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Project number:	PE 007
Project leader:	Dr Tim O'Neill, ADAS
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Location of project:	Commercial nursery, Essex, ADAS Boxworth, University of Warwick
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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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CONTENTS

GROWER SUMMARY

Headline

- Two fungicides and a biofungicide applied at flowering reduced Fusarium 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

Objectives 1-3 are reported in the Year 1 report (March 2012).

Objective 4 – Relative susceptibility of different varieties

Visibly healthy Class 2 peppers of six varieties collected from glasshouses in the Lee Valley on one day in May 2012 differed in their level of Fusarium internal fruit rot. After holding fruit at ambient temperature for 5 days, internal fruit rot ranged from 0.8% in Ferrari (green fruit) to 14.2% in Pele (yellow). Infection in Fiesta (8.3%), Spider (6.7%) and Boogie (5.8%) was also relatively high compared with Cupra (2.5%) and Ferrari. Two of the varieties that differed (Cupra and Spider) were from the same glasshouse. These results on varietal differences are supported by grower experience.

Class 1 peppers of the same six varieties were examined for their susceptibility to Fusarium fruit rot by inoculation of the inner wall with a standard inoculum of *F. lactis*. The diameter

of rot lesions after 10 days was greater in Pele, Spider and Cupra than in Ferrari (green) or Boogie, and was intermediate in Fiesta.

The effect of fruit sugar content (% Brix) on the rate of Fusarium rot development was examined. For 60 fruit (10 fruit x 6 colours), % Brix was determined using one third of the fruit tissue and rot development following inoculation with *F. lactis* was measured in the other two thirds of each fruit. Sugar content ranged from 4.0% (Ferrari green) to 7.2% (Cupra red). No relationship was found between sugar content and the rate of Fusarium fruit rot development.

Taken together, these results indicate:

- Pepper varieties differ in their susceptibility to Fusarium internal fruit rot, with Pele (yellow) very susceptible and Ferrari (green) less susceptible. The red variety Cupra is less susceptible than red Spider.
- Differences between varieties in the incidence of Fusarium internal fruit rot are not determined simply by fruit sugar content.
- The interval between fruit set and harvest may have some effect on incidence of Fusarium fruit rot, as green fruit, which show least infection, are harvested 10-14 days before coloured fruit. However, Pele showed the highest level of infection and yet is generally harvested 1 week earlier than other coloured fruit.
- Differences in varietal susceptibility are determined by factors other than, or in addition to, those noted above and may include, for example, flower characteristics or fruit chemical constituents.

Objective 5a – Effect of high humidity on flower infection

A replicated experiment was done in a commercial crop of peppers, variety Cupra, to determine the effect of imposing high humidity at flowering on the incidence of Fusarium internal fruit rot. In May 2012, flowers were loosely enclosed in small polythene bags for periods of 3, 6, 15 or 24 h after inoculation with *F. lactis*; moisture droplets on the inside of bags indicated very high humidity conditions were achieved. Only 10-18% of inoculated flowers developed to mature fruit. In this experiment, imposed high humidity for 3-24 h did not significantly increase the incidence of internal fruit rot (43-65%) compared with flowers inoculated and not enclosed in a polythene bag (71% with internal fruit rot).

This lack of an increase in internal fruit rot with high humidity duration is not consistent with grower observations which suggest the disease is worse during periods of high humidity. It is possible that artificial inoculation of flowers with a spray of *F. lactis* spores overrode any

humidity influence on infection success, or the experiment was insufficiently sensitive to detect humidity effects due to the relatively small number of fruit that developed to maturity in each treatment. Effect of humidity was further examined in Objective 6.

Objective 5b – Monitoring of condensation humidity and in commercial pepper crops

Air relative humidity (RH) and temperature and stem temperature in a pepper crop canopy were measured at three positions on two nurseries in the Lee Valley from March to October 2012. Potential condensation events were determined by calculation of dew point. The frequency and duration of potential condensation events differed between nurseries and monitoring points. At nursery 1 there were no condensation events longer than 5 minutes at positions 2 and 3; however, position 1 had many long condensation events, the longest over 3 hours. At nursery 2, position 3 had over 100 events of greater than 3 hours and over 500 condensation events of 15 minutes or less. Differences were due in part to differences in vent set points.

At nursery 1, RH rarely went above 85% for prolonged periods (>12h) until 23 September when values above 90% became quite common due to lowering of vent set point temperature to enhance colouring-up. Prolonged periods of high RH (>85%) were more common at nursery 2, occurring on average every other day throughout cropping. RH varied little between the three positions within the house at nursery 1; whereas two positions were similar to each other and the third position recorded a lower RH at nursery 2. Comparing the same variety across sites, Fiesta, incidence of Fusarium rot was greater at nursery 2 than nursery 1, indicating that the disease is favoured by greater occurrence of high humidity and condensation.

Objective 6 – Evaluation of potential control treatments applied to flowers

A replicated experiment was done in a commercial crop of peppers, variety Cupra, to determine the effect of four products approved for use on protected pepper on incidence of Fusarium internal fruit rot. Sprays of Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil), Serenade ASO (*Bacillus subtilis*) and Prestop (*Gliocladium catenulatum*) were applied to flowers in July and August 2012 one day before inoculation with *F. lactis*. Treated flowers were tagged and fruit that developed to maturity were assessed for internal fruit rot at the normal harvest stage. The incidence of Fusarium internal fruit rot was significantly reduced by Switch, Amistar and Serenade ASO (Figure 1).

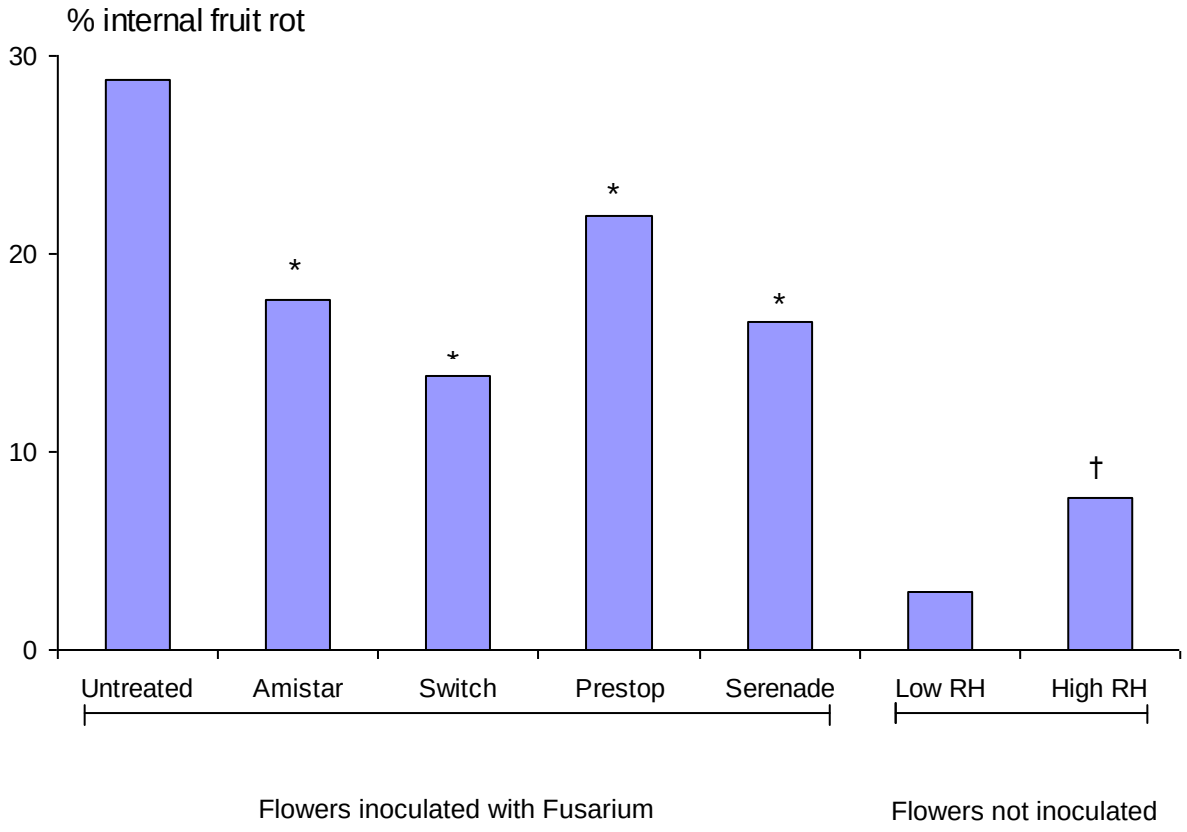

Figure 1. Effect of fungicides, biofungicides and imposed high humidity around flowers on Fusarium internal fruit rot of pepper, cv. Cupra – Lee Valley, 2012. * Significantly different from the untreated (water only) control; † significantly different from the low RH treatment.

An extra treatment was included in this experiment to further investigate the effect of high humidity on occurrence of Fusarium internal fruit rot (see Objective 5a). High humidity was created by loosely enclosing flowers in a polythene bag. In contrast to the previous work, no *Fusarium* inoculum was applied. This treatment significantly increased the level of Fusarium internal fruit rot, from 2.9% to 7.7%, supporting the hypothesis that Fusarium development is favoured by high humidity.

Objective 7 – Effect of season and fruit size on Fusarium species in pepper fruit

The identity of *Fusarium* species associated with pepper fruit at different times of the year and in fruit of different sizes was examined. This was to test the hypothesis that the incidence and range of *Fusarium* species in a pepper crop vary with time of year; and that the species associated with aborted fruit may differ from those found in mature fruit.

Small brown fallen aborted fruit and mature Class 2 fruit with symptoms of Fusarium fruit rot were collected from a commercial crop of variety Cupra at intervals between April and November 2012. The incidence of *Fusarium* was determined in 50 aborted fruit by culture on agar; identity of *Fusarium* species was determined by molecular tests (see Year 1 report) in 10-20 aborted and mature fruit.

The incidence of aborted fruit containing *Fusarium* spp. was 48%, 88%, 84% and 100% in April, June, August and November respectively. *Fusarium lactis* was the predominant species in all samples; *F. oxysporum* and *F. proliferatum* were each detected at a low incidence in both aborted fruit and mature fruit over most of the season.

The high incidence of *Fusarium* species capable of causing internal fruit rot found in aborted fruit from early in the year was surprising. The possible role of *Fusarium* spp. in causing fruit abortion may warrant investigation.

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

- Note that varieties differ in susceptibility to Fusarium internal fruit rot. Red Cupra is generally less susceptible than red Spider; yellow Fiesta is generally less susceptible than yellow Pele; green fruit (e.g. Ferrari) are less susceptible than the above named coloured fruit. The actual level of Fusarium internal fruit rot in a particular variety will also be affected by glasshouse humidity and condensation and the level of inoculum in a house.
- Remove fallen aborted fruit trapped in the canopy and from the floor as much as reasonably practical in order to reduce inoculum levels of *Fusarium*.

- Grower experience, nursery monitoring and some experimental evidence indicate that Fusarium internal fruit rot is favoured by high humidity; control the glasshouse environment to minimise prolonged periods above 85% RH and the risk of condensation events.
- In houses and varieties where there is a history of Fusarium internal fruit rot, consider application of preventative sprays to flowers of Amistar, Switch or Serenade ASO. The efficacy of such treatments will be further examined in Year 3 of this project.

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* spp. will be evaluated for their efficacy as preventative treatments applied to pepper flowers.

The project objectives in Year 2 were:

- To examine the relative susceptibility of different varieties to Fusarium internal fruit rot;
- To investigate the effect of high humidity around flowers on occurrence of Fusarium internal fruit rot;
- To evaluate some potential control treatments applied to flowers;
- To examine the effect of season and fruit size on *Fusarium* spp. associated with pepper fruit.

Objective 4 – Relative susceptibility of different varieties

Introduction

Grower observations suggest that varieties differ in susceptibility to Fusarium internal fruit rot. Varieties picked green (e.g. Ferrari) are reported to be less affected than coloured fruit, with Pele (yellow), Boogie (orange) and Spider (red) generally being affected to a greater extent. It is possible that time of year, glasshouse environment and how a crop is grown also influence the level of Fusarium internal fruit rot that develops in a crop. A series of three experiments was done to provide more information on the relative susceptibility of different varieties.

Materials and methods

Experiment 1 – Natural occurrence of Fusarium in different varieties

In order to minimise the influence of weather on the level of Fusarium infection, fruit were collected from six crops of different cultivars in a common area (Lee Valley), on one day, 17 May 2012. Four of the samples were from one nursery, with two of these from the same glasshouse. The other two samples were from nurseries within 10 miles distance. Samples

of 120 Class 2 fruit with no obvious symptoms of Fusarium or other damage, except for a low level of blossom end rot, were picked from crates of the different varieties. After storage in the dark at ambient temperature for 5 days at ADAS Boxworth, fruits were cut open and examined for Fusarium internal fruit rot, noting whether infection was on the internal wall and/or the seeds. Results were examined by Generalised linear modelling with logit transformation. The effect of blossom end rot on occurrence of Fusarium internal fruit rot was examined using the combined data for all varieties in a 2 x 2 chi-squared test.

Experiment 2 – Susceptibility of fruit tissue

Ten Class 1 fruit of each six cultivars were examined for susceptibility to Fusarium rot by inoculation of the inner wall surface with a standard inoculum of *F. lactis (ex* pepper). Fruit were cut to provide two flat sections of wall around 4 x 4 cm in size. A plug of *F. lactis* (8 mm diameter on potato dextrose agar) was applied to the centre of the inner wall of each piece; no wounding was done at the inoculation site.

Inoculated fruit pieces were placed on damp paper towel in sandwich boxes to create high humidity and incubated at 20ºC. The boxes of fruit were arranged in a randomised order on four shelves in an incubator, with one replicate block per shelf. The extent of tissue rot that developed from the plug of *F. lactis* was determined after 10 days by measuring the lesion diameter. Two diameters were measured at 90º to each other and the mean was calculated. Results were examined by Generalised linear modelling.

Experiment 3 – Effect of fruit sugar content on susceptibility to Fusarium rot

Sugar content (% Brix) was measured in 10 fruit of each of six varieties using around one third of each fruit. The rate of lesion spread following inoculation (as above) with *F. lactis* was determined in the second half of the fruit. Results were examined by regression analysis, plotting rate of lesion spread (two values/fruit) against Brix level (one value/fruit).

Results and discussion

Experiment 1 – Natural occurrence of Fusarium in different varieties

In order to obtain a wide range of varieties it was necessary to sample from different glasshouses and different nurseries. All of the crops sampled were grown on rockwool slabs, were in relatively close proximity to each other, and fruit were picked on the same day, 17 May 2012. Details of the crops and the levels of Fusarium internal fruit rot found are given in Table 1.

All of the fruit examined were suitable at picking for marketing as Class 2 fruit; none showed external symptoms of rotting, although some showed blossom end rot (calcium deficiency). After holding them for 5 days at ambient temperature, external symptoms of Fusarium rot were visible on some fruit. Fruit were stored for this period to allow opportunity for growth of any Fusarium within the fruit (i.e. increased expression of internal fruit rot symptoms). The incidence of internal fruit rot was much greater than external rot. It varied greatly, ranging from 0.8% in Ferrari (green) to 14.2% in Pele (yellow) (Table 1). The levels in Pele, Fiesta (8.3%), Spider (6.7%) and Boogie (5.8%) were all significantly greater ($p < 0.05$) than in Ferrari and Cupra (2.5%). The red cultivars Cupra and Spider were in the same glasshouse and differed significantly, indicating that this is very probably an inherent difference between the varieties, as the environment and infection pressure around the crops would probably be very similar. Grower observations also indicate that Cupra is a lot less susceptible than Spider.

The occurrence of *Fusarium* growth on seed was generally less (sometimes only slightly) than that on the internal fruit wall. This observation is inconsistent with the hypothesis that Fusarium infection in fruit arises first on the seed and then spreads to the internal wall. Possibly there was a greater incidence of infection on seed not visible to the eye. An alternative explanation is that infection was latent in the fruit part that develops into the wall from an early stage of fruit formation after infection of the flower.

The incidence of Fusarium internal fruit rot was above 2% in five of the six varieties examined, and over 14% in the worst affected. These are relatively high levels that one might expect to cause consumer complaint. It is likely they would be lower on fruit consumed within 1-2 days of purchase, and on fruit stored cool (e.g. in a fridge). Also, the persistent wet weather in the first half of 2012 may have contributed to a greater incidence of Fusarium internal fruit rot than in most years. Nevertheless, these results indicate the potential of Fusarium fruit rot to be a major cause of fruit rejection and/or complaint.

The proportion of fruit with blossom end rot differed significantly ($p < 0.001$) between varieties. It was high in Cupra (68%), Boogie (48%) and Spider (33%) with less in Fiesta (26%), Pele (7%) and Ferrari (6%). Combining the data for all varieties, the incidence of Fusarium internal fruit rot was significantly less ($p = 0.006$) in fruit with blossom end rot (3%) than in fruit without this disorder (8%). Possibly the presence of dry wall tissue due to blossom end rot makes the fruit internal environment less humid and less conducive to development of Fusarium.

Table 1. Details of commercial pepper crops where fruit was picked on 17 May 2012 and the level of Fusarium internal fruit rot found in them after ambient incubation for 5 days.

Nursery and house	Variety	Colour	Blossom end rot (%)	External fruit rot (%)	Mean % fruit affected	
					Internal fruit rot	Fusarium on seed
A 1	Cupra	Red	68	1.7 (1.2)	2.5 (1.4)	0.8 (0.8)
A 1	Spider	Red	33	3.3 (1.7)	**6.7** (2.2)	**5.8** (2.1)
A 2	Boogie	Orange	48	1.7 (1.2)	**5.8** (2.1)	**1.7** (1.2)
A 3	Fiesta	Yellow	26	4.1 (1.8)	**8.3** (2.5)	**4.2** (1.8)
B -	Pele	Yellow	7	10.8 (2.8)	**14.2** (3.2)	**15.8** (3.3)
G -	Ferrari	Green	6	0	0.8 (0.8)	0

( ) – standard error. Values in bold are significantly different ($p < 0.05$) from Ferrari.

Experiment 2 – Susceptibility of fruit tissue

Fusarium lactis applied as mycelium to undamaged tissue of the internal surface of fruit walls caused a rot in all varieties (Table 2). No rotting occurred on uninoculated areas of fruit. The mean lesion diameter after 10 days was significantly greater in cultivars Cupra, Pele and Spider (over 23 mm) than in Ferrari and Boogie (less than 18 mm). Although the relatively low rate of lesion spread in cv. Ferrari and the high rates in cvs Pele and Spider are consistent with the levels of rot found in commercial lots of these cultivars (Table 1), the high rate of spread in cv. Cupra and the low rate of spread in cv. Boogie are not consistent. This result indicates that although there are differences in the relative susceptibility of fruit wall tissue rot by *F. lactis*, this is not the only factor determining occurrence of Fusarium internal rot in fruit. It is possible that the level of rot found in fruit is also influenced by differences between cultivars at early stages of development (e.g. at flowering). The low levels of sugars in green fruits, significantly lower than in all other cultivars, may contribute to the low rate of lesion spread in cv. Ferrari but this cannot be the explanation for the low level of lesion spread in cv. Boogie. The effect of Brix level on lesion spread is further examined below (Experiment 3).

Table 2. Effect of pepper cultivar on susceptibility to Fusarium fruit rot (artificial inoculation) – 2012

Variety	Colour	Mean lesion diameter (mm) 10 days after inoculation with *F. lactis*	Mean Brix value (%)
Cupra	Red	**23.7**	**7.19**
Spider	Red	**26.5**	**6.64**
Boogie	Orange	16.7	**6.70**
Fiesta	Yellow	21.6	**5.64**
Pele	Yellow	**26.2**	**5.54**
Ferrari	Green	17.9	3.98
Significance (18 df)		0.001	<0.001
LSD		4.83	0.488

Values in bold are significantly different from Ferrari.

Experiment 3 – Effect of fruit sugar content on susceptibility to Fusarium rot

The data on rate of lesion spread in Experiment 2 was re-examined with regard to fruit total sugar content (% Brix) irrespective of fruit colour. As lesion spread was determined on two flat sections of fruit wall, and Brix % was determined in the remainder of each fruit, there were two lesion size values and one Brix value for each of the 60 fruit. Brix % had very little effect on lesion diameter, accounting for just 3% of the variation (Figure 2).

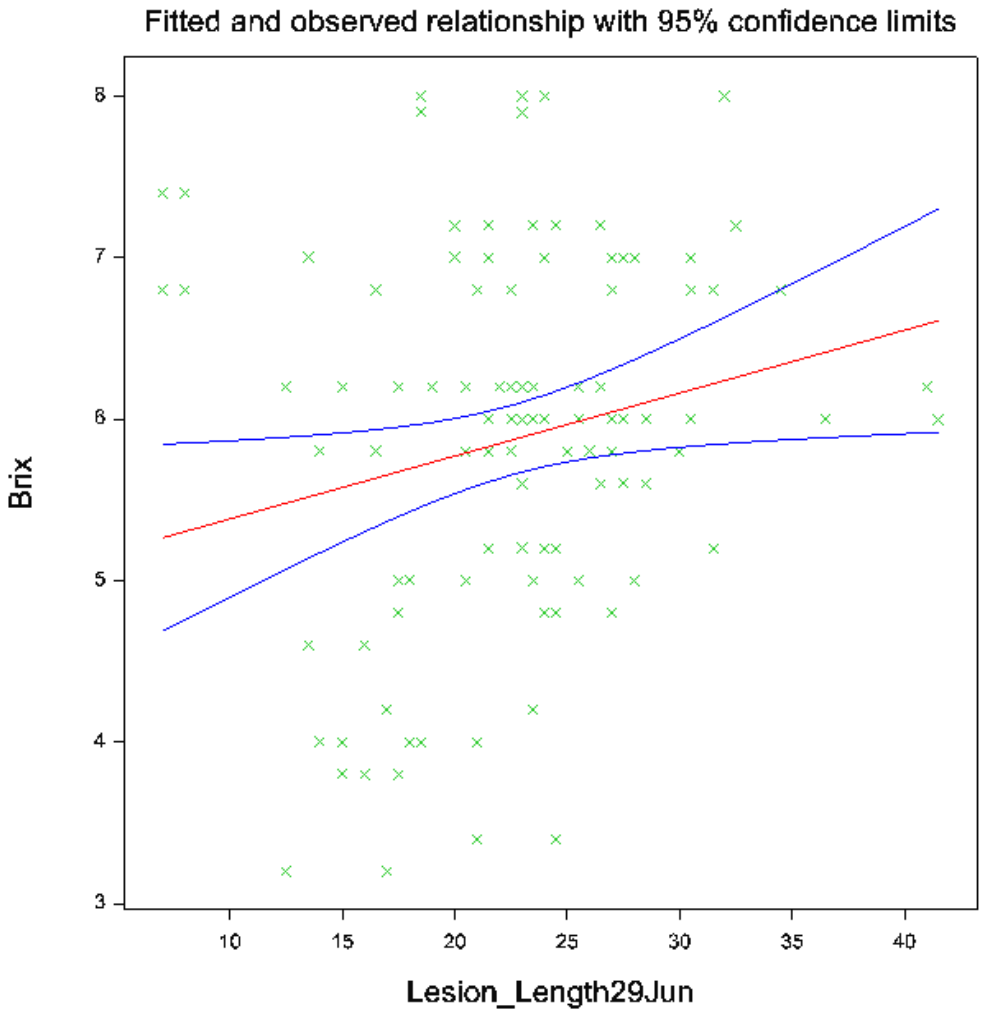

Figure 2. Association of Fusarium lesion diameter (mm) at 10 days after inoculation of fruit wall with fruit sugar content (% Brix)

Objective 5a – Effect of high humidity on flower infection

Introduction

Grower observations, work in PC 285, and reports from Belgium suggest that occurrence of Fusarium internal fruit rot is favoured by high humidity. However, there are no published reports of experiments with appropriate controls that substantiate this theory. An experiment was devised to test the hypothesis that imposing a high humidity around flowers increases the occurrence of Fusarium internal fruit rot. Additionally, humidity and stem temperature were measured in two pepper crops from April to September 2012 to record

the occurrence of high humidity periods and the variation within and between glasshouses (see Objective 5b).

Materials and methods

Site and crop details

The experiment was done in a commercial crop of cv. Cupra grown on rockwool slabs in the Lee Valley. Two adjacent pathways (four crop faces) in the centre of a block were used; plants from the central pathway to the first stanchion and from the last stanchion to the glasshouse wall were used as guard plants. The crop was grown to normal standards by the host nursery. No fungicides were applied to the crop. A trial diary is given in Appendix 1. Temperature and humidity in this area of crop were monitored by FEC (see Appendix 2).

Treatments

High humidity was imposed around flowers by loosely enclosing them in a small (around 3 x 3 cm) clear polythene bag. The time of polythene bag application and removal was done to create high humidity durations of 3, 6, 15 and 24 h; these were compared with nil imposed high humidity control (flowers left unbagged) (Table 3). All of the flowers were spray-inoculated with *F. lactis* (c. 0.8 ml of 5 x 10^5 spores/ml in water) immediately before bagging, using a handheld plastic sprayer with the nozzle adjusted to a fine spray; inoculum production and spray application is described fully in the Year 1 report.

Experiment design and data analysis

The experiment was done as a randomised block design with four replicates (crop rows). Plot length was at least 5 m with a gap of at least 2 m between adjacent plots. Thirty flowers at the fully open fresh white stage, on the main stem, were inoculated on 26 April and a further 30 flowers on 30 April. Inoculated flowers were marked by looping with an elasticated jewellery tag over the flower stalk. All fruit that developed from tagged flowers were harvested at maturity for assessment of internal fruit rot. Data for the two inoculations were combined as fruit numbers developing from each flower inoculated were low.

Assessments

All fruit that developed to full size from inoculated flowers were harvested on 2 July. Fruit were stored in the laboratory in the dark at ambient temperature for 5 days prior to assessment to increase expression of any internal fruit rot. Fusarium was recorded as external fruit rot, internal fruit rot and growth on seeds.

A sample of fruit with confirmed Fusarium internal fruit rot were sent to Warwick University to determine if the species present was the same as that (*F. lactis*) used to inoculate flowers.

Results and discussion

All fruit that developed to full size were harvested on 2 July, 63 and 67 days after the inoculation. The number of fruits harvested per treatment ranged from 24 (nil imposed high humidity) to 44 (15 h imposed high humidity) (Table 3). This means that only 10-18% of inoculated flowers developed into full size mature fruit. Most of the other flowers either never set, or the fruit aborted at an early stage. Failure of flowers to set and abortion of young fruit at an early stage both happen naturally in pepper crops and are reported to vary with plant condition (number of fruit already set), light level and other factors. It is not known if the high incidence of flowers that failed to develop to mature fruit in this experiment was normal for a crop grown under these conditions, or was different from normal because of the inoculation with *F. lactis* and/or imposed high humidity. The high incidence of *Fusarium* species (*F. lactis, F. oxysporum* and *F. proliferatum*) pathogenic to pepper found in aborted fruit (see Objective 7) suggests that Fusarium infection may be a cause of fruit abortion. Whatever the causes of failure to set fruit and abortion of young fruit, they represent a waste of plant energy.

The insides of the polythene bags applied over flowers were commonly seen to contain small water droplets, indicating the desired very high humidity conditions were created.

Duration of imposed high humidity was found to have no significant effect ($p > 0.05$) on the proportion of fruit with Fusarium internal fruit rot. The level of Fusarium internal fruit rot was high in all treatments, ranging from 45% of fruit (15 h imposed high humidity) to 71% of fruit (nil imposed high humidity).

The species present in a sample of affected fruit was identified as *F. lactis*, the same species as used to inoculate the flowers.

Temperature and RH recorded in the crop canopy in the area of this experiment are shown in Figures 3 and 4 for the three days immediately after flower inoculation (26 April and 30 April for the first and second inoculations respectively). On both occasions RH was above 80%, frequently close to 90%, for most of the three days after inoculation.

This lack of an increase in internal fruit rot with high humidity duration is not consistent with grower observations which suggest the disease is worse during periods of high humidity. This experimental result may indicate that high humidity is truly not a major determinant of internal fruit rot. Alternatively, it is possible that:

- Humidity in the glasshouse at the times of inoculation in the experiment was above the minimum required to permit infection;
- Artificial inoculation of flowers with a spray of *F. lactis* spores overrode any humidity influence on infection success;
- The experiment was insufficiently sensitive to detect humidity effects due to the relatively small number of fruit that developed to maturity in each treatment, resulting in large variation in the data.

Using the information from this experiment, a single high humidity treatment (24 h at high RH, no inoculation) was included in the work on potential control treatments (Objective 6). The aim was to determine if an imposed high humidity period at a low inoculum level, from natural spore dispersal in the glasshouse, affected the incidence of Fusarium fruit rot.

Table 3. Effect of imposed high humidity duration around flowers following inoculation with *F. lactis* on Fusarium internal fruit rot in pepper, cv. Cupra – July 2012

High humidity duration (h)	Applied from:	Total number fruit at harvest	Occurrence of Fusarium (% fruit)			
			External rot	Internal rot	On seed	Any symptom
1. Nil	-	24	50 (13.1)	71 (11.9)	29 (12.1)	71 (11.7)
2. 3	12:00–15:00h	42	52 (9.9)	60 (9.7)	36 (9.7)	62 (9.5)
3. 6	11.00–17.00h	31	58 (11.4)	65 (11.0)	32 (10.9)	65 (10.8)
4. 15	18.00–09.00h	44	36 (9.3)	43 (9.6)	27 (8.8)	45 (9.5)
5. 24	10.00–10.00h	36	33 (10.1)	53 (10.7)	28 (9.8)	56 (10.5)
	Total	177				
Significance (12 df)			NS	NS	NS	NS

( ) – standard error. NS – not significant.

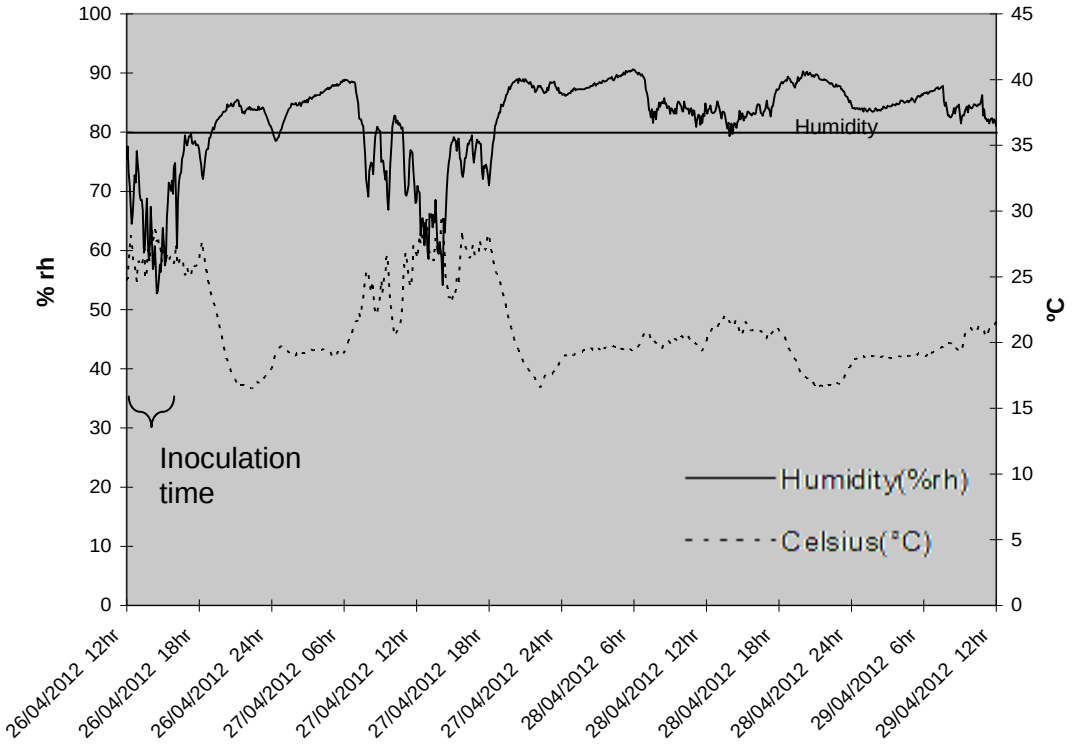

Figure 3. Relative humidity and temperature at time of flower inoculation with Fusarium and the following three days: Inoculation 1

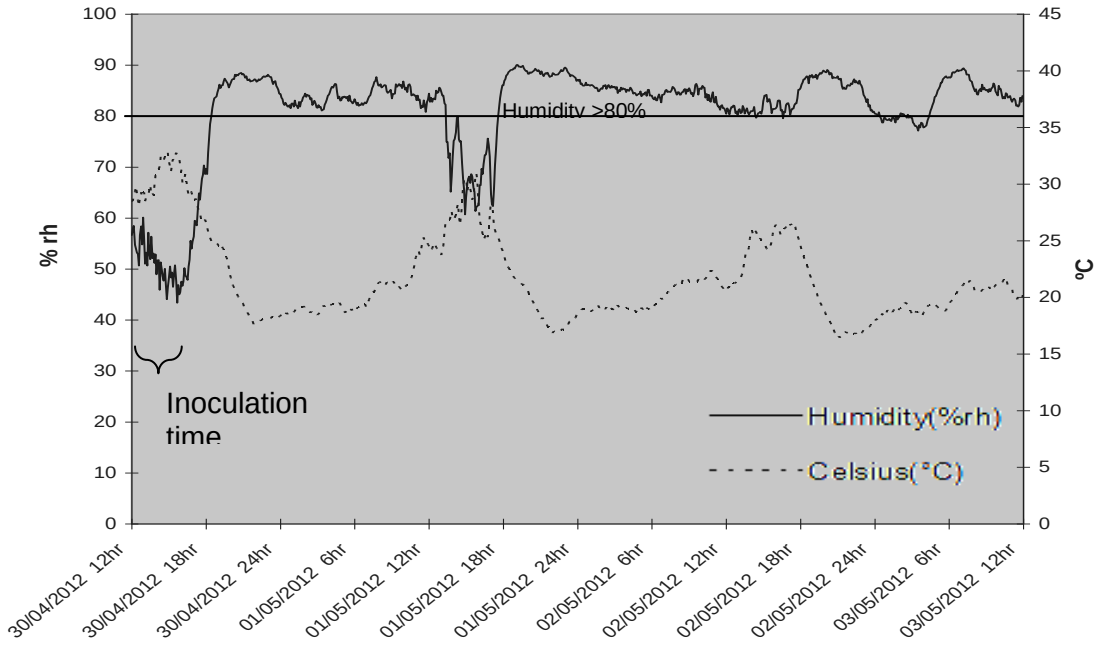

Figure 4. Relative humidity and temperature at time of flower inoculation with Fusarium and the following three days: Inoculation 2

Objective 5b – Monitoring of humidity in commercial pepper crops

Monitoring of humidity in pepper crops

The objectives of the work were to determine:

- Occurrence of condensation periods on stem tissue close to flowers;
- Occurrence of high humidity periods in the crop;
- Variation between three equivalent points in a house;
- Variation between two glasshouse blocks (two different nurseries).

This information, when combined with information on how humidity influences infection of pepper flowers by *Fusarium* spp., will help determine the frequency of potential infection events for *Fusarium* species on pepper.

Introduction

A full report on the monitoring of temperature and relative humidity in pepper crops at two nurseries in the Lee Valley by FEC Services from late March to 21 October 2012 is given in Appendices 2 and 3. Raw data were supplied as spreadsheets and are available from HDC on request.

Air temperature and relative humidity and stem temperature were measured at three positions in one house on each nursery. RH/temperature sensors were located in the crop canopy close to flowers and stem temperature sensors were located around the stem close to flowers; it was not possible to measure flower petal temperature with a contacting thermistor due to the delicate nature of flowers. Measurement of stem temperature close to a flower was done as the best option available. Conditions suitable for condensation occur when the dew point, calculated from air temperature and humidity, falls below the stem temperature.

Results

Condensation on stems

Nursery 1

Position 1 was close to the glasshouse entrance, position 2 was midway in the block of Cupra and position 3 was furthest from the glasshouse entrance. Position 1 saw the greatest number of potential condensation events and these occurred throughout the

cropping period with an increasing number from early October. Most events (79 in total) were of 15 minutes duration or less (Appendix 3, Figure 2). There were seven events of 15-30 minutes duration and a few events of longer duration, the greatest being more than 4.5 hours. At positions 2 and 3, condensation potential events were far fewer and, apart from one event at position 2, only occurred at the end of the crop life (from early October 2012). The high number of potential condensation events from early October coincided with stopping of the heads of plants and changing the environment set points to restrict venting and aid colouring-up of the peppers.

Nursery 2

Position 1 was close to the glasshouse entrance, position 2 was midway in the crop and position 3 was in the crop on the opposite side of the main pathway, midway in the crop. Potential condensation events were frequent throughout the cropping period at positions 1 and 3 and fewer at position 2 (Appendix 3, Figure 3). At nursery 1, these increased in frequency from early October when vent opening was restricted to encourage colouring-up of remaining fruit. Position 3 had far more potential condensation events (516) of 15 minutes duration or less than positions 1 and 2 (110 and 65 respectively). Position 3 had several potential condensation events of more than 10 h during August and September, before the environmental set points were changed. In total, position 3 experienced 102 potential condensation periods of greater than 3 hours, compared with 18 and 0 for positions 1 and 2. The low frequency of condensation events at position 1 could be explained by its proximity to the entrance. The glasshouse half where positions 1 and 2 sensors were located was reported to have had a history of humidity problems so the set points were set closer (i.e. venting occurred at a lower temperature) than in the opposite half of the glasshouse. This could explain why recorded potential condensation events were more frequent and of greater duration at position 3 than at positions 1 and 2.

Occurrence of high humidity periods

High humidity was examined as the number of occurrences of relative humidity above 80%, 85% and 90% for a continuous 12h period.

Nursery 1

From the end of March to 23 September, RH rarely went above 85%. From 23 September to 21 October, the frequency of periods of RH >80% more than doubled and values above 90% were quite common (around once every 3 days) – see Appendix 2.

Periods of high humidity were recorded at a very similar frequency at all three positions within a house. There was a slightly greater frequency of high RH periods at position 3, the furthest from the glasshouse entrance.

Nursery 2

12h periods of RH above 85% were more common than at nursery 1, occurring on average once every other day during the first 180 days of crop monitoring, compared with once every 5 days at nursery 1 during this period. As at nursery 1, there was again a trend for RH to increase in the final 30 days of crop monitoring, with 12 h periods at >90% RH recorded on four occasions.

Positions 2 and 3 recorded very similar frequencies of high humidity periods, whereas position 1, a similar distance to the entrance as position 3, rarely recorded values above 90%.

Data are further examined in Tables 4 – 6, where the frequency of daily humidity periods each week with at least 6 h continuously above 80%, 85% and 90% RH are given. The number of occasions each week when these 'critical values' are exceeded is very common for the 80% RH, common for 85% and occasional for 90%. If *Fusarium* sp. spores are able to germinate and initiate infection of flower petals under these conditions, even with the 6h at 85% threshold one might expect to see some Fusarium internal fruit rot develop throughout an extensive period of the cropping period.

Fusarium internal fruit rot occurred in fruit harvested from the crops at both nurseries at varying levels throughout the year. The incidence of fruit rot was reported to be worse at nursery 2 than nursery 1 comparing the same colours and varieties. For example, nursery staff reported fruit of cv. Fiesta had 80% more Fusarium at nursery 2 than nursery 1.

Discussion

Assuming that measurement of pepper stem temperature a few cm from flower petals is a reasonably accurate estimate of flower temperature, our data indicate that condensation events of short duration occur frequently throughout the cropping period. Also, that there can be large differences in the frequency and duration of condensation events at different positions in the same glasshouse. The occurrence of air humidity with frequent long periods above 80% RH and occasional long periods above 85% is consistent with occurrence of condensation events. Alternative approaches are required to confirm that condensation commonly occurs on flower parts in UK protected pepper crops grown to standard commercial practice; and to determine the ability of *F. lactis*, *F. proliferatum* and

F. oxysporum spores to germinate and infect pepper petals at different humidities and different wetness durations.

At nursery 2, sensors 1 and 2 were located in a crop of yellow variety Pele and sensor 3 was located in a crop of yellow variety Fiesta. Condensation events were greatest in the Fiesta at position 3, with a reported 30% more Fusarium than in the Pele at positions 1 and 2. Fruit inoculation studies under controlled conditions (Objective 2) found no significant difference in the susceptibility of Pele and Fiesta. This suggests the greater level of Fusarium in Fiesta at position 3 was due to greater humidity and condensation in this area.

Table 4. Number of daily periods each week with at least 6 h continuous >80% RH

Week number	Nursery 1			Nursery 2		
	Pos. 1	Pos. 2	Pos. 3	Pos. 1	Pos. 2	Pos. 3
30	9	11	11	5	13	13
31	10	11	11	6	15	15
32	13	14	14	10	15	15
33	13	12	12	11	13	13
34	16	16	14	10	15	15
35	16	19	16	12	19	16
36	13	14	13	4	15	13
37	15	14	14	12	19	14
38	21	22	22	19	25	25
39	21	21	20	20	23	23
40	22	23	23	23	26	23
41	22	23	23	23	22	24

Table 5. Number of daily periods each week with at least 6 h continuous >85% RH

Week number	Nursery 1			Nursery 2		
	Pos. 1	Pos. 2	Pos. 3	Pos. 1	Pos. 2	Pos. 3
30	3	5	3	0	5	6
31	6	6	6	2	6	8
32	6	9	5	1	9	9
33	5	6	4	1	8	10
34	9	8	7	1	10	13
35	9	8	6	1	7	13
36	4	2	4	0	5	5
37	5	4	4	0	6	5
38	9	11	10	3	15	13

Week number	Nursery 1			Nursery 2		
	Pos. 1	Pos. 2	Pos. 3	Pos. 1	Pos. 2	Pos. 3
39	15	18	16	9	20	16
40	14	18	19	10	21	21
41	15	22	21	9	23	22

Table 6. Number of daily periods each week with at least 6 h continuous >90% RH

Week number	Nursery 1			Nursery 2		
	Pos. 1	Pos. 2	Pos. 3	Pos. 1	Pos. 2	Pos. 3
30	0	0	0	0	0	0
31	1	1	1	1	3	3
32	1	2	1	0	2	2
33	0	0	0	0	1	0
34	1	1	1	0	3	1
35	1	1	1	0	1	1
36	0	0	0	0	0	0
37	0	0	0	0	0	0
38	2	3	3	0	1	1
39	2	5	5	0	7	5
40	1	9	7	1	7	9
41	3	9	8	0	9	10

Objective 6 – Evaluation of potential control treatments applied to flowers

Introduction

Experimental work in Canada identified some chemical and biological treatments that significantly reduced Fusarium internal fruit rot in pepper when applied to flowers. Effective treatments included products based on *Bacillus subtilis*, *Gliocladium catenulatum* and iprodione (Rovral WG). The aim of this experiment was to evaluate some chemical and biological products approved for use on protected pepper in the UK, and with known activity against Fusarium diseases, for control of Fusarium internal fruit rot. Additionally, opportunity was taken to further investigate the effect of an imposed high humidity period at flowering on development of the disease.

Materials and methods

Site and crop details

A replicated experiment was done in the same commercial crop of cv. Cupra described under Objective 5. The experiment was done a few rows away from the humidity experiment, and later in the year, starting on 26 July 2012. A full trial diary is given in Appendix 1.

Treatments

Fungicide and biofungicide treatments aimed at control of Fusarium internal fruit rot were applied to flowers one day before inoculation with a spore suspension of *F. lactis* (Table 7). A single 24 h high humidity period was applied at the same time to uninoculated flowers (T2). Results from both treatments were compared with uninoculated, unbagged flowers (T1) and inoculated unbagged flowers (T3). Each treatment was applied on three occasions at times when flower setting was considered by the grower to be good. Inoculum production and inoculation with *F. lactis* was as described previously except that only one squirt of *F. lactis* per flower was used. Fungicide and biofungicides were applied using new plastic hand-held sprayers, as used for *Fusarium* inoculation. High humidity around flowers was created using small plastic bags as described previously.

Table 7. Detail of fungicide, biofungicide and high humidity treatments applied to flowers in a crop of cv. Cupra – July and August 2012

Treatment		Active ingredients	Rate of use	Approval status
Applied	Inoculation			
1. Untreated	-	-	-	-
2. High RH for 24 h	-	-	-	-
3. Water	✓	-	-	-
4. Amistar	✓	Azoxystrobin (25%)	1 ml/L	Label
5. Switch	✓	Cyprodinil + fludioxonil (37.5+25%)	1 g/L	Label
6. Prestop	✓	*Gliocladium catenulatum* (32%)	5 g/L	EAMU
7. Serenade ASO	✓	*Bacillus subtilis* (13.96 g/L)	100 ml/L	EAMU

Experimental design and data analysis

The experiment was done as a randomised block design with four replicates (rows) of the seven treatments. Methods were as described previously except that 30 flowers per plot were inoculated on each of three occasions. Results were examined by Generalised linear modelling with logit transformation on combined data.

Results and discussion

This experiment was more successful than the previous ones in 2011 (Year 1) and 2012 in that a relatively large proportion of flowers developed into mature fruit (Table 8).

There was no significant difference between treatments in the number of flowers that developed into fruit ($p > 0.05$) (Table 8). The proportion of flowers that developed into mature fruit was much greater on 26 July (63%) than on 2 August (30%) or 29 August (9%).

There was a significant ($p < 0.05$) treatment effect on the incidence of *Fusarium* internal rot in fruit that developed after inoculation 1 (Table 9) and overall (Table 11); there appeared to be a treatment effect after inoculation 2 but this was not statistically significant ($p > 0.05$) (Table 10). Considering the overall results (Table 11), the incidence of Fusarium internal fruit rot on inoculated plants was reduced from 28.8% (untreated) to 13.8% by Switch. Treatments with Amistar (17.7%) and Serenade ASO (16.6%) were almost as effective, while Prestop did not reduce the disease significantly ($p > 0.05$). The incidence of *Fusarium* external fruit rot symptoms was consistently lower than the incidence of internal rot in the same treatment; the external fruit rot symptoms were almost always associated with severe internal Fusarium fruit rot at the corresponding position on the internal wall (i.e. external rots developed from internal infections). The occurrence of visible Fusarium sporulation on seed ranged from 2.9 to 10.7% with no significant differences between treatments (Table 11).

Imposition of a 24 h high humidity period on uninoculated fully open flowers significantly ($p < 0.05$) increased the incidence of Fusarium internal fruit rot, from 2.9% to 7.7%. The occurrence of Fusarium internal fruit rot in these two treatments indicates that there was natural dispersal of *Fusarium* sp. spores in the glasshouse, probably via air currents and/or on insects. A *Fusarium* isolate obtained from an uninoculated plot and examined by PCR was identified as *F. lactis.* The previous experiment (Objective 5, Table 3) failed to detect any effect of imposed high humidity duration in the range of nil to 24 h on inoculated flowers, when the level of Fusarium internal fruit rot was 71% in the nil humidity treatment. It seems likely that the experimental conditions in that previous experiment were too favourable to infection (e.g. due to the high inoculum and high background humidity) to permit detection of a humidity influence.

The temperature and humidity recorded in the crop at and immediately after inoculation on 27 July (10.30 –12.0 am), 3 August (10.00 – 11.30 am) and 31 August (10.00 – 11.30 am) are shown in Figures 5 – 7 respectively. The minimum night temperature was around 15ºC and the maximum day temperature was around 30ºC around the time of all three inoculations. For inoculation 1, RH was above 80% for around 7.5 hours the following night; for inoculation 2 it was above 80% for around 8 hours and for inoculation 3 for over 2 hours. The RH peaked at around 85% for a short period on each occasion. Given that a low incidence of Fusarium fruit rot developed in the uninoculated plants (T1) on each occasion (i.e. on plants where no water was applied and flowers were not enclosed in bags), it is possible that the temperature and humidity conditions shown are conducive to the establishment of Fusarium infection in flowers that leads to Fusarium internal fruit rot. Unfortunately the variability in the level of internal fruit rot between replicate plots on the same inoculation date is too large to attempt further interpretation of the effect of

environmental conditions on infection by comparison of the results of the three inoculations; also, the time at which flowers became naturally inoculated by *Fusarium* sp. is unknown, so it is not possible to identify the critical period when spore germination occurred.

Table 8. Number of mature fruit harvested following inoculation of pepper flowers on three occasions – summer 2012

Treatment	Mean number of fruit harvested per plot*			
	Inoculation 1 (26 July)	Inoculation 2 (2 Aug)	Inoculation 3 (29 Aug)	Total
Untreated	16.0	9.0	3.3	28.3
High RH	14.5	8.5	3.0	26.0
Water	15.3	6.5	1.0	22.8
Amistar	14.8	7.3	2.0	24.0
Switch	17.5	7.5	2.3	27.3
Prestop	17.0	8.0	1.8	26.8
Serenade ASO	15.8	5.5	1.5	22.8
Significance (18 df)	0.56	0.72	0.66	0.54

*Number of flowers per plot was 30, 30 and 28 for inoculations 1-3 respectively.

Inoculation was done on 25 flowers per plot on 26 July and 2 August and around 28 flowers per treatment on 29 August.

Table 9. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2012 (inoculation 1)

Treatment		Occurrence of Fusarium (% fruit)			
Applied	Inoculation	External rot	Internal rot	On seed	Any symptom
1. Untreated	-	1.7 (1.8)	1.7 (1.7)	0	5.1 (3.3)
2. High RH for 24 h	-	1.6 (1.8)	3.4 (2.4)	1.6 (1.5)	3.3 (2.7)
3. Water	✓	27.6 (5.9)	24.3 (5.5)	10.8 (3.7)	32.0 (6.7)
4. Amistar	✓	11.7 (4.5)	16.8 (5.0)	10.1 (3.7)	18.4 (5.8)
5. Switch	✓	11.6 (4.2)	15.6 (4.4)	8.6 (3.2)	14.2 (4.9)
6. Prestop	✓	7.6 (3.6)	19.9 (5.1)	9.2 (3.4)	19.9 (5.8)
7. Serenade ASO	✓	14.3 (4.8)	12.7 (4.3)	6.3 (2.9)	19.1 (5.8)
Significance (18 df)		0.007	0.009	0.045	0.018

( ) – standard error.

Table 10. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2012 (inoculation 2)

Treatment		Occurrence of Fusarium (% fruit)			
Applied	Inoculation	External rot	Internal rot	On seed	Any symptom
1. Untreated	-	0	6.1 (5.1)	6.0 (4.4)	9.1 (6.4)
2. High RH for 24 h	-	0	14.5 (8.2)	6.4 (4.7)	14.2 (8.6)
3. Water	✓	2.4 (1.6)	35.4 (9.6)	7.6 (4.5)	35.0 (10.0)
4. Amistar	✓	3.9 (2.5)	24.5 (9.7)	7.3 (5.3)	24.7 (10.4)
5. Switch	✓	2.9 (1.9)	6.5 (5.4)	0	6.6 (5.8)
6. Prestop	✓	7.4 (3.2)	32.9 (10.5)	14.8 (7.3)	36.2 (11.3)
7. Serenade ASO	✓	8.6 (3.7)	26.9 (11.4)	13.8 (7.8)	31.8 (12.7)
Significance (18 df)		0.067	NS	NS	NS

( ) – standard error. NS – no significant differences.

Table 11. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2012 (combined results of inoculation 1, 2 and 3)

Treatment		Occurrence of Fusarium (% fruit)			
Applied	Inoculation	External rot	Internal rot	On seed	Any symptom
1. Untreated	-	0.9 (1.0)	2.9 (1.3)	2.9 (1.6)	6.6 (2.7)
2. High RH for 24 h	-	1.1 (1.1)	7.7 (2.3)	4.2 (2.0)	7.7 (3.1)
3. Water	✓	16.5 (3.7)	28.8 (3.5)	9.3 (2.6)	33.0 (4.9)
4. Amistar	✓	8.4 (3.1)	17.7 (3.2)	9.5 (2.9)	19.8 (4.6)
5. Switch	✓	10.3 (3.2)	13.8 (2.7)	6.5 (2.3)	12.9 (3.6)
6. Prestop	✓	6.4 (2.5)	21.9 (3.3)	10.7 (2.9)	23.6 (4.6)
7. Serenade ASO	✓	12.5 (3.8)	16.6 (3.2)	7.9 (2.8)	22.3 (4.9)
Significance (18 df)		0.006	<0.001	NS	0.003

( ) – standard error.

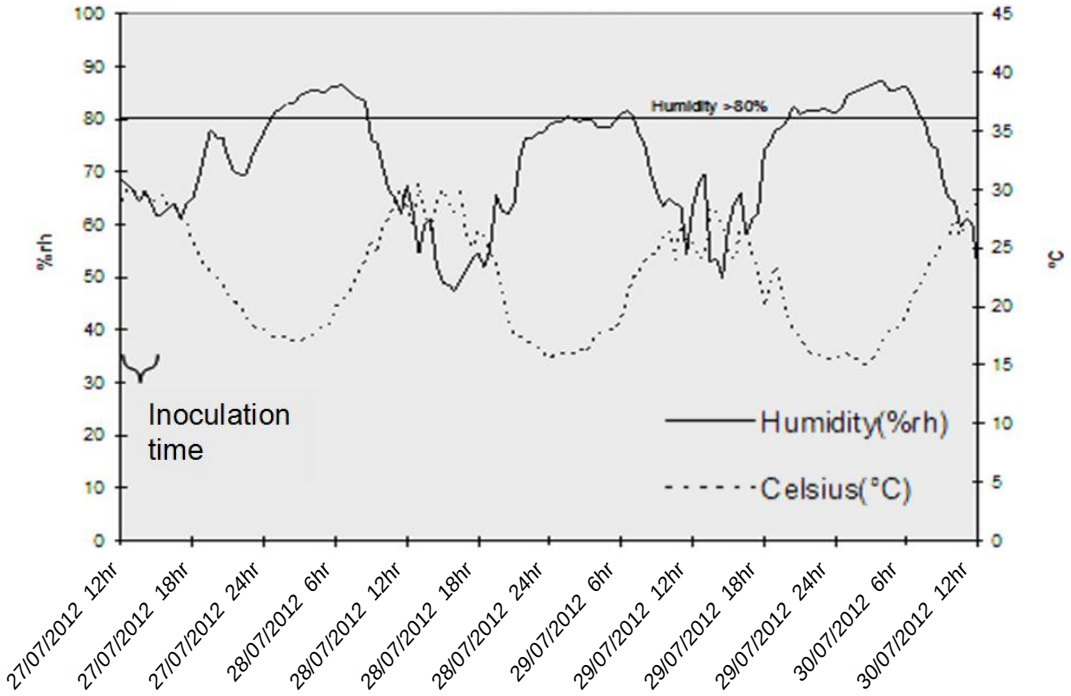

Figure 5. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 1 (27 July, 10.30 – 12 noon)

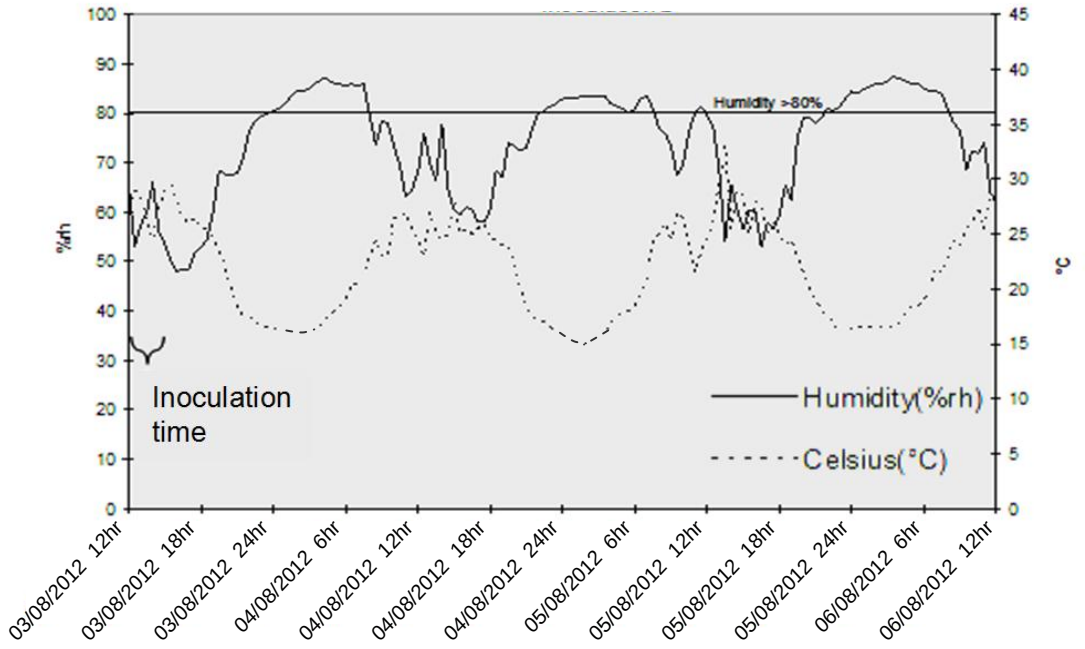

Figure 6. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 2 (3 August, 10 – 11.30 am)

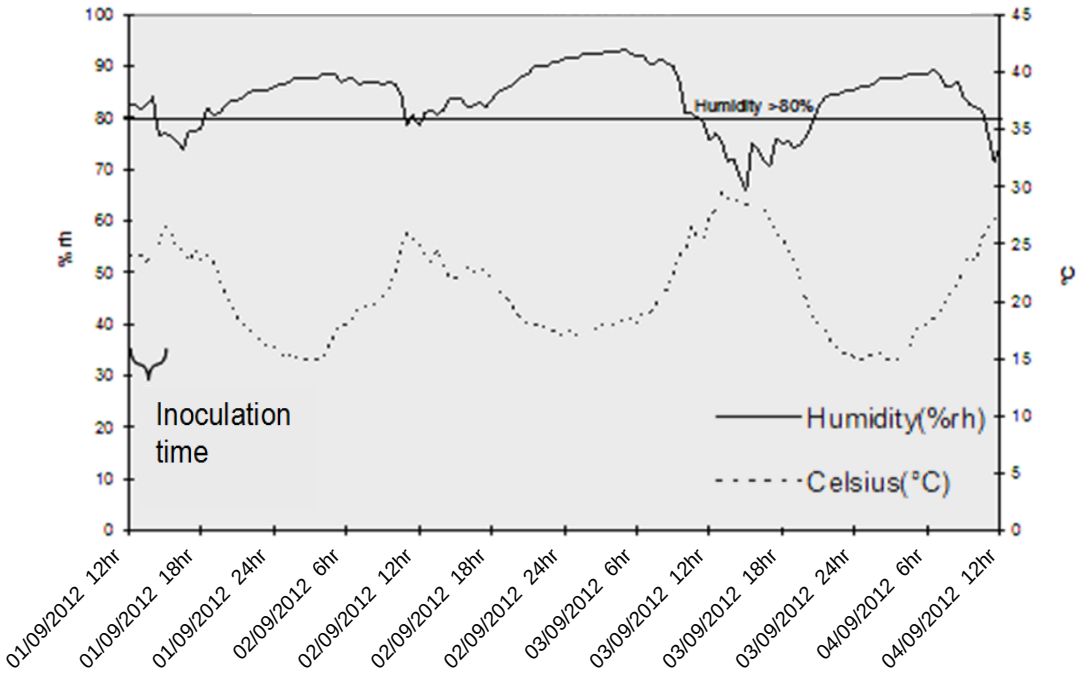

Figure 7. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 3 (31 August, 10 – 11.30 am)

Objective 7 – Effect of season and fruit size on *Fusarium* species in pepper fruit

Introduction

At least three species of *Fusarium* are associated with pepper internal fruit rot in the UK. Small brown aborted fruit can also contain *Fusarium* spp. pathogenic to pepper. It is unknown whether time of year or the type of fruit symptom affects the occurrence of different *Fusarium* species in pepper. Samples of fruit were collected from a commercial crop at intervals during 2012 and the incidence and identity of *Fusarium* infection was determined to examine these aspects.

Materials and methods

In April, June, August and November 2012, samples of fallen aborted fruit (70) and mature fruit with suspected early symptoms of Fusarium internal fruit rot (10-20) were collected from a crop of cv. Cupra in the Lee Valley. The mature fruit and 20 of the aborted fruit were sent to Warwick Crop Centre for determination of *Fusarium* species associated with them by molecular tests (see Year 1 report). The remaining 50 aborted fruit (1-2 cm diameter) were cut into quarters using a sterile blade and plated onto PDA to determine the proportion

of fruit affected by *Fusarium*. Plates were examined for colonies typical of *Fusarium* after incubation for 7-10 days at 21ºC. Identification of the species was not attempted.

Results and discussion

The incidence of aborted fruit containing *Fusarium* increased from 48% in April to 100% in November (Table 12). When a fruit was found to be infected, generally the fungus grew from all quarters.

Table 12. Occurrence of *Fusarium* spp. in small aborted pepper fruit on four occasions 2012

Date collected	% of quarters infected	% fruit infected
April 27	45	48
June 18	77	88
August 22	76	84
November 2	95	100

The identity of *Fusarium* species recovered from aborted and mature fruit as determined by molecular tests is given in Table 13. *Fusarium lactis* was predominant in both aborted and mature fruit, while *F. oxysporum* and *F. proliferatum* were both found at a low incidence. There was no evidence that time of year or the type of fruit symptom affected the occurrence of different *Fusarium* species in pepper.

Table 13. Identity and frequency of recovery of *Fusarium* species from pepper fruit collected from a commercial crop, cv. Cupra in 2012.

Date fruit collected	Number of fruit examined	Number in sample with any *Fusarium*	Number of fruit containing:			
			F. lac	F. pro	F. oxy	Other
Small aborted fruit						
April 27	12	12	7	1	3	1
June 20	20	14	8	2	0	4
July 2	20	16	11	2	2	1
August 30	20	12	12	0	0	0
November 2	20	13	12	0	0	1
Total	**92**	**67**	**50**	**5**	**5**	**7**
Mature fruit						
April 27	10	10	4	0	5	1
June 20	14	9	8	0	0	1
July 2	19	19	15	1	0	3
August 30	15	13	10	1	1	1
November 2	20	16	12	0	0	4
Total	**78**	**67**	**49**	**2**	**6**	**10**

F. oxy – *Fusarium oxysporum*; F. lac – *Fusarium lactis*; F. pro – *Fusarium proliferatum*; Other – unidentified *Fusarium* sp.

Conclusions

Years 1 and 2

1. Fusarium internal fruit rot is most commonly caused by *F. lactis* and 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 lactis* is frequently present in fallen aborted fruit.
4. *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.
5. 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.

6. Inoculation of pepper flowers with spores of *F. proliferatum* (2011) and *F. lactis* (2012) each resulted in mature fruit with Fusarium internal fruit rot.
7. Inoculation of pepper flowers by spray inoculation with *F. proliferatum* reduced the numbers that developed into mature fruit; small brown hard aborted fruit often contain Fusarium rot within them. The proportion of affected fruit increased with time from April to November 2012.
8. 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).
9. An imposed high humidity of around 100% RH around flowers for 24 h increased the proportion of fruit that developed Fusarium internal fruit rot from natural inoculum (i.e. flowers were not artificially inoculated with *Fusarium*).
10. No evidence was found to support the hypothesis that fruit sugar content (% Brix) alone determines the differing susceptibility of different coloured fruit.
11. High relative humidity (>85%) periods greater than 6 hours and potential condensation events greater than 15 minutes were commonly recorded in a crop on two nurseries in 2012; these conditions appear to be conducive to development of Fusarium internal fruit rot. There were large differences in those conditions between different monitoring points in the same house and between different houses.
12. Fusarium internal fruit rot is more likely to be found in more mature fruit (red stage) than less mature fruit (green stage) of the same variety.
13. Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil) and Serenade ASO (*Bacillus subtilis*) reduced Fusarium internal fruit rot when applied as a spray to open flowers.
14. The pepper varieties Cupra, Ferrari, Fiesta, Kelly, Pele, Special and Spider are all susceptible to Fusarium internal fruit rot. There is evidence that red Cupra is less susceptible than red Spider and that green Ferrari is less susceptible than yellow, orange or red fruited varieties.

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.

Acknowledgements

We are grateful to Gill Wardell, Alan Richardson and staff of Abbey View Nursery for help with this project and useful discussion.

Technology transfer

Articles

O'Neill T M and Barbara D (2012). Fusarium sneaks in from flower to fruit *HDC News* **182,** 18-19.

O'Neill T M (2013). The route to infection, the route to control. *HDC News* **191**, 24-25.

Appendix 1 – Experiment diaries

Sweet pepper: Natural infection of Fusarium internal fruit rot in mature – 2012 (Objective 4)

Date	Summary
18 May 2012	120 2nd class fruit of 6 varieties were collected from Abbey view Nursery (Picked by Alan on the 17/05/2012). Green Cupra was replaced with green Ferrari due to fruit availability. Fruit was checked for external signs of Fusarium and those with symptoms were removed. Fruit was put into crates of 40 and labeled. Crates were then stacked and left in the foyer of the pathology laboratory.
23 May 2012	Fruit was examined externally for Fusarium symptoms and was then cut in half and assessed for internal rot. External infection, internal infection, infection on seeds and the presence of blossom end was recorded. Fruit was then discarded.

Sweet pepper: Effect of high humidity duration on infection of flowers by *Fusarium lactis* and subsequent internal fruit rot – 2012 (Objective 5a)

Date	Summary
11 April 2012	3 strains of *Fusarium lactis* provided by Dez Barbara at Warwick University were subbed onto 27 PDA+S plates and 6 PDA+S slopes and put in the incubator at 20°C.
18 April 2012	*F.lactis* from plates subbed on 11 April was subbed onto fresh PDA+S plates and put in incubator at 20°C.
26 April 2012	A spore suspension was made up from 3 Fusarium isolates in the morning and kept in a cool box until use (consisted primarily of micro conidia). The trial was set up in 2 isles (4 rows) within bay 4. T he area was marked with hazard tape and signs to restrict access. Plots were 8 slabs each (3 plants per slab, totaling 24 per plot), with 2 slabs left at the end facing the pathway and 3 slabs at the far end. Data loggers

	were put out in each of the 2 rows amongst the crop. The area was marked with tape (on the crop and on the floor). 30 flowers were inoculated from each plot (with preference to large, open, up-ward facing flowers). Inoculation was done on a sunny day, so screens were put across the glasshouse roof. Timings were as follows: treatment 1: inoculated 3pm, treatment 2: inoculated 1pm, bag removed 4pm, treatment 3: inoculated 12pm, bag removed 6pm, treatment 4: inoculated 6.30pm (day 1), bag removed 9.30am (day 2), treatment 5: inoculated 11am (day 1), bag removed 11am (day 2). Each treatment was done separately. All plots of a given treatment were labeled and then 1 person proceeded to inoculate and the other person followed, bagging inoculated flowers. This was done in number order of each treatment. Water collected in bags (large droplets in bags left for 24hours). This led to some flowers dying and aborting, and petals falling off once bags had been removed. This was mainly seen in treatment 5.
27 April 2012	Bags were removed from treatments 4 and 5 at times specified above.
30 April 2012	The experiment was repeated. Data loggers were downloaded. A further 30 flowers from each plot were inoculated. Timings were as follows: treatment 1: inoculated 3pm, treatment 2: inoculated 1pm, bag removed 4pm, treatment 3: inoculated 12pm, bag removed 6pm, treatment 4: inoculated 6.30pm (day 1), bag removed 9.30am (day 2), treatment 5: inoculated 11am (day 1), bag removed 11am (day 2). Each treatment was done separately. All plots of a given treatment were labeled and then 1 person proceeded to inoculate and the other person followed, bagging inoculated flowers. This was done in number order of each treatment. A small number of flowers had aborted which had been infected on the 1st experiment. This was mainly observed in treatment 5, though flowers were also dying on un-inoculated plants. This could have been down to lower light levels leading to plants wilting (as advised by Alan). However, this was only seen in small numbers (approx 2 per plot).

01 May 2012	Bags were removed from treatments 4 and 5 at times specified above.
18 May 2012	The experiment was examined for fruit strangulation by trapped labels and where appropriate, labels were loosened. A large number of fruit have aborted; approximately 10 fruit/ plot remain.
02 July 2012	All fruit was harvested. Each plot was picked and put directly into bags marked by plot. Where labels were found on aborted fruit, these were also collected. Only approximately 10 fruit were collected per plot, due to the rest of the fruit aborting. Fruit was stored in the laboratory foyer in loosely tied bags. Data loggers were downloaded and reset.
06 July 2012	Fruit was assessed for presence of Fusarium. External symptoms and internal symptoms (on the seed and wall) were recorded. High levels of Fusarium were observed though numbers of fruit and fruit maturity varied between plots. A sample of fruit was sent to Dez Barbara at Warwick University to identify whether isolates were the same as the isolates that we inoculated with.

Sweet pepper: Effect of fungicides and biofungicides applied to flowers on occurrence of Fusarium internal fruit rot – 2012 (Objective 6)

Date	Summary
17 July 2012	*F. lactis* from 11 April cultures was subbed onto fresh PDA+S plates and left in the incubator at 20 degrees.
26 July 2012	**Replicate 1** Chemicals were weighed up and stored in sealed tubes. Water was measured and put into labeled brand new hand-held sprayers. These were transferred to Abbey View Nursery in a large, sealed container. Rates were as follows: Treatment 4 (Amistar) was applied at 500 ml water + 0.5 ml Amistar. Treatment 5 (Switch) was applied at 500 ml

	water + 0.5 g. Treatment 6 (Prestop) was applied at 500 ml water + 2.5 g Prestop and treatment 7 (Serenade ASO) was applied at 500 ml water + 50 ml Serenade ASO.
26 July 2012	Experiment was set up at Abbey View Nursery. The trial was set up in 2 isles (4 rows) within bay 4 (opposite to experiment 2). The area was marked with hazard tape and signs to restrict access. Plots were 5 slabs each (3 plants per slab, totaling 15 per plot), with 2 slabs left at the end facing the pathway and 5 slabs at the far end. Data loggers recording temperature and relative humidity were attached in delta T traps between the rows at the start of plot 10 and 13 amongst the crop. The area was marked with tape. Each plot was marked with hazard tape and labels were written on the bag where the plot began. 25 flowers were tagged from each plot with 1 written on the tag representing 1st replicate. In each treatment, 1 was written in a different colour. 1=red, 2=black, 3=black biro, 4=pink, 5=blue biro, 6=blue, 7=green. Weather during spray application was hot and sunny though vents were open and shades were pulled over. Chemicals were mixed up and sprays were applied in a hand-held sprayer at a rate of 1ml per plant (1 squirt of the hand sprayer). Treatments 2 and 3 were completed first (start: 1.30pm, temperature: 34 degrees, relative humidity: 51%, finish 3.00pm, temperature: 33 degrees, relative humidity: 53%) and then sprays were applied in order of the plots from 1 to 28 (start: 3.00pm, temperature: 33 degrees, relative humidity: 53%, finish: 6.00, temperature: 29 degrees, relative humidity: 28%), in all cases excepting treatment 4 (Amistar) which was applied last, so to meet spray conditions of below 30 degrees. This was sprayed at 6.30 at temperature: 28 degrees, relative humidity: 52% and finished at 7.30pm: temperature: 24.5 degrees and relative humidity: 52%.
27 July 2012	A spore solution of *F.lactis* was made up at a concentration of 1×10^5 and was stored in a cool box for transportation to Abbey View Nursery. The inoculum was applied to each tagged flower (except for treatments 2 and 3) at a rate of 1ml per flower (1 squirt of hand-held sprayer).

	At the start of inoculation (10.30am) temperature was: 24.4 degrees and relative humidity was 76% and at finish (12.00) temperature was 29 degrees and relative humidity was: 70.1%. All bags were then removed from flowers in treatment 3. A small proportion of petals were removed during this process, though the majority remained on the flower.
02 August 2012	**Replicate 2** Chemicals were weighed up and stored in sealed tubes. Water was measured and put into labeled hand-held sprayers. These were transferred to Abbey View Nursery in a large, sealed container. Rates were as follows: Treatment 4 (Amistar) was applied at 500 ml water + 0.5 ml Amistar. Treatment 5 (Switch) was applied at 500 ml water + 0.5 g. Treatment 6 (Prestop) was applied at 500 ml water + 2.5 g Prestop and treatment 7 (Serenade ASO) was applied at 500 ml water + 50 ml Serenade ASO.
02 August 2012	Labels were made up as before, though marked with a 2 for replicate 2. Chemicals were mixed up at Abbey View Nursery and were applied as before, though Amistar was applied first before the temperature increased. Application began at 10.45am: temperature: 27 degrees, relative humidity; 46%, finish: 12pm, temperature; 25.4 degrees, relative humidity; 51%. Treatments 2 and 3 were then completed, (start: 12pm: temperature; 25.4 degrees, relative humidity; 52%, finish; 2pm; temperature: 27.6 degrees, relative humidity; 44%). All other treatments were then applied (start; 2pm, temperature; 27.6 degrees, relative humidity; 44%, finish: 5pm, temperature; 27.4 degrees, relative humidity: 40%. Not all plots had sufficient numbers of flowers so tags were kept in bags labeled by the plot number so that additional flowers could be labeled in the next replicate.
03 August 2012	A spore solution of *F. lactis* was made up at a concentration of 1 x 10 (5) and was stored in a cool box for transportation to Abbey View

	Nursery. The inoculum was applied to each tagged flower (except in treatments 2 and 3) at a rate of 1ml per flower (1 squirt of hand-held sprayer). At the start of inoculation (10am) temperature was; 20.8 degrees and relative humidity was: 76%. When finished (11.30pm) temperature was 30.6 degrees and relative humidity was 52%. Bags were then removed from treatment 3 and loggers were downloaded. Some fruit that had been tagged in replicate 1 had aborted, though only a small proportion.
20 August 2012	*F. Lactis* from 17 July cultures was subbed onto fresh PDA+S plates and left in the incubator at 20 degrees.
22 August 2012	Data loggers were downloaded and reset.
29 August 2012	**Replicate 3** Chemicals were weighed up and stored in tubes (using balance: box/b57 and test weight: box/b66). Water was measured and put into hand-held sprayers. These were transferred to Abbey View Nursery in a large, sealed container. Rates were as follows: Treatment 4 (Amistar BX-F951) was applied at 500ml water + 0.5ml Amistar. Treatment 5 (Switch BX-F1028) was applied at 500ml water + 0.5g. Treatment 6 (Prestop BX-FR024) was applied at 500ml water + 2.5g Prestop and treatment 7 (Serenade ASO BX-F1012) was applied at 500ml water + 50ml Serenade ASO.
30 August 2012	Labels were made up as before, though marked with a 3 for replicate 3. Chemicals were mixed up at Abbey View Nursery and were applied as before. Application began at 11am: temperature: 25.6 degrees, relative humidity; 56%. Treatments 2 and 3 were completed first. All other treatments were then applied in plot order. The temperature remained the same throughout the day, never exceeding 28 degrees. Treatments were finished at 5.30, temperature 23 degrees, relative humidity: 68%. As many flowers were treated as possible. This reached 28 in all plots except plot 9: 23 flowers, plot 13: 22 flowers, plot 15: 25 flowers, plot 24: 25 flowers, plot 27: 23 flowers and plot 28: 24 flowers, due to fewer open flowers. Fruit from previous replicates

	was checked and tags were loosened where they were beginning to strangle fruit. Fruit set was about 15 per plot (a high level of abortion). Fruit was about 2-3 weeks from harvest (1st replicate).
31 August 2012	Fusarium inoculum was made up at a concentration of 1 x 10^5 and was stored in a cool box for transportation to the nursery. The inoculum was applied to each tagged flower (except in treatments 2 and 3) at a rate of 1ml per flower (1 squirt). Bags were then removed from treatment 3.
26 August 2012	Fruit from replicate 1 harvested.
1 October 2012	After being picked and left for 5 days, fruit from replicate 1 were assessed for Fusarium levels externally, internally and on the seeds. Fruit discarded once assessments completed.
3 October 2012	Fruit from replicate 2 harvested. Data loggers were downloaded and reset.
8 October 2012	After being picked and left for 5 days, fruit from replicate 2 were assessed.
11 October 2012	Replicate 3 and all remaining fruit were harvested. Trial finished so all kit removed from site.
16 October 2012	After being picked and left for 5 days, fruit from replicate 3 were assessed. Disease levels were low. Many fruit from the third inoculation had not set or were still green, probably due to being later in the season.

Sweet pepper: Effect of fruit sugar level on susceptibility to fruit rot caused by *Fusarium lactis* - 2012 (Objective 4)

Date	Summary
11 April 2012	3 strains of *Fusarium lactis* provided by Dez Barbara at Warwick University were subbed onto 27 PDA+S plates and 6 PDA+S slopes and put in the incubator at 20°C.
10 May 2012	*F. lactis* from plates subbed on 11 April was subbed onto fresh PDA+S plates and put in incubator at 20°C.

18 May 2012	**Replicate 1** 15 1st class fruit of 6 varieties were collected from Abbey View Nurseries (all fruit was picked on 17/05/2012 and collected on 18/05/2012). Green Cupra was replaced by green Ferrari. Fruit was returned to the laboratory and cut in half. Half of the fruit was placed in a sealed lunch box with 2 plugs of *F. lactis* placed on the flesh. Each plot consisted of a lunch box containing 2 halves of pepper (a total of 10 pepper halves from each variety within 5 plots). Containers were placed in an incubator set at 20 degrees, 12L:12D according to the trial design. The other half of the fruit was used to measure the Brix. Juice was squeezed out of each half and measured separately.
21 May 2012	Inoculated fruit was assessed for the presence of Fusarium growth on and around the plugs. This was recorded as a presence/absence since growth was not abundant enough to measure the lesion size.
28 May 2012	Inoculated fruit was assessed. Lesions were measured from the longest length (mm).
29 May 2012	*F. Lactis* was subbed onto fresh PDA+S plates and left in the incubator at 20 degrees
11 June 2012	*F. Lactis* was subbed onto fresh PDA+S plates and left in the incubator at 20 degrees
18 June 2012	**Replicate 2** Fruit was collected from Abbey View Nursery (picked on 18/06/2012 and collected on 18/06/2012 and stored in the path lab foyer overnight).
19 June 2012	Plastic ventilated boxes were lined with damp paper roll, along with damp paper roll taped to the lid. Two large flat squares were cut out of each pepper and were used for the inoculation experiment. The other half was used to measure the Brix. The two squares, along with two squares from another pepper (same

	variety) were positioned apart in the plastic box. Boxes were labeled by plot number and fruit was labeled 1-10 and a and b. This would allow for comparison between the Brix measurements of fruit 1-10. An 8mm cork borer was used to cut circular pieces of agar around the leading edge of the Fusarium culture. A needle was used to pick up these pieces and transfer them to the pepper. One plug was placed mycelium down onto the central area of the pepper square. Containers were sealed and placed in an incubator (20 12L:12D). Two incubators were used as they would not all fit in one.
22 June 2012	3 day assessment was done on the inoculated peppers, recording the presence/absence of mycelium and any other fungi present. Plugs were still plump and mycelium was developing into the pepper flesh. Mucor was found on some peppers.
25 June 2012	6 day assessment was done on the inoculated peppers recording the diameter of Fusarium lesions on each pepper. Many plots had mucor on them.
29 June 2012	10 day assessment was done on the inoculated peppers recording the diameter of Fusarium lesions on each pepper again. Most peppers covered in mucor, making the Fusarium difficult to see.

Sweet pepper: Effect of season and symptom type on occurrence of Fusarium species associated with internal fruit rot and aborted fruit – 2012 (Objective 7)

Date	Summary
23 April 2012	PDA+S was made and agar was poured into 50 plates and stored in the fridge.
27 April 2012	60 brown aborted Cupra (red) fruit was collected from Abbey View Nursery from 10 rows (approx 0.5-1cm diameter). Rows were chosen at random, though were at a distance from the trial site. 13 mature fruit (3 picked from the crop with external signs of Fusarium and 10 with external mycelium and broken skin) were also collected. Fruit was packaged up and stored in the fridge over the weekend.

27 April 2012	Of the 60 aborted fruit, 50 were plated up on PDA+S plates. These were cut into quarters in a sterile environment and distributed around the plate. Plates were stored upside down in an incubator set to 20 degrees, 12L:12D. Some had mycelium clearly present internally.
30 April 2012	The remaining 10 aborted fruit and 13 mature fruit were packaged up in separate sealed bags and were sent to Dez Barbara at Warwick University by courier.
04 May 2012	The 1st assessment was done on the aborted fruit. 50 percent had extensive growth of *F. lactis*, which was confirmed under the high power microscope. 50% had slight growth (visible mycelium) which was also confirmed as *F. lactis* under the microscope.
11 May 2012	The 14 day assessment was done on the aborted fruit. Mycelium was examined under the microscope for identification. 6 of the plates containing Fusarium were sent by courier to Dez Barbara and the remaining plates were discarded.
18 June 2012	50 brown aborted Cupra (red) fruit was collected from Abbey View Nursery from 5 rows (approx 0.5-1cm diameter). Rows were chosen at random, though were at a distance from the trial site.
20 June 2012	The 50 aborted fruit were plated onto PDA+S plates as before. Fruit was cut up into quarters and plated on PDA+S. Plates were stored upside down and incubated at 20 degrees 12L:12D.
27 June 2012	The 7 day assessment was done on the aborted fruit. The majority of the plates had mucor in though Fusarium could still be observed. Plates were then discarded.
02 July 2012	20 aborted fruit and 20 mature fruit with external signs of Fusarium was collected from the nursery. This was packaged up and sent to Dez Barbara by courier.
22 August 2012	50 brown aborted Cupra (red) fruit was collected from Abbey View nursery from 5 rows (approx 0.5-1 cm diameter). Rows were chosen at random, though were at a distance from the trial site. Fruit was cut up into quarters and plated on PDA+S. Plates were

	stored upside down and incubated at 20 degrees 12L:12D.
29 August 2012	7 day assessment was done on the aborted fruit. The majority of plates had Fusarium growth, though most also had Penicillium and some had mucor.
30 August 2012	20 brown aborted Cupra (red) fruit was collected from Abbey View Nursery from 5 rows (approx 0.5-1 cm diameter). Rows were chosen at random, though were at a distance from the trial site. 15 mature fruit with external signs of fruit rot were also collected. Fruit was packaged up and stored in the fridge over the weekend to be sent to Clare Grant at Warwick University by courier on Monday 3 September.
2 November 2012	70 aborted fruit and 20 mature fruit with Fusarium symptoms were collected. 50 of the aborted fruit were cut in quarters and plated on PDA+S agar. Other 20 aborted and 20 mature peppers sent to John Clarkson at Warwick University.
9 November 2012	Agar plates from 2/11/12 assessed. 94.5% of quarters exhibited signs of Fusarium. Most had contracted mucor and Penicillium too.

Appendix 2 – Nursery monitoring (temperature and RH)

Nursery 1 and Nursery 2 pepper crop monitoring

Accompanying notes to spreadsheets

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Farm Energy is a trading name of FEC Services Ltd Company Reg No: 04056474 VAT Reg No: 754 1246 42

This report is intended as an accompanying document to the 'High humidity data' spreadsheets

1. Summary

Temperature and Relative Humidity (RH) monitoring equipment was installed in two glasshouses, Nursery 1 and Nursery 2. This was to determine periods where pepper plants may be susceptible to condensate build up and thus disease. ADAS are interested in prolonged 'wet periods' i.e. where humidity levels are above 80% for a long period of time. In this report, the raw collected data has been analysed for the following:

- RH Level: >80%, >85%, >90%
- Time duration at RH level: 6 hrs, 9 hrs, 12 hrs

2. Equipment

The monitoring equipment installed at Nursery 1 and Nursery 2, consisted of:

Name	Description	Specification	Measurement	Quantity
HC2-SH High precision humidity probe	Measures humidity and temperature (mounted in a protective tube and hung amongst the crop)	Accuracy: ± 0.5 %RH ± 0.1 K Range: -50...100 °C 0...100 %RH	Recording at 5 min intervals for period: Nursery 1 (26/03/12 – 21/10/12) Nursery 2 (24/03/12 – 21/10/12)	6
Minco TS665 NTC thermistor	Measures plant temperature, mounted on plant stem	Time constant: 0.8 sec Range: -50...125 °C		6
t-mac data logger	The above sensors were connected to the data logger, which was mounted at a central location in each glasshouse	Collected data transmitted over wireless GPRS to central web based server		2

Figures for all equipment and locations in the glasshouse can be seen in Section 6.

3. Data correction and calibration

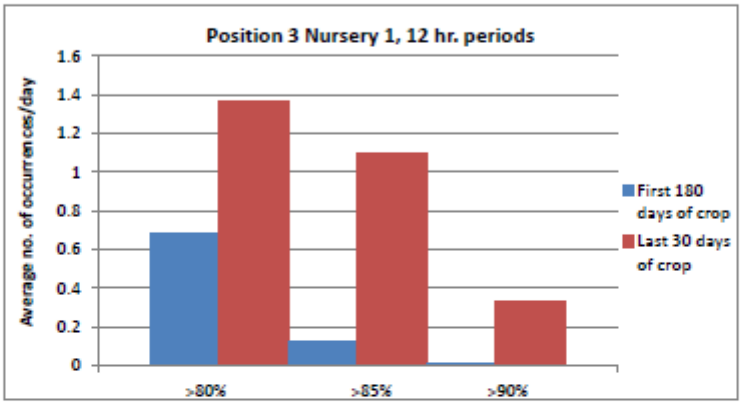
Calibration tests were carried out in a controlled environment before the equipment was installed on site. As such, calibration factors were applied to each temperature and humidity value. The calibration factors applied to the raw data can be seen in the spreadsheet *'Calibration factors'*

Raw data and data correction undertaken can be seen in the spreadsheets *'AV/BG data download'*. There were occasional instances where data intervals were missed, double counted or gave an error message. To reduce the impact of these errors on later data processing, they were removed.

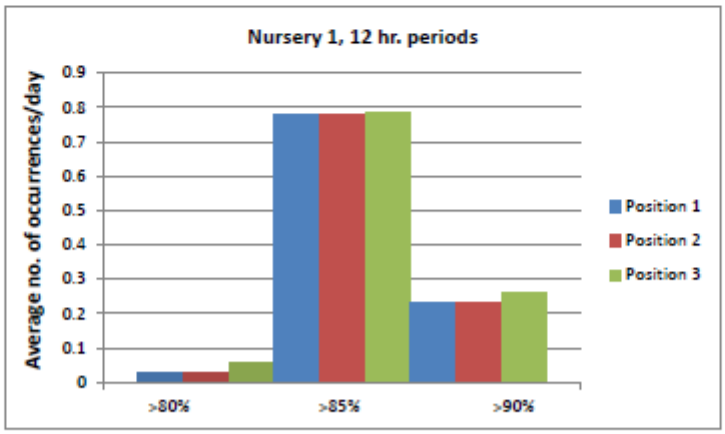
4. Nursery 1 Data Analysis *(from spreadsheet 'High Humidity data AV')*

All three positions experience a period of high RH early in the cropping cycle for March 27/28th. From the end of March to September 23rd, RH remained stable in the glasshouse and rarely went above 85%. From September 23rd to October 21st (end of cropping cycle), the frequency for periods of high RH more than doubled and >90% RH was commonplace, especially for long 12 hour periods (Graph 1).

Interestingly positions 1 & 2 experienced similar frequencies of high RH, whereas position 3 (the furthest from glasshouse entrance) was subject to a slightly greater frequency of high RH periods (Graph 2).



Graph 1

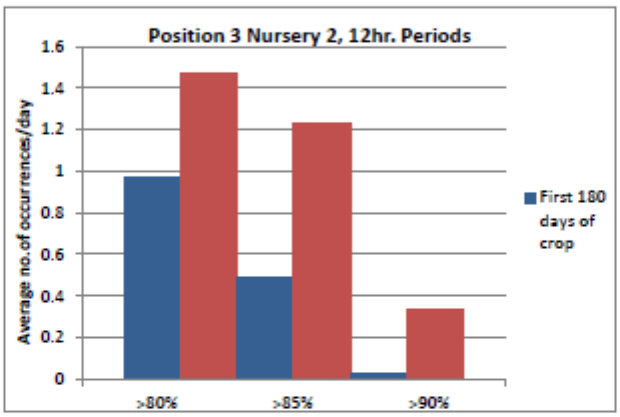


Graph 2

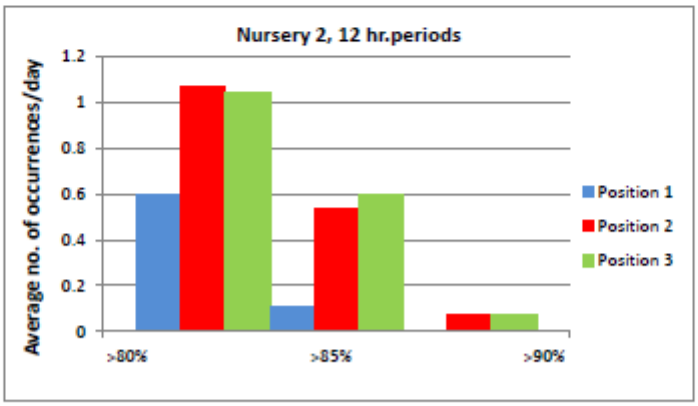
5. Nursery 2 Data Analysis *(from spreadsheet 'High Humidity data BG')*

The Nursery 2 humidity data is markedly different to Nursery 1. For position 2 and 3 there appears to be less of an obvious trend for when high humidity periods occur, these do however increase in frequency towards the end of cropping (Graph 3) as with Nursery 1. Position 1 on the other hand performs well, with humidity levels, especially for any length of time of above 90% very rare (only once on the 8th August and four instances at very end of cropping).

Nursery 2 follows Nursery 1 in that positions 2 and 3 (further from the glasshouse entrance) experience higher humidity than position 1 (Graph 4).



Graph 3



Graph 4

6. Figures

Figure 1: RH/temp units

Figure 2: Plant temp sensors

Figure 3: Nursery 1 sensor locations

Appendix 3 – Nursery monitoring (condensation events)

Nursery 1 and Nursery 2 pepper crop monitoring

Condensation conditions

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Following an email request from Tim O'Neil on 16/01/13, this brief report is intended as additional information to the main report 'Details of project' sent on 09/11/12.

Summary

Nursery 1

Position 1 had highest frequency of condensation potential events; this position is closest to the glasshouse entrance which may explain why conditions were right for condensation. Nursery 1 on the whole had far fewer events than Nursery 2.

Nursery 2

Condensation potential events were frequent throughout the cropping period for positions 1 and 3 and fewer at position 2. The frequency at position 1 could well be explained by its proximity to the entrance. But for 3, which is well inside the glasshouse, there must be some other underlying cause.

Introduction

Temperature and Relative Humidity (RH) monitoring equipment was installed in two glasshouses, Nursery 1 and Nursery 2. This was to determine periods where pepper plants may be susceptible to condensate build up and thus disease. Conditions suitable for the forming of condensation occur when the dew point (calculated from the air temperature and humidity) falls below the plant stem temperature. Details of occurrences and duration of such conditions for both greenhouses are as follows.

Nursery 1

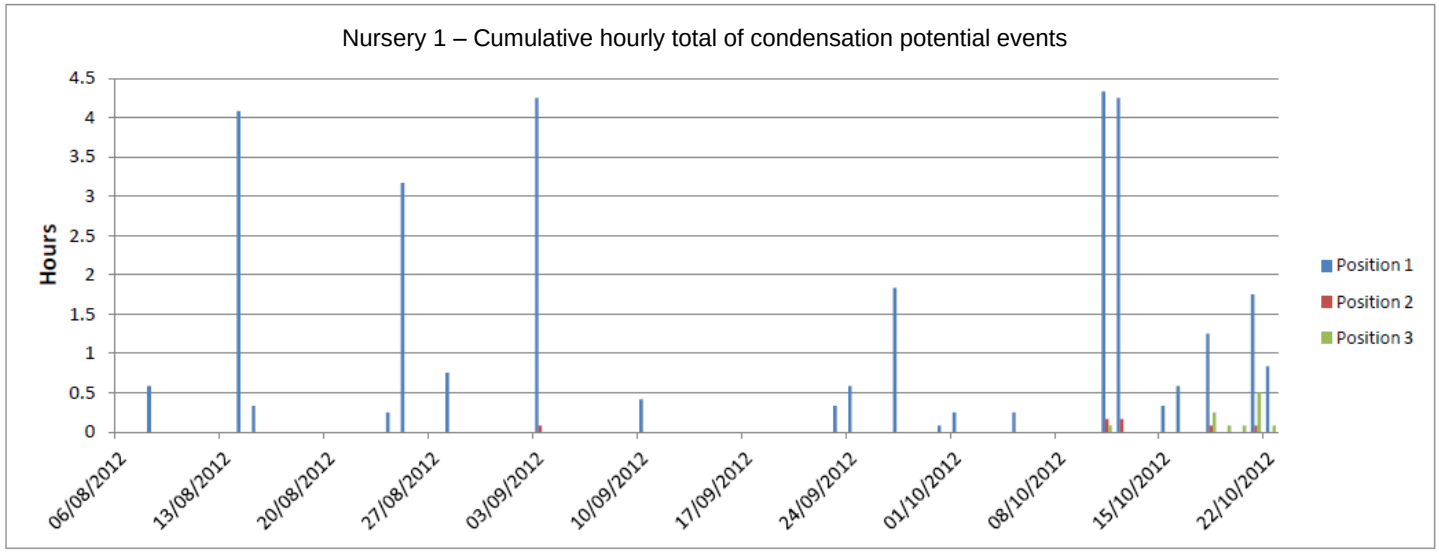

Figure 1: Nursery 1 5 minute periods

Position 1 saw the highest frequency of 'condensation potential' events. These periods were spread throughout the cropping cycle. At positions 2 and 3, 'condensation potential' events were far fewer and apart from one event at position 2, only occurred at the end of the crop life. – *see figure 1 above*

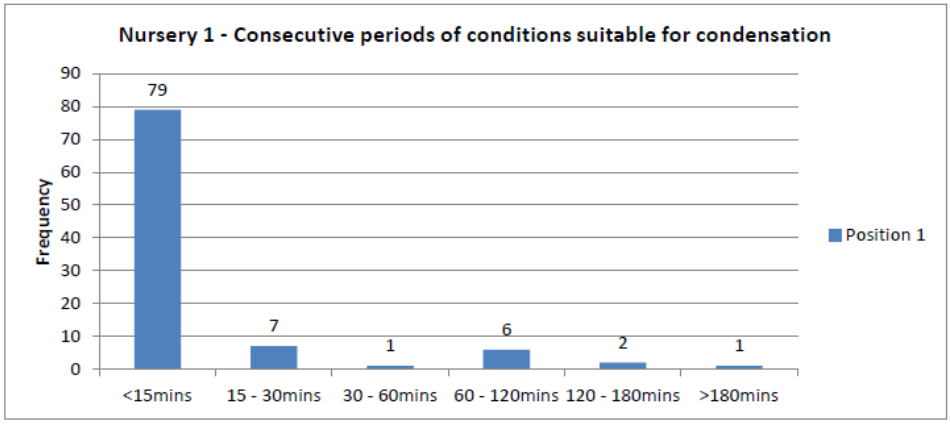

Figure 2: Frequency distribution for consecutive events at Nursery 1

There were no consecutive condensation events longer than 5 minutes at position 2 or 3. Figure 2 above shows that Position 1 however had many long condensation events, the longest of which was over 3 hours. In general shorter, sub 15 minute periods were most common.

Nursery 2

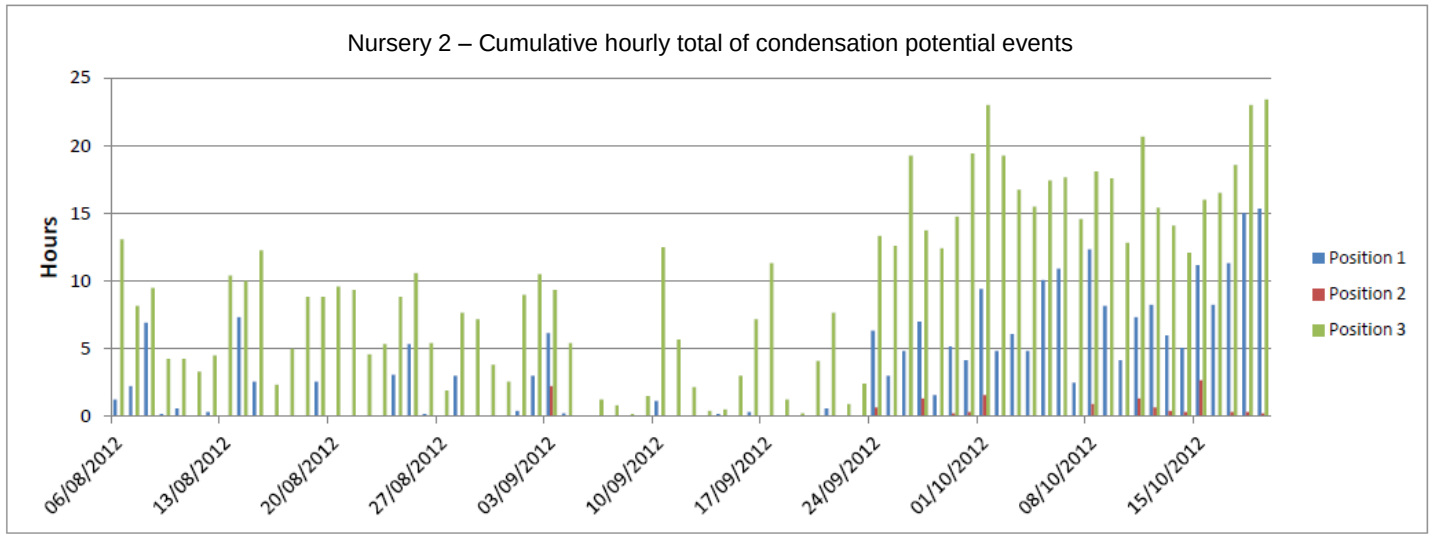

Figure 3: Nursery 2 5 minute periods

Position 3 saw the highest number of condensation potential events. These occurred throughout the crop life, increasing in frequency towards the end. Position 1 saw slightly fewer of these periods and they were more localized towards the last 10 days of cropping. Position 2 had relatively few events, again localized towards the end of the cropping period. – *see figure 3 above*

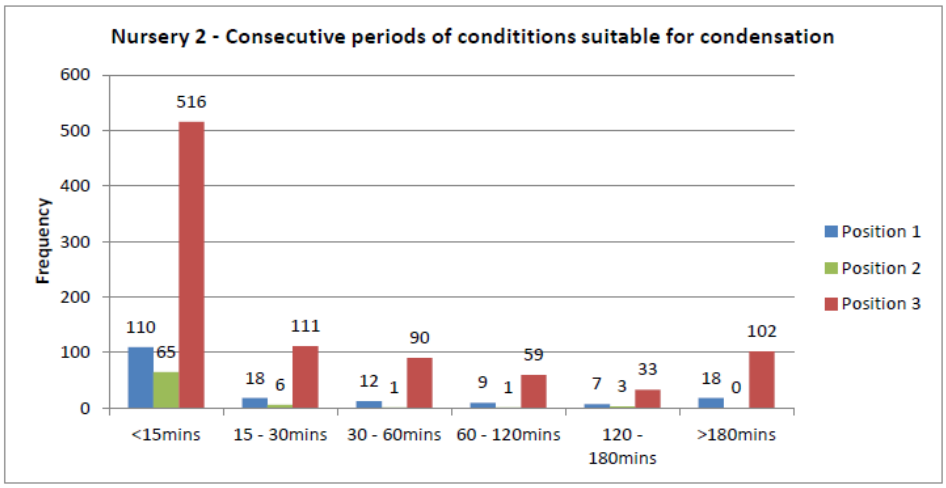

Figure 4: Nursery 2 consecutive periods

Position 3 also has the greatest number of events longer than 5 minutes. Sub 15 minute periods are exceedingly frequent throughout crop. Figure 4 above illustrates just how many more events there were at position 3 compared to 1 and 2.