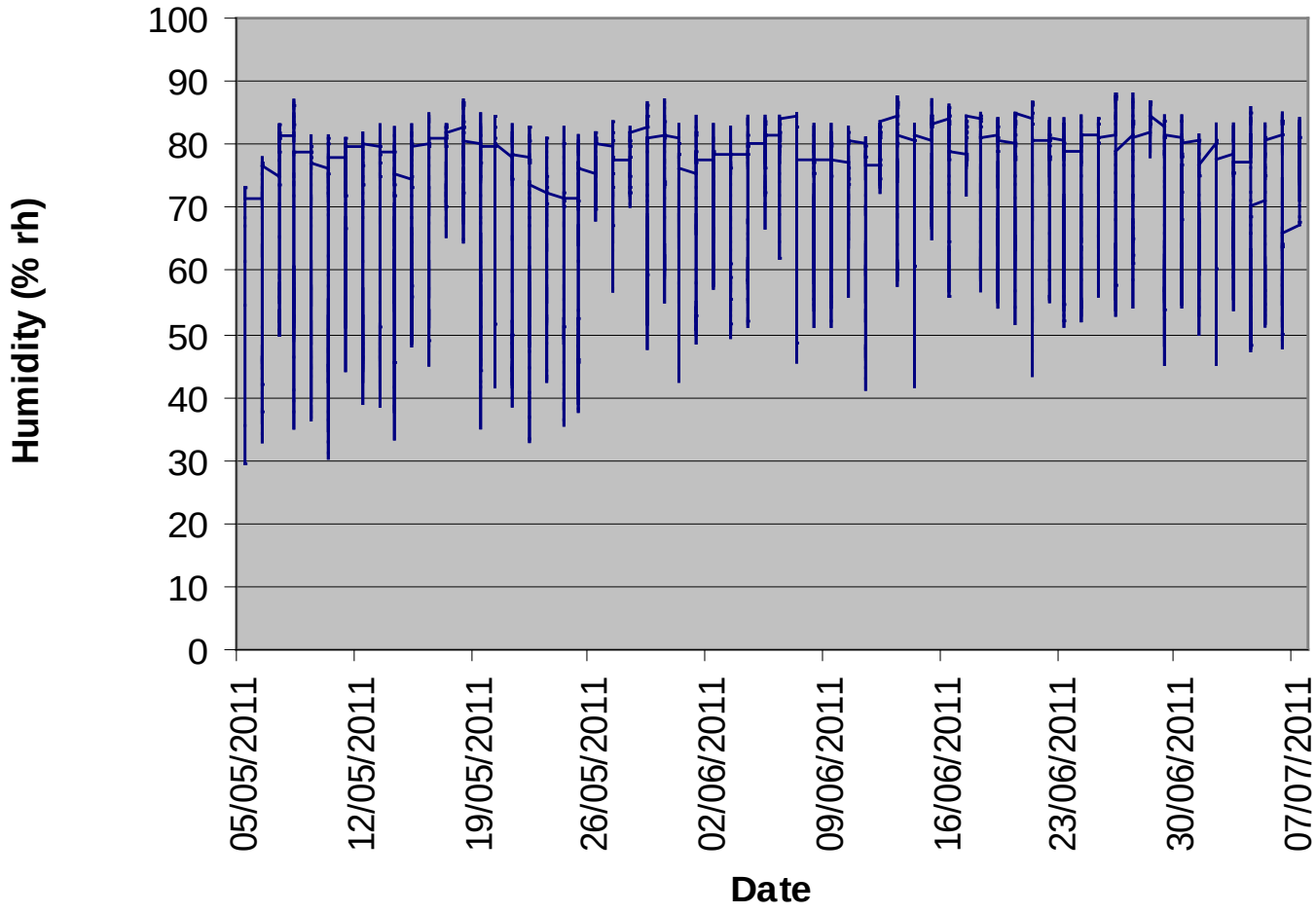

Figure 3.2. Experiment 1: Humidity recordings from a logger placed in the pepper crop in the greenhouse from 5 May to 7 July 2011 (flowers inoculated 5 May).

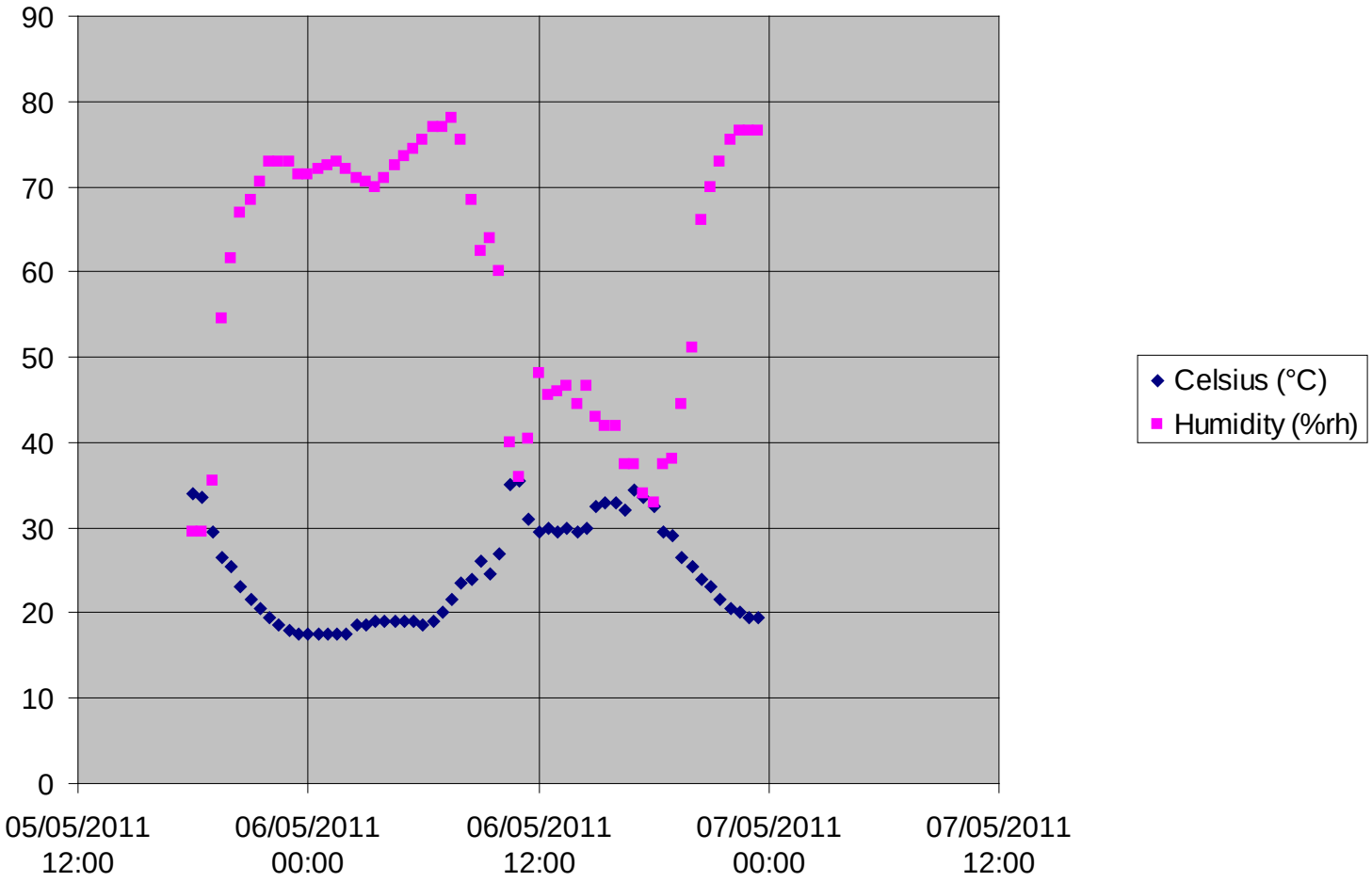

Figure 3.3. Experiment 1: Temperature and humidity recordings at time of inoculation and the following 24 hours, from a logger placed in the pepper crop in the greenhouse from 5 May to 7 July 2011 (flowers inoculated 5 May).

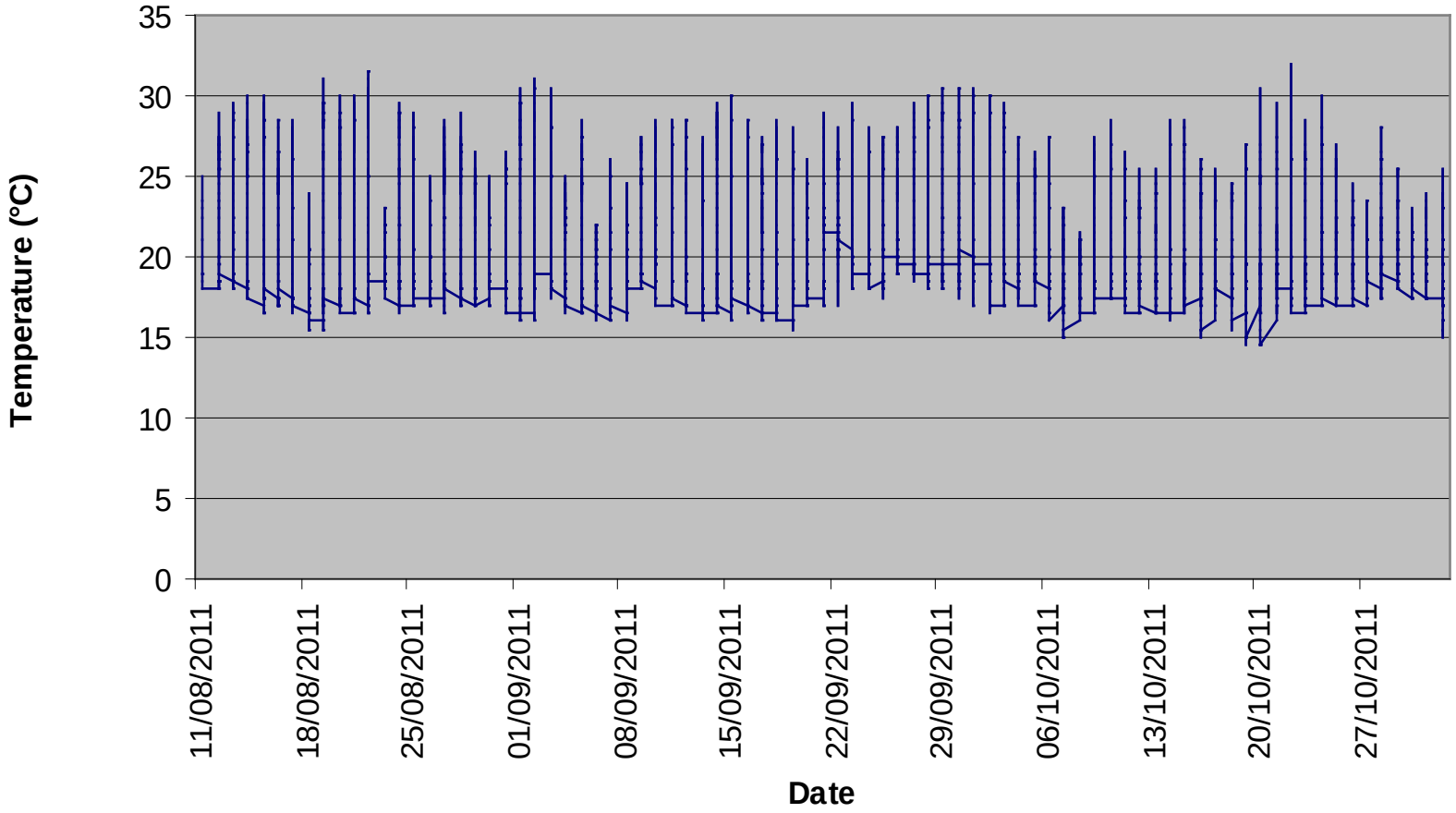

Figure 3.4. Experiment 2: Temperature recordings from a logger placed in the pepper crop in the greenhouse from 11 August to 2 November 2011 (flowers inoculated 11/12 and 24 August).

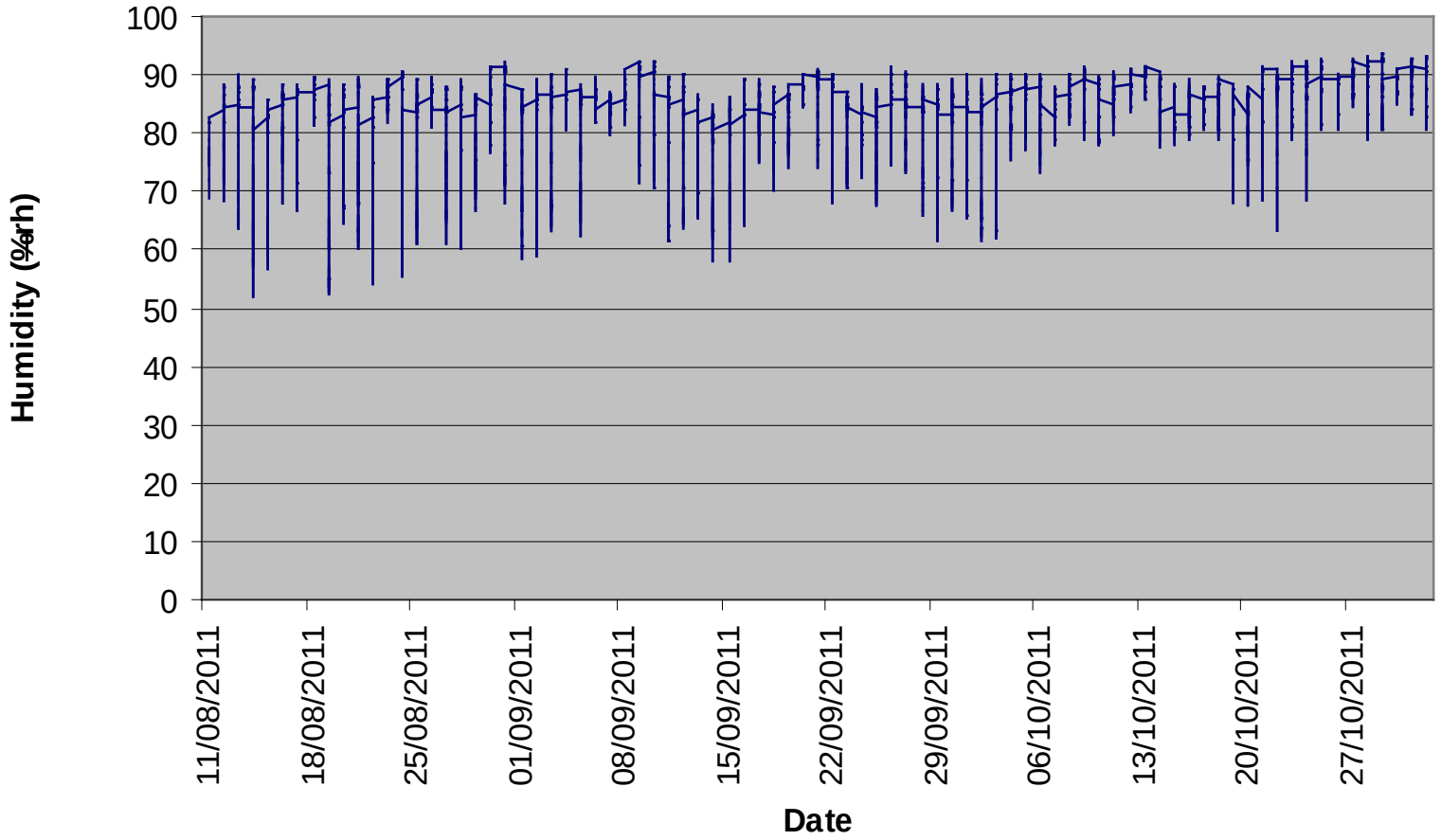

Figure 3.5. Experiment 2: Humidity recordings from a logger placed in the pepper crop in the greenhouse from 11 August to 2 November 2011 (flowers inoculated 11/12 and 24 August).

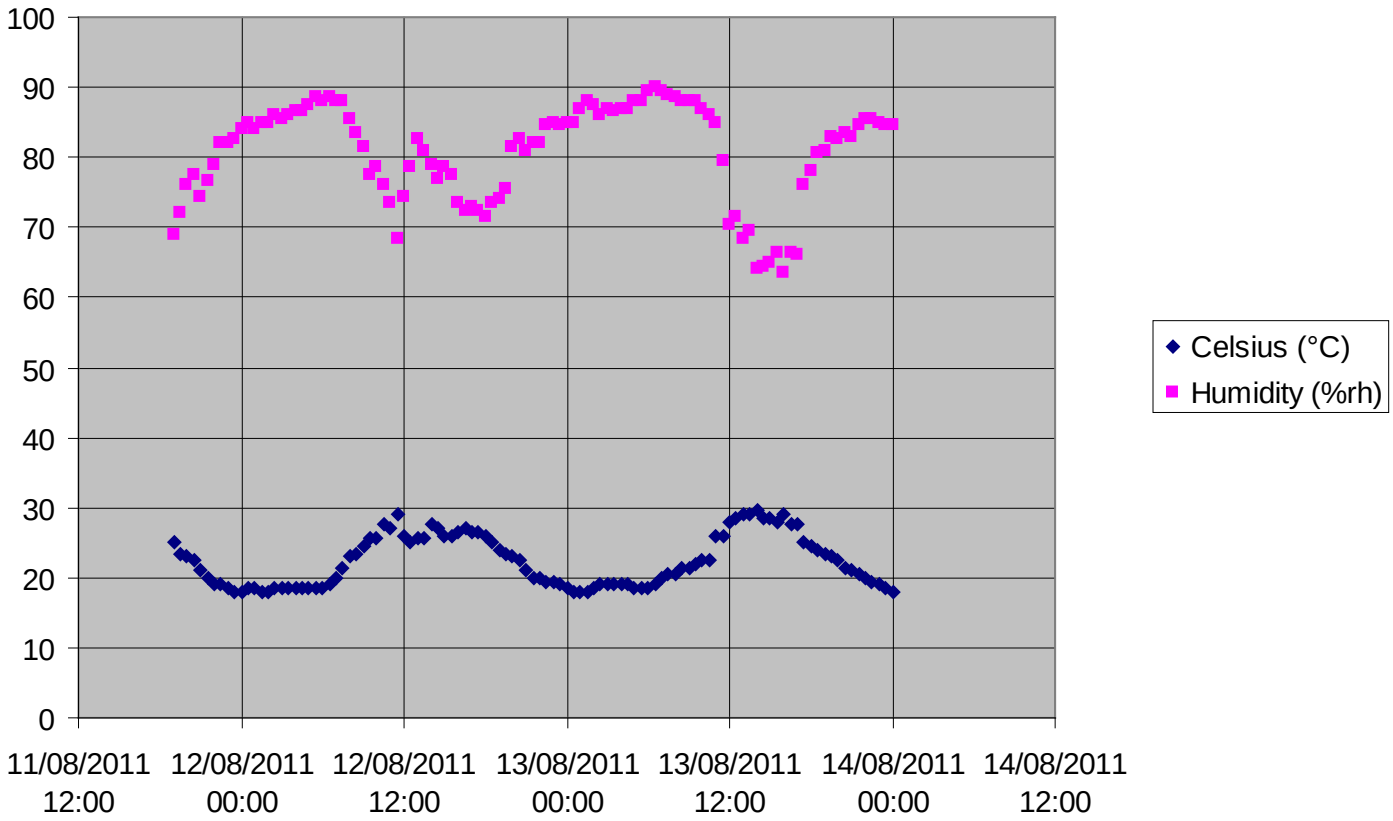

Figure 3.6. Experiment 2: Temperature and humidity recordings at time of inoculation and the following 24 hours, from a logger placed in the pepper crop in the greenhouse from 11 August to 2 November 2011 (flowers inoculated 11 and 12 August).

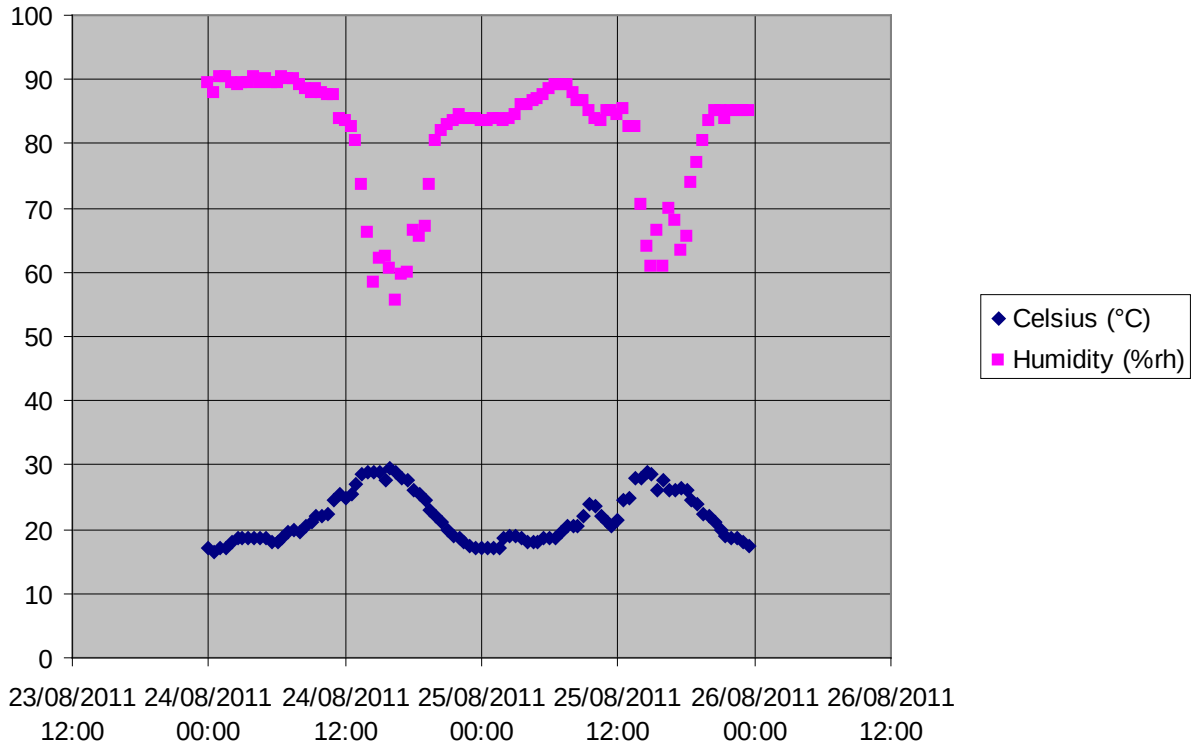

Figure 3.7. Experiment 2: Temperature and humidity recordings at time of inoculation and the following 24 hours, from a logger placed in the pepper crop in the greenhouse from 11 August to 2 November 2011 (flowers inoculated 24 August).

Conclusions

From this project:

1. Fusarium internal fruit rot is most commonly caused by *F. lactis* and somewhat less frequently by *F. oxysporum* and *F. proliferatum*.
2. Fruit on a particular nursery may be affected by one of these species or variously by all three species.
3. *Fusarium oxysporum* and *F. proliferatum* are closely related to each other and more distantly related to *F. lactis*; *F solani* is only very distantly related to these three species.
4. Colony colour on potato dextrose agar, although sometimes consistent for a particular species, is not a reliable feature for identification of the three *Fusarium* species that cause pepper internal fruit rot.
5. Inoculation of pepper flowers with spores of *F. proliferatum* resulted in mature fruit with Fusarium internal fruit rot.
6. Inoculation of pepper flowers by spray inoculation with *F. proliferatum* reduced the numbers that develop into mature fruit; small brown hard aborted fruit often contain Fusarium rot within them.
7. Pepper flowers are more susceptible to development of Fusarium internal fruit rot if inoculated when young (fresh white and fully open) than when old (off white and flaccid).
8. Long periods (> 8 hours) of high relative humidity (> 85%) were recorded in the crop canopy in August 2011; these may increase the risk of Fusarium internal fruit rot.
9. Fusarium internal fruit rot is more likely to be found in more mature fruit (red stage) than less mature fruit (green stage) or the same age.
10. The pepper varieties Ferrari, Fiesta, Kelly, Special and Spider are all susceptible to Fusarium internal fruit rot.

From recent overseas research

1. Pepper Fusarium internal fruit rot is an emerging disease that has occurred simultaneously in major sweet pepper growing regions (northern Europe and Canada) over the last 10 years.

2. The causes of pepper Fusarium internal fruit rot are the same in Belgium, Canada, the Netherlands and the UK.
3. *F. lactis* is a weak pathogen that grows down the style and develops slowly on the placenta and seeds as fruit swell.
4. Symptomless internal seed infection is one pathway by which *Fusarium lactis* can be introduced into a glasshouse; limited spore trapping in a glasshouse tomato crop showed that *F. lactis* and *F. proliferatum* can occur in the air and may be introduced into a crop this way.
5. *F. lactis* can develop on pepper pollen grains suggesting that bees and other pollinating insects can transfer the fungus between flowers during transfer of pollen.
6. Mycotoxins (beaumaricin and a fumonisin) were produced in pepper fruit inoculated with *F. lactis* and *F. proliferatum*.
7. The mycotoxin beaumaricin did not migrate beyond the fungal affected tissue; a fumonisin migrated to some extent (at least 15 mm) into surrounding healthy tissue.
8. Mycotoxins in surrounding healthy fruit tissue were mostly at lower levels than in lesions.

Acknowledgements

We are grateful to Kris van Poucke of the Institute for Agriculture and Fisheries Research, Merelbeke, Belgium for identification of some UK *Fusarium* isolates.

Technology transfer

Article

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Appendix 1 – Crop diaries

Experiment 1 Fusarium fruit rot of sweet pepper

Trial Task	**Date completed**
First visit to Essex Nursery. Aborted fruit with fusarium were collected.	13/04/2011
The trial was set up in the new glasshouse block using the pepper variety Spider. All flowers were inoculated with fusarium inoculum (made from cultured fusarium from the nursery).	05/05/2011
Return to Essex Nursery to tag all pepper stems where a flower had been inoculated.	06/05/2011
Week 1 assessment of inoculated flowers.	12/05/2011
Week 2 assessment of inoculated flowers.	19/05/2011
Experiment 1 pepper harvest.	07/07/2011
Pepper assessed for fusarium.	11/07/2011

Experiment 1 Temperature and humidity records for peppers

	Overall mean Temp °C		Overall mean % Humidity	
	Day	night	day	night
Logger data **5th May to 7th July**	26.4	19.9	64.8	79.7

Experiment 2 Fusarium fruit rot of sweet pepper

Trial Task	Date completed
Experiment laid out using the pepper variety Ferrari. Block one and two tagged with pink tags and inoculated. Dataloggers places in trial.	11/08/2011
Block three and four set up, tagged and inoculated.	12/08/2011
Second part of experiment using blue tags set up, flowers tagged and inoculated.	24/08/2011
Fruit with pink tags were harvested.	19/10/2011
Pink tagged fruit assessed for fusarium.	25/10/2011
Fruit with blue tags were harvested.	02/11/2011
Blue tagged fruit assessed for fusarium	07/11/2011

Experiment 2 Temperature and humidity records for peppers

	Overall mean Temp °C		Overall mean % Humidity	
	Day	Night	Day	Night
Logger data 11th August to 2nd November	23.9	18.37	79.39	86.74

Appendix 2 – Raw data from inoculation experiments

Experiment 1 – July 2011

Treatment	Total harvested	Number of fruit with:		
		External rot	Internal rot	Seed Fusarium
1. Untreated	14	0	0	0
2. Water control	6	0	0	0
3. Spore droplet	12	2	2	1
4. Dry spore transfer	7	2	2	1
5. Spray – low (1 x 10^3/ml)	8	5	5	2
6. Spray – medium (1 x 10^5/ml)	6	4	4	4
7. Spray – high (1 x 10^7/ml)	3	1	1	1
Total	56	14	14	9

Experiment 2 – November 2011

Treatment	Total harvested	Number of fruit with:			
		External rot	Internal rot	Seed Fusarium	Any symptom
Harvest 1					
1. Untreated	25	0	0	0	0
2. Water control	23	0	0	0	0
3. Mist	20	2	7	4	8
4. Dry spore transfer	12	2	3	5	5
5. Spray – low (1 x 10^3/ml)	11	4	6	4	6
6. Spray – medium (1 x 10^5/ml)	7	1	3	3	4
7. Spray – old flowers (1 x 10^5/ml)	10	3	4	1	4
Total	108	12	23	17	27
Harvest 2					
1. Untreated	8	0	0	0	0
2. Water control	4	0	0	0	0
3. Mist	6	1	1	1	1
4. Dry spore transfer	6	0	0	1	1
5. Spray – low (1 x 10^3/ml)	5	3	3	2	3
6. Spray – medium (1 x 10^5/ml)	7	3	3	2	3
7. Spray – old flowers (1 x 10^5/ml)	11	0	0	0	0
Total	47	7	7	6	8