Project title:	Pepper: Improved control of Fusarium internal fruit rot through increased knowledge exchange with the Netherlands and Belgium and targeted application of plant protection products.
Project number:	PE 022
Project leader:	Dr Tim O'Neill, ADAS ADAS Boxworth, Boxworth, Cambridge, CB23 4NN
Report:	Final report, March 2015
Previous report:	None
Key staff:	Sarah Mayne, ADAS Dr John Clarkson, Warwick Crop Centre
Location of project:	ADAS Boxworth Three commercial pepper nurseries, Essex
Industry Representative:	Gary Taylor, Valley Grown Nursery, Nazeing, Essex EN9 2EX
Date project commenced:	1st August 2014
Date project completed:	31st March 2015

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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.

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

- A high proportion (mean 59%, range 6-100%) of small (pea-sized) pepper fruits examined on three nurseries in August-October 2014 were found to contain *F. lactis*
- No clear evidence was found to link the incidence of *Fusarium* infection in small unripe pepper fruit with that found in the corresponding cohort of flowers

Background and expected deliverables

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

In PE 007 we showed that *Fusarium lactis* was the predominant cause of Fusarium internal fruit rot throughout the year. A small proportion of rots were caused by *F. oxysporum* and *F. proliferatum.* We found a significant reduction in fruit rot when a single spray of Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil) or Serenade ASO (*Bacillus subtilis* strain QST 713) was applied to flowers one day before they were artificially inoculated with *F. lactis* spores. Furthermore, in a whole row comparison study, a single spray of Serenade ASO applied to the crop face (flowers, leaves and stems), to rockwool cubes and floor (to treat fallen debris) was associated with around a 50% reduction in Fusarium internal fruit rot of fruit developing from open flowers at the time of the spray.

Fusarium internal fruit rot also remains a problem in the Netherlands and research on the disease is ongoing at Wageningen University. In 2014, Dutch growers reported a lower incidence of fruit rot, as was also reported initially in the UK, following a mild spring and good summer, which may have allowed better control of glasshouse humidity. Work in the Netherlands has highlighted temperature as a key determinant in disease development. As *Fusarium* has been found to survive in water, Dutch work recommends the use of UV-C and heat treatments to disinfect irrigation water. Efforts focusing on disease control have also focused on control of environmental conditions and on the use of biological plant protection products.

The aim of this project was to reduce fruit wastage due to Fusarium internal rot through knowledge exchange and by targeted application of plant protection products. The specific objectives were:

1. To liaise with the Dutch and Belgian researchers working on Fusarium internal fruit rot, exchange information and develop a coordinated, applied research programme
2. To examine rockwool cubes as a source of *F. lactis* and/or *F. oxysporum*
3. To examine the relationship between flower infection by *Fusarium* spp. and Fusarium infection in small fruit

Summary of the project and main conclusions

Objective 1 - To liaise with the Dutch and Belgian researchers working on Fusarium internal fruit rot, exchange information and develop a coordinated, applied research programme

At the start of the project, following a visit to the Netherlands and a presentation of UK pepper Fusarium results to Dutch research contractors by Tim O'Neill, Jantineke Hofland-Zijlstra, a research plant pathologist based at Bleiswijk Research Station, Wageningen University, was contacted regarding her future work on Fusarium internal rot of pepper. It was agreed, subject to successful applications for funding and agreement with funders, that work carried out in 2015 on pepper Fusarium internal fruit rot by both UK and Wageningen University researchers would seek to be complementary, and that results would be shared. Wageningen's links to researchers in Belgium, also studying *F. lactis*, were also explored.

In early 2015, following agreement of their respective funders, ADAS and Wageningen University shared their results obtained in 2014 through exchange of PowerPoint presentations and conference calls to discuss them. Wageningen University (at Bleiswijk Glasshouse Crops Research Station) have now secured funding from the Dutch Produce Association and the Dutch Growers Association for continued work on the disease in 2015. These groups have also agreed to continued exchange of results with ADAS in the UK.

In 2015 the Dutch team will focus on the effect of individual climatic factors (humidity, light levels and carbon dioxide), plant quality (nutrition, salicylic acid, flower development), spore load in glasshouse air and the induction of systemic acquired resistance on levels of Fusarium internal fruit rot. Subject to continued funding from HDC, (proposal submitted March 2015), ADAS will focus on potential control treatments using plant protection products, undertake plant quality measurements in UK crops to feed into Dutch research,

and further examine aspects of the disease biology (e.g. seed infection; transmission from seed to rockwool cube; occurrence of *F. lactis*, potential antagonists and levels of internal fruit rot in organic crops).

Key findings from the Dutch work in 2014 were:

- Incidence of Fusarium internal fruit rot at harvest varied greatly between six growers (0-4% in weeks 21-37); but all nurseries were affected to a similar degree by weeks 44-45 (1-2% fruit rot).
- Levels of Fusarium internal fruit rot were generally lower in 2014 than in 2013.
- One grower who managed crop growth in 2014 on dew point control had no internal fruit rot until week 38, when it was necessary to lower night temperature (and risk condensation) in order to encourage generative growth.
- Monitoring of *Fusarium* spore occurrence in flowers showed they were present throughout the year (weeks 24-41).
- Application of Serenade ASO (*Bacillus subtilis* QST713) to flowers prior to inoculation with *F. lactis* spores reduced Fusarium fruit rot by 50%; Prestop (*Gliocladium catenulatum* J1446) and Trianum (*Trichoderma harzianum* T-22) were ineffective.
- At more than three hours after application of spores to flowers, it is not possible to reduce fruit infection; this result is consistent with Canadian work showing rapid growth of *Fusarium* down the style.
- In experimental work at Bleiswijk, application of the Natugro system (e.g. addition of Trianum products and growth stimulating products such as ProParva, a root stimulant) or hydrogen peroxide through drip lines appeared to reduce Fusarium fruit rot. Hydrogen peroxide and these growth stimulating products are not registered as Plant Protection Products at present.
- None of the 10 products applied to plants as potential stimulators of SAR (Systemic Acquired Resistance) significantly reduced Fusarium fruit rot.
- Addition of supplementary ammonium sulphate in the feed appeared to reduce Fusarium fruit rot; however, a high incidence of flower/fruit abortion occurred in the experiment and the incidence of Fusarium fruit rot in control uninoculated plants was very low (0.2%).
- Increased molybdenum nutrition in peppers was associated with less fruit rot.

Objective 2 - To examine rockwool cubes as a source of *F. lactis* and/or *F. oxysporum*

On three occasions, at monthly intervals, three commercial pepper nurseries in the Lea Valley, Essex were visited. An assessment of 50 slabs per row, over five rows, was carried out to establish the occurrence of suspect fungal growth on rockwool cubes across the nursery (Table 1) on three occasions. The majority of cubes without visible fungal growth were those covered with moss or fern.

Table 1. Occurrence of visible fungal growth on rockwool cubes in three pepper crops – Essex, 2014

Assessment date	Presence or absence of visible fungal growth (%) on rockwool cubes								
	Nursery 1			Nursery 2			Nursery 3		
	Definite	Suspect	None	Definite	Suspect	None	Definite	Suspect	None
11 Aug	6	18	76	21	33	46	2	14	84
8 Sep	4	12	84	42	27	31	10	21	69
6 Oct	6	16	78	34	32	34	17	15	68

Levels of fungal growth appeared to remain relatively constant within each nursery over the period observed. Nurseries 1 and 3 appear to have similar levels of fungal growth on their slabs, whereas Nursery 2 has higher levels of visible fungal growth present.

An extreme example of a rockwool cube that was assessed and sampled as having fungal growth present – Nursery 2, Essex, 2014

On the same three occasions, rockwool cubes were sampled to check for *Fusarium* species, and a high incidence was confirmed at all three nurseries, with the highest being at Nursery 1 (Table 2). It was notable that even cube pieces with no visible fungal growth often contained *Fusarium* spp., sometimes at a higher incidence than where growth was visible. A representative set of *Fusarium* isolates from the nurseries were identified by PCR tests at Warwick Crop Centre. 13 isolates were identified as *F. lactis*, two as *F. equiseti* and one as *F. culmorum*. *F. culmorum* is a root and foliar pathogen of cereal crops, but can cause disease across a wide range of plant species. Similarly, *F. equiseti* is a root pathogen of cereal crops, but is capable of foliar infection and has been implicated in diseases of a diverse range of crops. Based on these results, it is possible that *Fusarium* species occuring on rockwool represent a significant source of inoculum that may lead to Fusarium internal fruit rot.

Table 2. Incidence of *Fusarium* spp. recovered from rockwool cubes – 2014, Essex

Sampling date	Incidence of suspect *F. lactis* (% cubes affected) from rockwool pieces with or without visible fungal growth on three pepper crops					
	Nursery 1		Nursery 2		Nursery 3	
	Suspect *Fusarium*	No fungal growth	Suspect *Fusarium*	No fungal growth	Suspect *Fusarium*	No fungal growth
11 Aug	55	10	35	40	30	15
8 Sep	75	65	10	55	35	40
6 Oct	5	15	0	0	15	5

The relationship between visible fungal growth on rockwool cubes on the nursery, and fungus isolated and identified as *F. lactis* differed between each nursery. When statistically analysed as nine sets of data, no significant relationship was observed. However, as viable *F. lactis* was regularly recovered from samples of rockwool cube, this confirms the surfaces of rockwool cubes as a source of inoculum in UK glasshouses. It is clear that a variety of other fungal species are also present on slabs; and the growth of viable *F. lactis* is highly dependent on site-specific factors, which likely includes competition with other fungi.

Objective 3 - To examine the relationship between flower infection by *Fusarium* spp. and Fusarium infection in small fruit

On three occasions at monthly intervals, flowers were sampled from three commercial pepper nurseries in Essex. Flowers were removed from the crop and incubated, not touching one another, and checked for presence of Fusarium by culture tests. Table 3 shows the incidence of *Fusarium* spp. recovered from these flowers from each nursery over the growing season.

Table 3. Incidence of *Fusarium* spp. isolated from pepper flowers in three crops – 2014, Essex

Sampling date	Suspect *F. lactis* incidence (% flowers infected)		
	Nursery 1	Nursery 2	Nursery 3
11 August	10	14	16
8 September	26	24	32
6 October	6	4	86

Incidence of *Fusarium* spp. varied between the three nurseries sampled and over the course of the season. Levels recovered from flowers at each nursery were broadly comparable at the first two sampling occasions, but differed at the last. In Nurseries 1 and 2 incidence was highest mid-season, whereas in Nursery 3 levels gradually increased over the season, to reach their highest levels in October.

Two weeks after flowers were sampled, the nurseries were re-visited and 50 small, green fruit sampled from the same crop rows. A tagging system was used to ensure that the fruit sampled had developed from the cohort of fully open flowers sampled 2 weeks previously. Table 4 shows the incidence of *Fusarium* spp. recovered from the fruit sampled.

Table 4. Incidence of *Fusarium* spp. isolated from small pepper fruit sampled – 2014, Essex

Sampling date	*F. lactis* incidence (% infected)		
	Nursery 1	Nursery 2	Nursery 3
26 August	46	6	94
22 September	40	50	96
20 October	72	30	100

The incidence of *F. lactis* in small green fruit showed high levels of variation between nurseries. Levels at nurseries 1 and 3 appeared to follow a similar pattern, being relatively constant at the first two sampling dates, and peaking towards the end of the season. Nursery 3 had consistently higher levels recovered from fruit than the other two nurseries, reaching 100% infection by the end of the season. Nursery 2 had initially low levels, which climbed to a peak at 50% in September and then fell slightly to 30% in October. *Cladosporium* spp. were commonly isolated from flowers at this nursery (34%, 50%, 70%), but not at the other two nurseries. Possibly there is a biocontrol effect, as was also suggested by Dutch researchers when it was consistently isolated in the Netherlands in 2013.

The occurrence of Fusarium infection in flowers and fruit was compared (Fig. 1). Statistically, no relationship was observed between the incidence of flowers infected by *Fusarium* spp. and the incidence infection of small green fruit infected. The level of flower infection accounted for only 19.4% of the variance in the level of fruit infection.

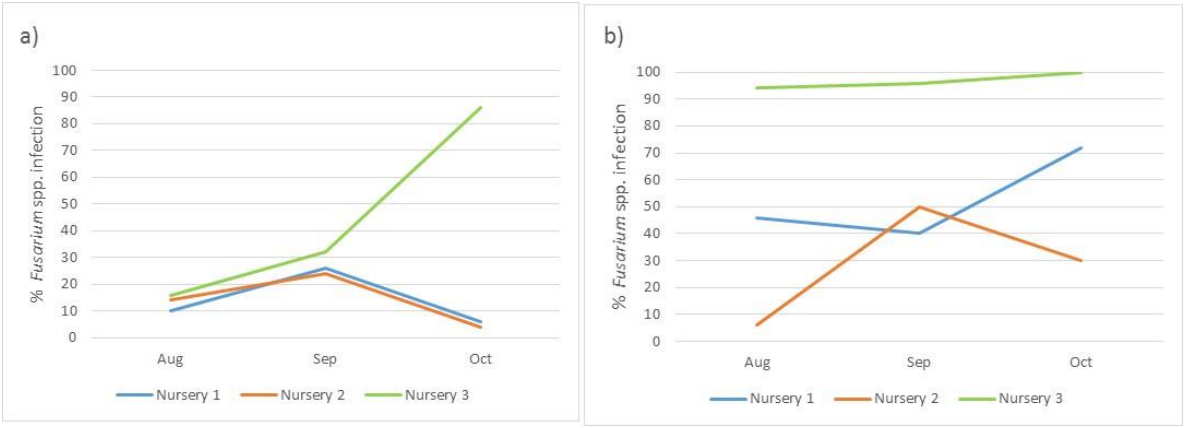

Figure 1. Levels of *Fusarium* spp. present in: a) fully open flowers; b) in small fruit developed from the same cohort of flowers

Though a large percentage of small fruit had *F. lactis* present, discussion with the growers indicated this infection was not carried through to grade-out in the packhouse or supermarket rejections. Therefore, it is possible that development of the infection to visible symptoms is slow, and/or that the plant-pathogen interaction is dependent on environmental conditions. In 2012, it was found that small, brown, aborted fruit had 100% *F. lactis* infection, and it follows that many of the small green fruit with high levels of infection may not have persisted in the crop through to harvest.

Financial Benefits

The development of a robust platform for future collaborative, joint-funded work with Dutch and/or Belgian growers on protected edible crop diseases is likely to increase knowledge on pepper Fusarium internal fruit rot through coordinated work with Dutch and Belgian researchers. Pooling resources and knowledge will also ensure that research is more cost effective, and not repeated unnecessarily. Development of an effective method to predict risk of Fusarium fruit rot through measurement of flower infection would provide the potential to apply targeted treatments to reduce Fusarium internal fruit rot through use of biofungicides and fungicides in periods when flower infection is increasing/high. However, results in this project indicate that a simple relationship is unlikely and that a high percentage of flowers and fruit may be infected with F. lactis, and yet relatively few fruit develop visible rot. It is suggested that future work seeks to confirm the above findings and examine factors that may influence the transition from latent infection to visible rot in mature fruit.

Action Points

- Consider preventative applications of plant protection products according to glasshouse environmental conditions (optimum temperature 22-30°C, high relative humidity) reported most favourable to the disease.
- Keeping rows, floors and slabs as clean as possible of crop debris to prevent *F. lactis* growing and sporulating is good hygiene practice and may reduce the inoculum present in the glasshouse and thereby reduce disease risk. However, evidence for the effect of good crop hygiene on this disease is conflicting.
- Further work is required to investigate aspects of the disease biology which are not well understood, including for example: a) can seed infection result in growth of *F. lactis* on rockwool cubes; b) if and by what mechanism does *F. lactis* on rockwool cubes result in flower infection; c) what factor(s) determine the transition of *F. lactis* from a latent infection in developing fruit to a fruit rot; d) can plant manipulation (e.g. nutrition, resistance inducers) be used to delay development on Fusarium rot in fruit; e) the role of *Cladosporium* and other fungi occurring naturally in flowers on the ultimate incidence of Fusarium fruit rot.

SCIENCE SECTION

Introduction

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide for approximately the last 14 years. 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 packhouses and product returns from supermarkets. Losses vary greatly between crops and seasons. Several weakly pathogenic *Fusarium* species are associated with the disease, notably *F. lactis* and *F. oxysporum*. Work in Canada showed that these pathogens are 'weak' in the sense that symptoms of disease (fruit rot) do not appear immediately following infection, and sometimes not at all (Yang *et al.,* 2010). *Fusarium* spores deposited on the stigma during flowering grow down through the style resulting in infection of seeds and the internal fruit wall (Yang *et al.,* 2010). Work in PE 007 demonstrated that a single spray of Serenade ASO applied to a crop during flowering can reduce the incidence of infection in fruit developing from treated flowers by around 50%. The optimum time to apply sprays of plant protection products during the year is unknown. The potential benefit of treating rockwool cubes and crop debris is unknown. The harvest period over which a single spray to flowers provides continued protection is unknown. This project aims to reduce losses to Fusarium internal fruit rot through: agreed information exchange and a joint work programme with Dutch/Belgian researchers; examination of rockwool cubes as a source of *F. lactis* and *F. oxysporum*; devising experiments to determine if the level of flower infection can be used to predict risk of fruit infection; communication of results to growers.

Materials and methods

Objective 1 - To liaise with the Dutch and Belgian researchers working on Fusarium internal fruit rot, exchange information and develop a coordinated, applied research programme

Following previous conversations regarding the study of Fusarium rot of pepper, Dr Jantineke Hofland-Zijlstra, Wageningen UR Glastuinbouw, was contacted in early 2014 and a number of conference calls set up to establish the progress of ongoing research in the Netherlands, the potential for results sharing and collaboration of research efforts in the 2015 cropping season.

Objective 2 - To examine rockwool cubes as a source of *F. lactis* and/or *F. oxysporum*

Three nurseries in the Lea Valley, Essex were visited three times for rockwool cube sampling during the growing season. Nurseries were visited on 11th August, 8th September and 6th October 2014. At each visit to sample flowers, a site visit sheet was completed detailing the weather surrounding the sample date, and the quality of flower setting present in the nursery (Appendix 1). Samples were taken from areas of pepper crop containing varieties of pepper known to be susceptible to Fusarium internal fruit rot, or from glasshouses with a history of the disease (Table 5).

Rockwool cubes were assessed and sampled over five rows of crop, using the same five rows at each visit. In the nursery, 50 rockwool cubes along each row were assessed for the presence or absence of fungal growth on their surface (Figure 2). The assessment started two slabs into a row, and cubes on each row were assessed as having either no visible fungal growth, suspect fungal growth, or definite fungal growth.

Rockwool was sampled from these crop rows, pulling off a small piece (a pinch between thumb and index finger) from the upper surface of 20 cubes showing no visible fungal growth, and 20 cubes showing suspect or definite fungal growth. In each nursery, these were collected in separate grip seal, polythene bags, and were brought back to the pathology laboratory at ADAS Boxworth. Samples with no visible growth were collected first at each visit.

Figure 2. An extreme example of a rockwool cube that was assessed and sampled as having fungal growth present – Nursery 2, Essex, 2014

Rockwool pieces were arranged in clean plastic containers, not touching, and were damp-incubated at room temperature for 7 days to encourage fungal growth (Figure 3). Pieces were then plated onto Potato Dextrose Agar + Streptomycin (PDA+S) plates. Plates were assessed for fungal growth after 7 days of incubation at 22°C. At assessment, plates were scored for growth of *Fusarium* spp. that were likely to be *F. lactis* or *F. oxysporum*, and the presence of bacteria, *Penicillium* spp. and other fungi such as *Trichoderma* or *Colletotrichum* spp. were recorded. Segregation of *Fusarium* species prior to sequencing was based on colony colour and microscope examination. A representative set of isolates were sent to Dr John Clarkson at Warwick Crop Centre for identification by PCR.

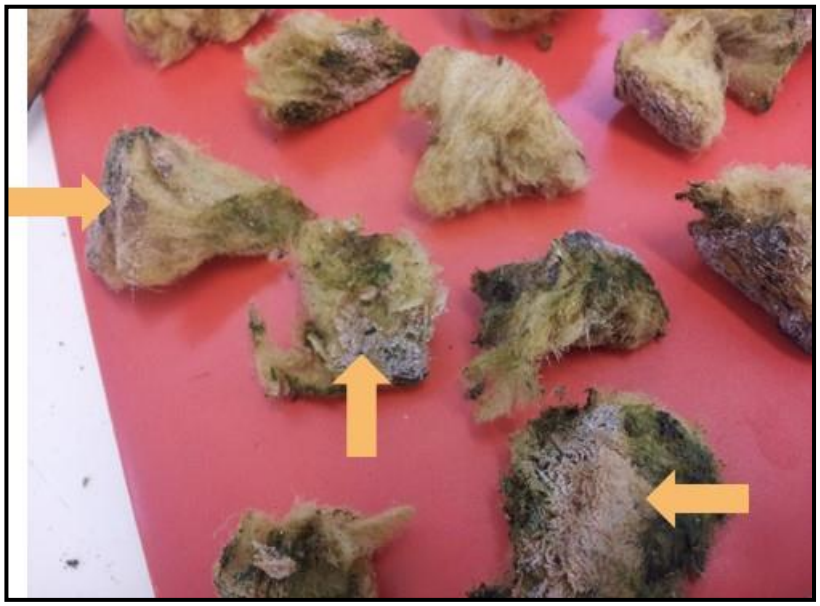

Figure 3. Sampled rockwool cube pieces after incubation showing pinkish/off-white sporulation of probable *Fusarium* spp.

Objective 3 - To examine the relationship between flower infection by *Fusarium* spp. and Fusarium infection in small fruit

The same nurseries as used in Objective 2 were used in Objective 3. Nurseries were visited on 11th August, 7th September and 6th October 2014, for flower sampling, and again after 2 weeks on 26th August, 22nd September and 20th October for fruit sampling. Samples were taken from areas of pepper crop containing varieties of pepper known to be susceptible to Fusarium internal fruit rot, or from glasshouses with a history of the disease (Table 5).

Table 5. Varieties of pepper and history of Fusarium internal root rot on nurseries, 2014

Nursery	Variety	Nursery history of Fusarium internal fruit rot
1	Jorrit	More towards end of house, and from certain propagators
2	Nagano RZ	Very little
3	Cupra	More than in previous years due to high humidity

Fifty visibly healthy, fully open flowers were sampled along a single row at each visit, and any flowers with discolouration or wilting were avoided. Flowers from the top of the crop were favoured, if agreeable to the grower. In some instances flowers had to be taken from lower in the crop if those at the top were too few in number. The same row was sampled at each nursery throughout the project. Flower sample 3 at Nursery 1 was across numerous rows in order to achieve a sample of 50 flowers, as the top of the crop had already been removed. If possible, flowers from side shoots were favoured over those on the main stem, so that the fruit loss to the grower could be negated. Flowers were collected in grip seal polythene bags and brought back to the ADAS Boxworth pathology laboratory. Before leaving each nursery, ten flowers remaining in the sampled row were tagged with jewellery tags labelled with the date, so that subsequently, fruit that had developed from this flower cohort could be recognised.

On arrival in the pathology laboratory, the flowers from each nursery were arranged on moist paper towel in clean plastic boxes so that they were not touching one another, and were damp incubated for 24 hours at 22°C. Following this incubation period, the style and some stamens were removed from each flower, surface sterilised in a 2% solution of sodium hypochlorite for one minute, and placed onto PDA+S agar plates. These plates were then incubated at 22°C for 7 days, after which they were assessed.

At assessment, plates were scored for growth of *Fusarium* spp. that were likely to be *F. lactis* or *F. oxysporum* from colony appearance, and the presence of bacteria, *Penicillium* spp. and other fungi such as *Botrytis* spp. were recorded (Appendix 2).

Figure 4. Fully open flower left in crop tagged with flower sampling date (left); Small green fruit 2 weeks later, tagged with flower sampling date (right) – Nursery 3, Essex, 2014

At visits to sample small green fruit 2 weeks later, the ten jewellery tags left on flowers in the appropriate row were used to determine the correct size of fruit to sample (Figure 4), so that it would correspond to the flower set sampled at the previous visit. Fruit were collected in grip seal, polythene bags and brought back to the ADAS Boxworth pathology laboratory. Fruit from each nursery were laid out, not touching one another, on trays on tissue paper, and were left to dry out (Figure 5). Two weeks after sampling, the fruit that had dried sufficiently, and turned brown in colour were surface sterilised in 2% sodium hypochlorite for one minute, and were plated onto PDA +S. After a further two weeks, one month after sampling, isolations were taken from all remaining fruit, whether they had become dry and brown or not. These isolations were assessed after seven days of incubation at 22°C. At assessment, plates were scored for growth of *Fusarium* spp. that were likely to be *F. lactis* or *F. oxysporum*, and the presence of bacteria, *Penicillium* spp. and other fungi such as *Botrytis* species were recorded. A representative set of isolates were sent to Dr John Clarkson at Warwick Crop Centre for identification by PCR.

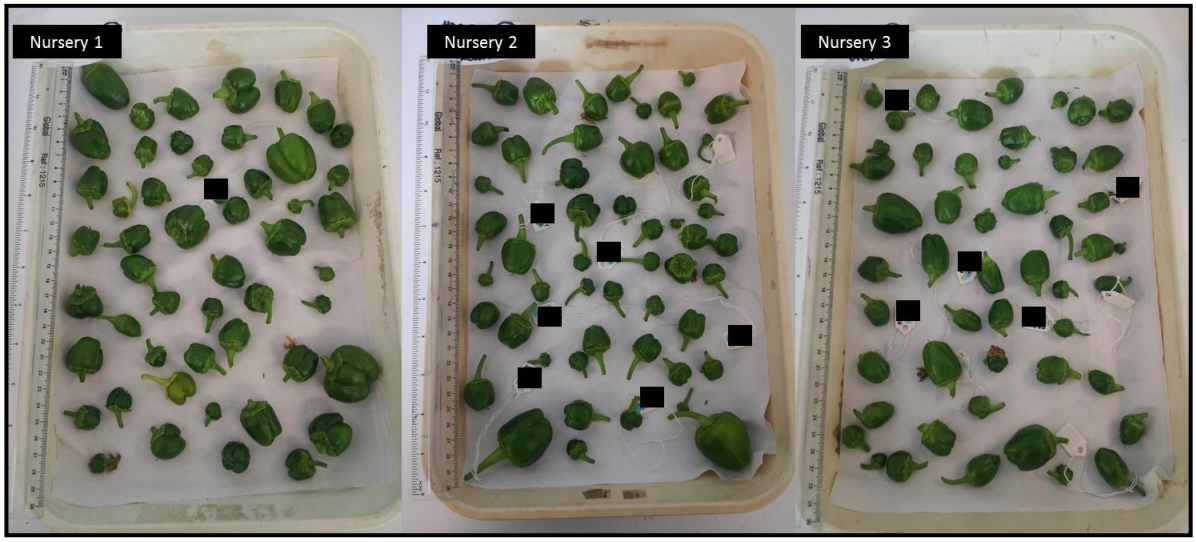

Figure 5. An example of sampled small green fruit as left to dry out; labels blacked out – ADAS, 2014

Results and discussion

Objective 1 - To liaise with the Dutch and Belgian researchers working on Fusarium internal fruit rot, exchange information and develop a coordinated, applied research programme

Work carried out on Fusarium internal fruit rot at Wageningen University in 2014 involved closely monitoring six commercial nurseries. A lower incidence of Fusarium internal fruit rot was observed in 2014 than in 2013. Trials on small pepper plants and detached flowers were also carried out. The key results from this work are summarised below.

Glasshouse environment – observations from crop monitoring

- Hygiene was found to be important in reducing Fusarium internal fruit rot, but the most hygienic grower did not have the lowest levels in fruit
- Increasing humidity, for example with the use of screens, was associated with increased levels of Fusarium fruit rot
- Avoiding the dew point appears to be key to avoidance of internal fruit rot; lower night temperatures were associated with increased fruit rot
- Post-harvest, a higher temperature increases development of Fusarium internal fruit rot
- It was found that *Fusarium* spores were always present in flowers over the growing season

- Fusarium internal fruit rot occurred on all the commercial sites monitored – it was suggested it is not a case of avoiding the disease, but retarding its development for as long as possible
- *Fusarium* has been found to survive in water, Dutch work recommends the use of UV-C and heat treatments to disinfect irrigation water

Plant protection products

- Growers who used a *Trichoderma* product (Trianum P), either applied at propagation or in the irrigation water generally had lower levels of Fusarium internal fruit rot
- Application of Serenade ASO to flowers, prior to artificial inoculation with a concentration of $1x10^4$ *Fusarium* spores per ml, reduced internal fruit rot by 50%. When a higher concentration of inoculum ($1x10^6$ *Fusarium* spores per ml) was used, no significant differences were observed
- Growers who applied hydrogen peroxide and sodium hypochlorite through drippers or directly onto plants (neither of which is an approved plant protection product in the Netherlands of the UK) generally had less internal fruit rot
- Spraying floors, rockwool and pathways with hydrogen peroxide also appeared to affect internal fruit rot incidence, but results were variable
- 1% hydrogen peroxide caused no damage to flowers when applied as a foliar spray and was effective in preventing Fusarium internal fruit rot up to 3 hours after inoculation
- Applying plant protection products weekly may not be sufficiently frequent, as germinating spores of *Fusarium* can grow down the flower style in approximately 3 hours

Nutrition

- Drip and run-off were measured in a glasshouse trial to effectively calculate nutrient uptake by the crop
- Some aspects of pepper nutrition appear to influence incidence of internal fruit rot, e.g. increased molybdenum was associated with less fruit rot

Work in 2015 will concentrate on screening for natural immune response (through measurement of salicylic acid and jasmonic acid) and investigate the effect of climate conditions on plant quality (inherent basal resistance).

Dr Jantineke Hofland-Zijlstra and her research team secured funding in early 2015 for further work on *F. lactis*, and it has been agreed that results of this research, and of our HDC funded work will be shared. The research programme has been discussed to avoid repeating work, and to make the work complementary where possible. Proposed work in the Netherlands will run for 4 years, from 2015 to 2018, with a break point at the end of each year. The five areas the work will focus on are:

1. Screening for robust, disease resistant, plant material
2. Hygiene practices to reduce *Fusarium lactis* inoculum in glasshouses
3. Effect of climate, nutrition and substrate on *F. lactis* development
4. Salicylic acid/jasmonic acid (SA/JA) inducers, biological control and endophytes for control of *F. lactis*
5. Curative treatments

Objective 2 - To examine rockwool cubes as a source of *F. lactis* and/or *F. oxysporum*

From the in-crop assessments of fungal growth present on rockwool cubes, it became clear that Nursery 2 had greater amounts of visible fungal growth than Nurseries 1 or 3 (Table 6). After incubation at high humidity, it was shown by microscope examination that the fungal growth on rockwool pieces from all three nurseries was producing microconidia and microconidia suggestive of *Fusarium* species. The in-crop assessments did not match the results from the fungal isolations completely, as the slabs sampled from Nursery 2 had an incidence of *Fusarium* spp. comparable to Nursery 3, and lower than Nursery 1 (Table 7 and Figure 6) rather that being the highest of the three nurseries.

Results show that although *F. lactis* is capable of growing and sporulating on rockwool slabs in UK nurseries, any visible sporulation may not always be *F. lactis*. Visible sporulation on the rockwool slab may be more closely linked to glasshouse humidity and/or cleanliness than occurrence and severity of *F. lactis* on rockwool.

Table 6. Average occurrence of visible fungal growth on five rows of rockwool in three pepper crops – 2014, Essex

Assessment date	Presence or absence of fungal growth (%)								
	Nursery 1			Nursery 2			Nursery 3		
	Definite	Suspect	None	Definite	Suspect	None	Definite	Suspect	None
11 Aug	6	18	76	21	33	46	2	14	84
8 Sep	4	12	84	42	27	31	10	21	69
6 Oct	6	16	78	34	32	34	17	15	68

Following assessment of the cultures growing from the sampled pieces of rockwool, it became apparent that at the initial sampling, Nursery 3 had a lower incidence of *Fusarium* spp. growing on rockwool slabs. In September, incidence increased at Nursery 3 to a level more comparable with Nursery 2. At this point, the incidence of *Fusarium* spp. found on slabs at Nursery 1 peaked at 70% of those sampled, the highest slab incidence recorded in this study. Incidence of *Fusarium* spp. found in slabs dropped to the lowest recorded at each site throughout the season at all three nurseries in October. The levels of viable pathogen present on/in rockwool cubes could be linked to environmental conditions such as moisture, temperature and humidity, with changes at the end of the growing season as heating is reduced. Notably, in September, the dematiaceous, endophytic fungus *Chaetomium globosum* was isolated from every piece of rockwool sampled from Nursery 2. Levels of *F. lactis* fell in September in Nursery 2, whilst they increased everywhere else, and a natural biocontrol effect may have been observed.

Table 7. Incidence of *Fusarium lactis* / *F. oxysporum* recovered from rockwool cubes sampled from three nurseries in Essex – 2014

Incidence of suspect *F. lactis* (% cubes affected) from rockwool pieces with or without visible fungal growth on three pepper crops			
Date	Nursery 1	Nursery 2	Nursery 3
11 August			
Cubes sampled with suspect *Fusarium*	55	35	30
Cubes sampled with no fungal growth	10	40	15
All cubes sampled	32.5	37.5	22.5
8 September			
Cubes sampled with suspect *Fusarium*	75	10	35
Cubes sampled with no fungal growth	65	55	40
All cubes sampled	45	32.5	37.5
6 October			
Cubes sampled with suspect *Fusarium*	5	0	15
Cubes sampled with no fungal growth	15	0	5
All cubes sampled	10	0	10

There was no sustained link between observable fungal growth on cubes at the nurseries and the amount of *Fusarium* spp. recovered on agar (Figure 6).

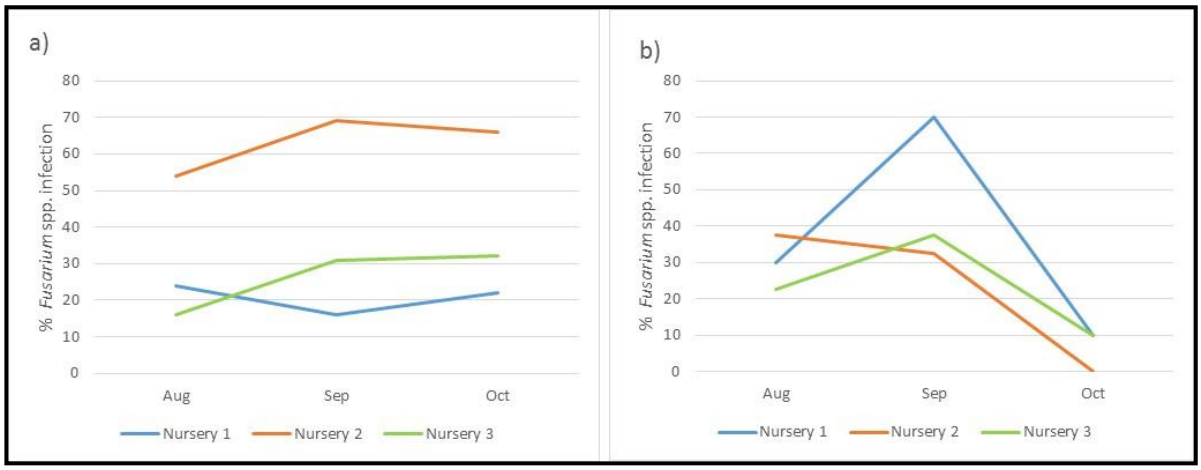

Figure 6. a) Suspected fungal growth on rockwool cubes in the nursery; b) Growth of *Fusarium* spp. from rockwool on agar plates

Additionally, when the incidence of *Fusarium* species recovered from rockwool cubes was compared to the incidence of *Fusarium* spp. recovered from flowers (Figure 7) there was no evidence of a link between the two sets of data. A regression analysis of presence in rockwool cube vs flower infection found no significant correlation between them (P=0.848).

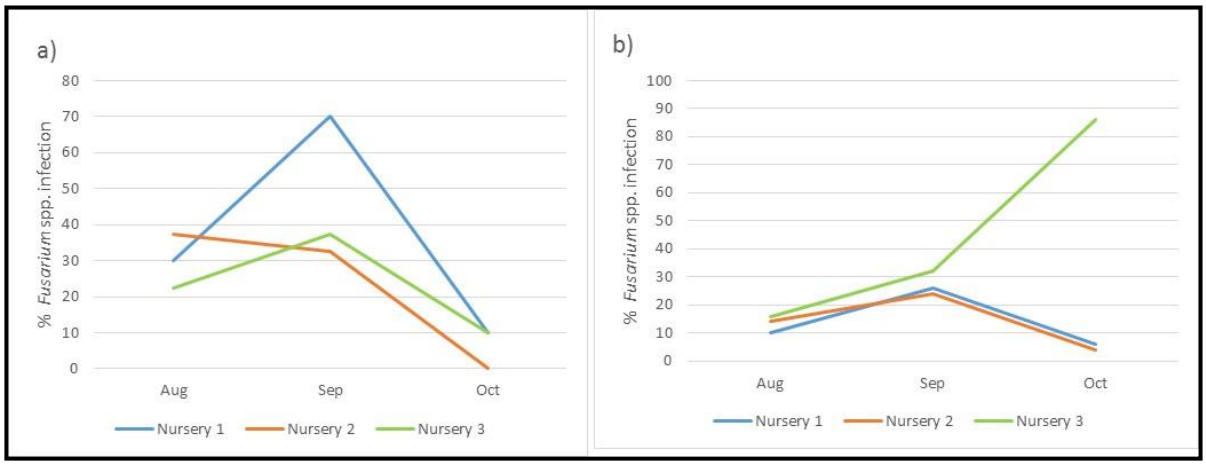

Figure 7. a) Growth of *Fusarium* spp. from rockwool on agar plates b) Growth of *Fusarium* spp. from flowers on agar plates

The cultures isolated from slab samples taken from each of the three nurseries were identified to species level by Dr John Clarkson, Warwick Crop Centre (Table 8). A representative set of *Fusarium* spp. were sent, as well as some fungi that were consistently isolated from slab samples and that did not appear typical of *F. lactis* or *F. oxysporum*.

Table 8. Results of PCR tests to identify a representative sample of suspect *Fusarium* spp. isolates taken from rockwool slabs to species level by PCR tests – Warwick Crop Centre, 2014

Isolate number	Isolate code	Colony colour	Tissue isolated from	Origin nursery	Result
3	95a	Peach	Slab	Nursery 1	*F. equiseti*
4	95b	Peach	Slab	Nursery 1	*F. equiseti*
9	98	Orange	Slab	Nursery 2	*F .lactis*
10	121	Unknown	Slab	Nursery 2	*Chaetomium globosum*
15	101	Buff	Slab	Nursery 3	*F .lactis*
16	122	Yellow-brown	Slab	Nursery 3	*Ceratobasidium*

F. lactis was confirmed for two of the samples sent, but *Fusarium equiseti* was also recovered. *F. equiseti* has been recorded causing pepper fruit rots previously in Nigeria and causing a rot of tomatoes in Egypt (Adisa & Lekunze, 1986). *Chaetomium globosum* and *Ceratobasidium* spp. are not known plant pathogens, and live endophytically. *Chaetomium globosum* is currently being researched as a potential biocontrol agent (Soytong *et al.,* 2001) and plant growth stimulant (Khan *et al.,* 2012). The lower levels of *F. lactis* found on Nursery 2 could possibly be linked to the presence of this naturally occurring biocontrol agent.

Objective 3 - To examine the relationship between flower infection by *Fusarium* spp. and Fusarium infection in small fruit

A representative number of *Fusarium* isolates obtained from flowers and small fruit were sent for identification by PCR. The majority of *Fusarium* spp. isolated from flowers and fruit were *F. lactis*, though one isolate of *F. culmorum,* also a plant pathogen, was identified from flowers at Nursery 1 (Table 9).

Table 9. Results of PCR tests to identify a representative sample of isolates taken from flowers and small green fruit to species – Warwick Crop Centre, 2014

Isolate number	Isolate code	Colony colour	Tissue isolated from	Origin nursery	Result
1	96a	Pink	Flower	Nursery 1	*F. culmorum*
2	96b	Peach	Flower	Nursery 1	*F. lactis*
5	134	Peach	Fruit	Nursery 1	*F. lactis*
6	112b	Buff	Fruit	Nursery 1	*F. lactis*
7	138a	Peach	Flower	Nursery 2	*F. lactis*
8	138b	Peach	Flower	Nursery 2	*F. lactis*
11	135a	Peach	Fruit	Nursery 2	*F. lactis*
12	135b	Peach	Fruit	Nursery 2	*F. lactis*
13	102a	Peach	Flower	Nursery 3	*F. lactis*
14	102b	Peach	Flower	Nursery 3	*F. lactis*
17	136a	Peach-orange	Fruit	Nursery 3	*F. lactis*
18	136b	Peach	Fruit	Nursery 3	*F. lactis*

There was no clear pattern linking flower infection and fruit infection (Figure 8). At Nursery 1 incidence of *Fusarium* spp. in flowers had an inverse relationship with the incidence found in fruit (Table 10). The strongest link between flower incidence and fruit incidence was apparent at Nursery 2, but this was not emulated at the other sites. At Nursery 3 flower infection markedly increased over the season, whilst fruit infection remained at relatively constant, high levels (Table 10). *Cladosporium* species were commonly isolated (34% of flowers at sampling date 1; 50% at sampling date 2; 70% at sampling date 3) from flowers sampled from Nursery 2, but only occasionally at the other two sites. This suggests a possible biological control effect, as was suggested by Dutch researchers when it was consistently found in flowers in the Netherlands in 2013.

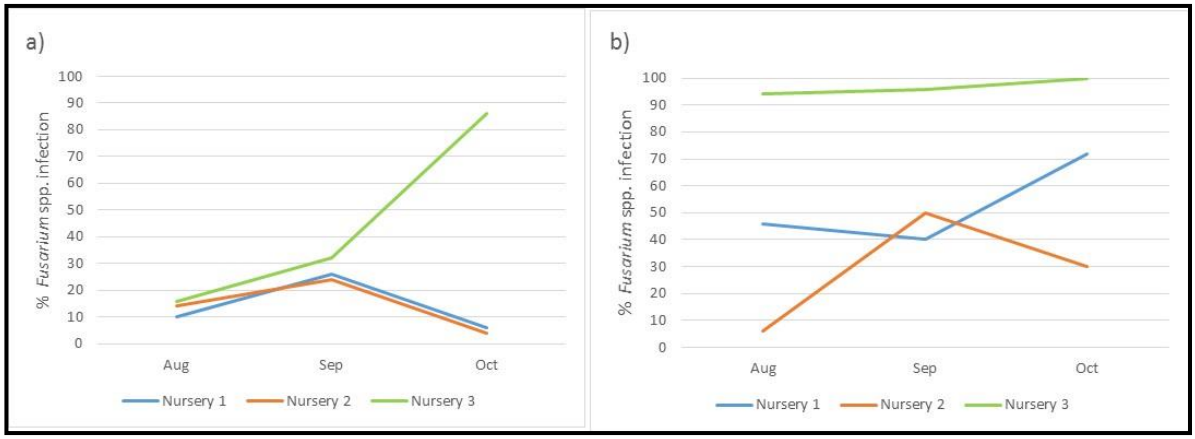

Figure 8. Levels of *Fusarium* spp. present in: a) fully open flowers; b) in small green fruit

Table 10. Incidence of *Fusarium* spp. (primarily *F. lactis*) isolated from flowers and small green fruit sampled over the 2014 growing season from three nurseries in Essex

Sampling date	*F. lactis* incidence (% infected) in pepper flowers and small green fruit		
Flowers	Nursery 1	Nursery 2	Nursery 3
11 Aug	10	14	16
8 Sep	26	24	32
6 Oct	6	4	86
Fruit	Nursery 1	Nursery 2	Nursery 3
26 Aug	46	6	94
22 Sep	40	50	96
20 Oct	72	30	100

A regression analysis of fruit infection vs flower infection found no significant correlation between them (P=0.131); flower infection accounted for only 19.4% of the variance in fruit infection.

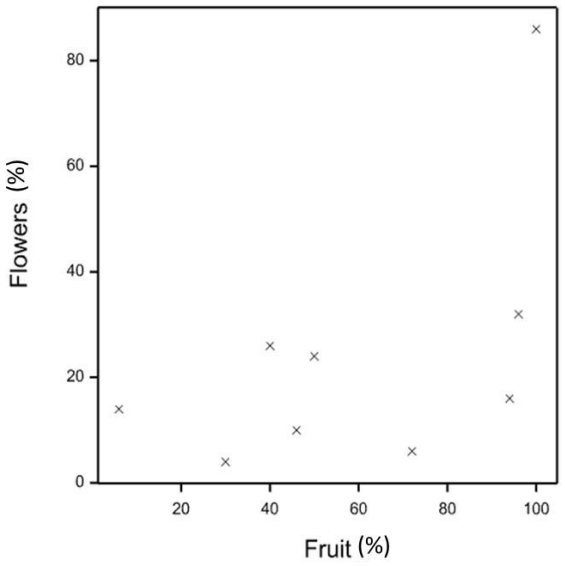

Figure 9. Scatter plot showing lack of statistical relationship between incidence of *Fusarium* spp. in flowers and incidence in from small green fruit – Lea Valley, 2014

General discussion

Fusarium species were successfully isolated from all tissue types and all nurseries at each sampling date. Initially, it was planned that fungal isolations from flower tissue would be performed by centrifuging washings of sterile distilled water from the sampled flowers. However, this was attempted with some preliminarily sampled flowers and the method was found to introduce too much contamination to plates in the form of bacteria, such that no *Fusarium* species were recovered. Following this, more conventional isolation techniques were used.

When assessing slabs on-site for visible fungal growth, as expected, it was not possible to determine if the growth was due to Fusarium lactis, another Fusarium species, or another fungal species altogether. In addition, the presence of algae and weeds such as ferns had a large effect on what fungal growth was observed on slabs – whether the algae was preventing fungal growth, or just preventing it from being visible is also unclear, as Fusarium was isolated from both slabs with obvious growth and those which appeared clean. Dutch experiments have found F. lactis to be capable of growth in anoxic conditions, up to 20% CO2, and able to survive for long periods outside a host plant (Hofland-Zijlstra, J, pers. comm.), so finding F. lactis where there appears to be no visible fungal growth is not surprising. The glasshouses sampled on Nursery 1 and Nursery 3 were kept very clean

and tidy, and crop waste was quickly removed from the glasshouse. The glasshouse sampled on Nursery 2 was older glass, and was generally less tidy, with waste fruit often present between rows. However, despite this, Nursery 2 generally had a lower incidence of Fusarium recovered from rockwool cubes. At sampling date 2, a Colletotrichum species was consistently isolated from rockwool cubes on this site, and there was always much more fungal growth present on the slabs when this was assessed. Algae and ferns were also more common on this nursery. Possibly there is a naturally occurring biocontrol effect acting on Nursery 2 to suppress levels of Fusarium compared to Nurseries 1 and 3 which were much cleaner and had less visible fungal growth on cubes; or possibly the difference between nurseries is associated with environmental conditions. This would be consistent with Dutch work where F. lactis has been found to be a relatively weak competitor.

Fusarium species, highly likely to be *F. lactis* as representative isolates were identified as such, were consistently isolated from rockwool cubes, flowers and small green fruit. This confirms the surface of rockwool cubes as a potential reservoir for inoculum in epidemics of Fusarium internal fruit rot in UK pepper glasshouses. However, the levels at which *Fusarium lactis* isolates were recovered did not show an obvious pattern. Rockwool, though possibly contributing to the spore load of *F. lactis* in glasshouses, must not be the only contributor to infection, as nurseries were still experiencing moderate to very high infection levels in fruit following periods (2 weeks earlier when fruit were at the flower stage) where none or very little *F. lactis* was being recovered from the cube surface. As such, it is likely that spores are arriving in glasshouses from sporulating infected fruit in the crop or fallen fruit on the floor or trapped in the crop, or possibly from the outside environment. In the case of Nursery 3, where the majority of fruit became infected, it is also likely that inoculum levels in the glasshouse was so consistently high that it became impossible to resolve any patterns or relationships present.

Interestingly, Fusarium sp., highly likely F. lactis, was found at a much higher incidence in fruits than in the flowers (8 out of the 9 paired samples). As flower infection is the only documented infection pathway leading to Fusarium internal fruit rot, it seems likely that this unexpected result may be due to a difference in recovery efficiency of Fusarium from flowers and fruit in our tests (e.g. possibly Fusarium is able to grow out more readily from the higher nutrient base of fruit walls than flower parts, and away from saprophytic competitor microorganisms suppressing outgrowth (e.g. Cladosporium spp.); or the cohort of flowers developing into young fruit was not tracked with sufficient accuracy.

The incidence of Fusarium internal fruit rot in the small green fruit sampled from all three nurseries was generally much higher than the incidence of fruit reported to have been rejected during grading. For example, 100% of small green fruit from Nursery 3 sampled in October produced *Fusarium* spp. on agar in our tests, but 100% of fruit picked from that cohort were not rejected because of Fusarium internal fruit rot. It is possible that *F. lactis* is present in all, or nearly all, fruit on heavily infected nurseries after invading through the flower. However, the fungus may need the correct environmental conditions, host physiology and/or time scale to cause visible fruit symptoms that would cause rejection. Additionally, some sampling bias may have influenced results, as we purposely sought areas of glasshouses with a history of problems with Fusarium internal fruit rot, and those with varieties known to have high susceptibility. Similarly, Nursery 2 had less of a problem with Fusarium internal fruit rot historically than Nurseries 1 and 3, so inoculum is less likely to have been carried over from a previous season.

Latent infection with *F. lactis* is an area Dutch work is focussing on, and has found temperature to be the most important determining factor in dictating whether latent infection will progress to a visible rot or not. Infection can enter through the pistil, and more commonly results from flowers with intact pistils compared to those where it is missing and only stamens remain.

Conclusions

- Coordinated research programmes across Europe are likely to improve our understanding of Fusarium internal fruit rot of pepper by allowing information sharing, and optimising resource use efficiency
- *F. lactis* is capable of growing and sporulating on the surface of rockwool cubes in UK glasshouses
- This on-cube sporulation may or may not be visible during crop walks, and any fungal growth that is apparent may not be *F. lactis*
- A high proportion of small green pepper fruit in crops in August-October may be infected by *F. lactis*
- No statistically significant relationship ($p>0.05$) was observed between the incidence of flower infection and the incidence of small green fruit infected by *Fusarium* sp.
- Results indicate that fruit may be infected with *F. lactis*, but may not develop symptoms of internal fruit rot

Knowledge and Technology Transfer

Mayne S & O'Neill TM (2015) Pepper: coordinated approach to control of Fusarium internal fruit rot. HDC News (in preparation)

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Appendices

Appendix 1 – Weather conditions and flower setting on nurseries when flowers were sampled

	Nursery 1	Nursery 2	Nursery 3
Weather conditions			
11 Aug	21°C, clear and sunny. Rainy and stormy over the preceding weekend. The rest of the week was sunny with a few showers.		
8 Sep	21°C, clear and sunny. A morning of 9°C but quickly warmed up. Cloudy and humid over the preceding weekend. Nice for the rest of the week.		
6 Oct	Cloudy and rainy on the days surrounding the sampling visit. A morning of 8°C, followed by breaking sunshine and a high of 20°C.		
Flower setting			
11 Aug	Light levels consistently good, setting spontaneously.	Very well.	Just had a very large fruit set, lots of flowers present but fewer than in Nurseries 1 and 2.
8 Sep	Very well, most flowers fully open and white.	Better than first visit, 3-4 flowers per plant. Flowers fully open, white and healthy looking. Many lateral shoots remain.	Fewer flowers than Nurseries 1 and 2. About the same as the first visit. Not every plant had a flower at the suitable stage.
6 Oct	Tops removed from crop– finding 50 flowers proved difficult. Crop had missed a number of sprays and looked in bad condition, lots of blossom end rot/rotten fruit on plants.	Really well, lots of flowers and smaller fruit. Many lateral shoots remain.	Really well, more than previous visits.

Appendix 2 – Record of fungal species found associated with rockwool cubes, pepper flowers and fruit – 2014

	Nursery 1	Nursery 2	Nursery 3
Rockwool			
11 Aug	*Penicillium* (H)	*Penicillium* (M)	*Penicillium* (M)
	Bacteria (L)	*Bacteria* (M)	*Bacteria* (M)
	Trichoderma (L)	*Trichoderma* (L)	*Trichoderma* (M)
	Botrytis (M)	*Botrytis* (M)	*Botrytis* (L)
	Mucor/Rhizopus (L)	*Mucor/Rhizopus* (L)	
8 Sep	*Penicillium* (M)	*Penicillium* (M)	*Penicillium* (M)
	Bacteria (M)	*Bacteria* (M)	*Bacteria* (M)
	Trichoderma (L)	*Trichoderma* (L)	*Cladosporium* (L)
	Pythiacious spp. (M)	*Botrytis* (L)	*Mucor/Rhizopus* (L)
	Mucor/Rhizopus (M)	*Cladosporium* (L)	*Pythiacious* spp. (H)
	Botrytis (L)	*Pythiacious* spp. (M)	
	Cladosporium (L)		
6 Oct	*Penicillium* (M)	*Penicillium* (M)	*Penicillium* (M)
	Bacteria (M)	*Bacteria* (M)	*Bacteria* (M)
	Trichoderma (H)	*Trichoderma* (H)	*Trichoderma* (M)
	Pythiacious spp. (H)	*Mucor/Rhizopus* (M)	*Mucor/Rhizopus* (L)
	Cladosporium (L)	*Pythiacious* spp. (L)	*Cladosporium* (L)
		Cladosporium (L)	*Pythiacious* spp. (M)
Flowers			
11 Aug	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Bacteria (L)	*Bacteria* (L)	*Bacteria* (L)
	Botrytis (L)	*Cladosporium* (H)	*Cladosporium* (L)
	Cladosporium (L)	*Botrytis* (L)	*Botrytis* (L)
		Yeast (L)	

8 Sep	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Bacteria (L)	*Bacteria* (L)	*Bacteria* (L)
	Yeast (L)	*Cladosporium* (H)	*Cladosporium* (L)
	Cladosporium (L)	*Botrytis* (L)	*Botrytis* (L)
6 Oct	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Bacteria (M)	*Bacteria* (L)	*Bacteria* (L)
	Cladosporium (L)	*Cladosporium* (H)	*Botrytis* (M)
	Botrytis (L)	*Botrytis* (M)	*Cladosporium* (M-H)
			Mucor (L)
Fruit			
26 Aug	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Bacteria (L)	*Bacteria* (L)	*Bacteria* (L)
	Botrytis (L)	*Cladosporium* (M)	*Trichoderma* (L)
	Trichoderma (L)	*Trichoderma* (M)	*Botrytis* (L)
	Mucor (L)	*Botrytis* (M)	
	Yeast (L)	*Mucor/Rhizopus* (L)	
22 Sep	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Bacteria (L)	*Bacteria* (L)	*Bacteria* (L)
	Botrytis (L)		*Cladosporium* (L)
			Botrytis (L)
20 Oct	*Penicillium* (H)	*Penicillium* (H)	*Penicillium* (H)
	Mucor (H)	*Bacteria* (L)	*Cladosporium* (L)
		Yeast (L)	
		Cladosporium (L)	
		Botrytis (L)	

L- low; M-moderate; H-high frequency of recovery