



Grower Summary

PE 022

Pepper: Improved control of Fusarium

Final 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.

AHDB Horticulture is a Division of the Agriculture and Horticulture Development Board.

Project Number: PE 022

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 Leader: Dr Tim O'Neill, ADAS

Contractor:

Industry Representative: Gary Taylor, Valley Grown Nursery

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Previous report/(s): None

Start Date: 01 August 2014

End Date: 31 March 2015

Project Cost: £20,619.00

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 occurring 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 <i>F. lactis</i> (% cubes affected) from rockwool pieces with or without visible fungal growth on three pepper crops					
	Nursery 1		Nursery 2		Nursery 3	
	Suspect <i>Fusarium</i>	No fungal growth	Suspect <i>Fusarium</i>	No fungal growth	Suspect <i>Fusarium</i>	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 <i>F. lactis</i> 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	<i>F. lactis</i> 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.

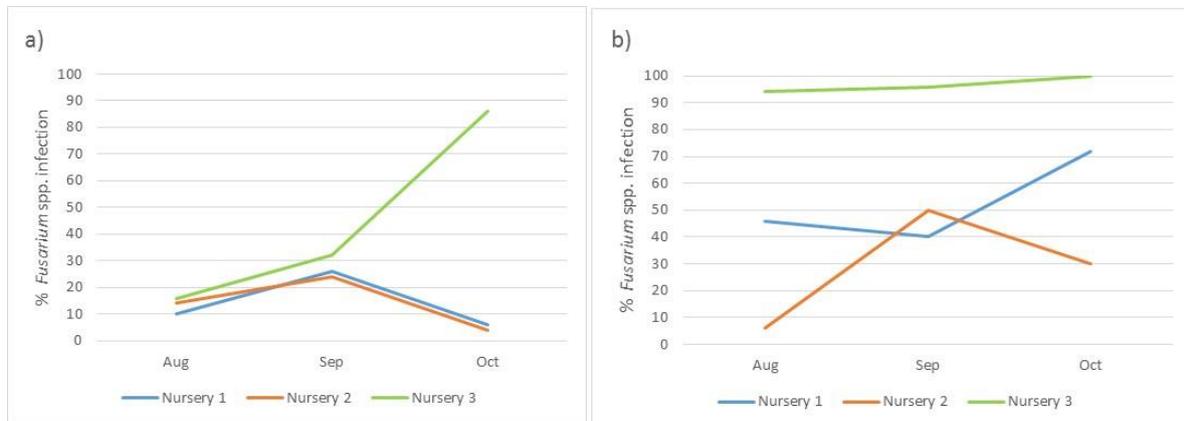


Figure 1. Levels of *Fusarium* spp. present in: a) fully open flowers; b) in small fruit developed from the same cohort of flowers

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.