Grower Summary

PE 022a

Pepper: Improved control of Fusarium internal fruit rot through increased knowledge exchange with the Netherlands and targeted application of plant protection products – phase 2

Annual report, March 2016

Project title:	Pepper: Improved control of Fusarium internal fruit rot through increased knowledge exchange with the Netherlands and targeted application of plant protection products – phase 2
Project number:	PE 022a
Project leader:	Dr Tim O'Neill, ADAS, Cambs CB23 4NN
Report:	Annual report, March 2016
Previous report:	None
Key staff:	Sarah Mayne, ADAS Jonny Kerley, ADAS
Location of project:	ADAS Boxworth, commercial sites – Herts & Somerset
Industry Representative:	Neal Ward, Cantelo Nurseries, Bradon Farm, Isle Abbots, Somerset, TA3 6RX
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The results and conclusions in this report are based on an investigation conducted over a nine month period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

- Nursery trials confirm Serenade ASO can reduce Fusarium internal fruit rot; there was a trend for greater reduction with three sprays than with a single spray.
- There was evidence that a treatment benefit persists after the final spray.

Background

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide, including the UK, for the last 15 years. 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. We have shown several weakly pathogenic *Fusarium* species are associated with the disease, notably *F. lactis* and *F. oxysporum* in the UK. Fusarium spores deposited on the stigma during flowering grow rapidly through the style resulting in infection of seeds and internal fruit wall. 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%. In PE 022 (phase 1) we showed *F. lactis* commonly occurs on rockwool propagation blocks in production glasshouses, a previously unknown source of the fungus. We also found that a high proportion of flowers and young fruits (1-2 cm diameter) in commercial crops were infected with *F. lactis*, yet only a relatively small proportion of fruit develop internal fruit rot. Results from 2014 and plans for 2015 were shared between the pathology teams at Bleiswijk Research Station (Wageningen University) and ADAS.

This project aims to reduce losses to Fusarium internal fruit rot. Specific objectives in Year one were:

1. Continued information exchange and discussion on the disease with Dutch researchers;
2. Examination of pepper seeds as a source of *F. lactis* and *F. oxysporum* that leads to growth of the fungi in rockwool propagation cubes;
3. Determining the reduction in fruit infection provided by one and several applications of Serenade ASO to a crop row, cube surface and floor;
4. Determining if use of biopesticides / plant resistance inducers applied preventatively provide protection to flowers and/or fruit against infection and/or fruit rot development;

5. Monitoring occurrence of *F. lactis* in flowers, young fruit and mature fruit in an organic pepper crop and a conventional crop.

Objectives 1 and 3 were completed in this first reporting year (July 2015 – March 2016). Objectives 2, 4 and 5 commenced in Year 1 and will conclude in Year 2.

Summary

1. Information exchange with Dutch researchers

Information was exchanged with Jantineke Hofland-Zijlstra, Plant Pathologist at Wageningen UR Greenhouse Horticulture in Bleiswijk, Glasshouse Crop Research Station, as well as hosting a visit to ADAS Boxworth and two pepper growers in the Lee Valley to facilitate discussion on the disease.

Fusarium internal fruit rot remains a major concern in the Netherlands. In 2014 it was found that incidence of the disease can vary greatly between individual crops, including between crops of the same variety on different nurseries. From detailed monitoring of growers' crops and practices, factors that appeared to reduce the disease were: hygiene, reduced humidity (by careful use of screens), avoiding dew point, cool storage of fruit post-harvest, use of Trianum P and Serenade ASO and increased molybdenum nutrition.

In 2015, levels of Fusarium internal fruit rot in Holland were generally lower than in previous years. Experimental work at Bleiswijk in 2015 showed a reduction in Fusarium infection in young fruit with Serenade ASO and antagonism of *F. lactis* by an experimental product.

Growers in the Netherlands were interested in measuring spore levels of Fusarium in a crop to help assess risk of fruit rot. In 2016 work is commencing to examine Fusarium spore loads on bumble bees introduced into the crop to assess risk of Fusarium fruit rot; the bees are washed to remove spores and then released. Other areas being examined this year are the effect of plant water relations on fruit susceptibility, and further experiments on antagonists and use of Serenade ASO.

2. Pepper seeds as a source of *F. lactis*

When Fusarium internal fruit rot first emerged as a problem around 2000, examination of commercial seed lots revealed that some were infected with a low level of *F. lactis* and other *Fusarium* species (see AHDB Horticulture Project PC 260). It was also shown that the sodium hypochlorite seed treatment greatly reduced seed infection. The aim of work in this project was i) to determine if there is any evidence that *Fusarium* continues to be a potential source of infection in a crop and ii) to determine if seed infected with *F. lactis* results in growth of the

fungus on rockwool cubes (in PE 022 we showed that *F. lactis* was present on the surface of rockwool cubes in some commercial crops).

Commercial seed lots of five varieties were obtained from two seed companies in July 2015. One lot had been treated with thiram and the others appeared untreated. Seeds were plated either directly or after surface disinfection in sodium hypochlorite onto a fusarium selective agar growth medium. When plated directly, many seeds of all varieties were found to be contaminated with saprophytic fungi, especially species of *Aspergillus* and *Penicillium*; some were contaminated with *Mucor* and bacteria. *Fusarium* species grew out from seeds of two of the varieties, at a low incidence ($^{5}/_{300}$ and $^{1}/_{300}$). The appearance of these fungi in culture was not typical of *F. lactis*. Isolates are currently being tested by PCR to determine species identity. When plated after surface disinfection in sodium hypochlorite, there was no fungal or bacterial growth from the majority of seeds of all batches.

In order to examine transfer of *F. lactis* from seeds to rockwool, a batch of seeds was contaminated with the fungus by soaking them in a spore suspension followed by drying them back. When plated onto agar, *F. lactis* grew out from all seeds. The artificially contaminated seed and uncontaminated seed were sown in rockwool plugs, resultant seedlings were transferred to rockwool cubes, and plants grown on in a glasshouse. This experiment is continuing and will be reported in greater detail in the final report.

To further examine the possible introduction of *F. lactis* into glasshouses at planting, two nurseries were visited within 24 h of plant delivery on 12 January 2016 and samples of rockwool were collected from the edge of cubes from 6 crops. Additionally, at a propagation nursery, plants of cv.s Ferrari and Fiesta were grown specifically for this project and the edges of rockwool cubes were sampled when plants were ready for dispatch in early March. From the commercial nursery samples, *Fusarium* sp. grew out of rockwool pieces from one out of three varieties at site one, and from two out of three varieties at site two at incidences of 2, 22 and 48% respectively. The work on propagation nursery samples is continuing and will be reported next year. A sample of the suspect Fusarium isolates obtained from rockwool were examined under the microscope and confirmed as Fusarium sp. No colonies were obtained in clean culture so it was not possible to determine if any were *F. lactis* by PCR test.

3. Efficacy of Serenade ASO sprays in reducing Fusarium internal fruit rot

In July 2015 a trial was established in a commercial crop of cv. Cupra to determine the effectiveness of a single spray of Serenade ASO and three sprays of the product applied at weekly intervals in reducing Fusarium infection in fruit, compared with an untreated control. Serenade ASO mixed with Codacide was applied to the crop face as a fine mist in a single pass with a boom sprayer and to the pathway and slab surface using a lance. At weekly

intervals for five weeks after the first spray application, 90 small green fruit were sampled per plot and examined for Fusarium infection. Additionally, at weekly intervals for five weeks after the flowers at the first spray timing had developed into harvestable fruit, all fruit in each plot were examined to determine the proportion with external symptoms of Fusarium internal fruit rot; and 50 marketable fruit per plot were incubated at ambient temperature in the laboratory for one week (to enhance Fusarium development, where present) and then destructively assessed for Fusarium internal fruit rot; and all unmarketable fruit per plot (e.g. misshapen; small size) were similarly assessed.

In small green fruit, the incidence of Fusarium for the fourth and fifth samples combined was significantly reduced ($p<0.05$) by three sprays of Serenade ASO to 6.4%, compared with 18.7% infection in untreated plots. A single spray of Serenade ASO appeared to give a slight reduction, to 16.9%, compared with untreated plots. Levels of Fusarium were nil or virtually nil in harvests one to three, and reached 21.5% and 14.4% in fruit from untreated plots at harvests four and five respectively.

In mature fruit, for the five harvests combined, there was a trend for a reduced incidence of Fusarium infection in the incubated marketable fruit (6.9, 5.0 and 3.2% infection in untreated, Serenade ASO x one and Serenade ASO x three respectively). The proportion of fruit at harvest with external symptoms of Fusarium internal fruit was so low that firm conclusions cannot be drawn (0, 0.06 and 0.06% respectively). This reflects the difficulty for growers given that fruit appearing healthy at harvest can progress to show internal rots.

Averaged across all harvests where infection was present, a higher incidence of Fusarium infection was detected in small green fruit (6-19% for samples four and five) than in mature fruit (7-8%). As *F. lactis* may be present as a latent infection in small green fruit but never progress to a rot in mature fruit, this is not surprising. Whereas infection was rarely detected in small green fruit for harvests one to three, it was detected at an incidence of 6-10% in mature fruit from these harvests. Reductions in level of Fusarium infection in mature fruit were statistically significant ($p <0.05$) at harvest five (Table 1).

Table 1. Effect of Serenade ASO sprays on incidence of Fusarium infection in pepper fruit, cv. Cupra – 2015

	% fruit infected with Fusarium			
	External symptoms at harvest	Incubated small green fruit	Incubated mature fruit	
			External symptoms	Any symptom
Harvest 5				
1. Untreated	0	4.8	8.8	14.3
2. Serenade ASO x 1	0	3.9	4.0	8.3
3. Serenade ASO x 3	0	0	0	7.1
All harvests				
1. Untreated	0	2.4	5.4	7.9
2. Serenade ASO x 1	0.06	2.1	3.6	5.8
3. Serenade ASO x 3	0.06	0.5	4.7	4.6

4. Effect of some biological products (biopesticides and resistance inducers) on Fusarium internal fruit rot of pepper

An experiment was established in February 2016 to determine the effect of two biofungicides (Serenade ASO and T 34 Biocontrol) applied as protectant sprays to flowers and five treatments applied to plants in propagation as potential resistance inducers/plant strengtheners (sodium chloride, Serenade ASO, T 34 Biocontrol, Trianum P and a coded product), for their effect on Fusarium internal fruit rot. This experiment is continuing and results will be reported in the Final report.

5. Monitoring occurrence of Fusarium fruit rot in organic pepper crops

Grower comments in the UK and the Netherlands suggest that Fusarium internal fruit rot is not a problem in pepper crops grown organically. To examine this suggestion, samples of flowers, young green fruit and mature fruit were collected at monthly intervals in July, August and September 2015 from two nurseries where crops were grown to organic standards. The same samples were collected from a conventional crop at each site. Both organic crops were cv. Artega while the conventional crop was cv. Sapporo at site one and cv. Falco at site two (no variety common to both organic and conventional production was available at either site). *Fusarium* sp. was isolated and symptoms of Fusarium internal fruit rot were recorded at a low incidence in all crops at one or more of the sample dates (Table 2). Generally, levels found in flowers and small green fruit were greater than those found in mature fruit, supporting previous observations. In addition to *Fusarium* sp., there were relatively high levels of

Cladosporium, Penicillium and *Mucor* on flowers and of *Penicillium* and *Botrytis* on small green fruit. There was no consistent difference in the levels of these fungi between organic and conventional crops. Site one suffered obvious infection of *Mucor* soft rot in mature fruit. This monitoring work is continuing in 2016 and, providing pure cultures are obtained, a sample of *Fusarium* isolates will be examined by PCR to determine species identity.

Table 2. Occurrence of *Fusarium* sp. and symptoms of Fusarium internal fruit rot in organic and conventional pepper crops – 2015

Site and sample month	Incidence of Fusarium (%)					
	Isolated from flowers		Present in small green fruit		Present in mature fruit[a]	
	Organic	Conventional	Organic	Conventional	Organic	Conventional
Site 1						
July	6	0	2	0	2	2
Aug	8	30	0	2	0	0
Sep	12	2	22	10	14	2
Site 2						
July	6	12	0	2	0	2
Aug	12	2	16	24	0	0
Sep	0	2	2	6	2	0

[a] External symptoms after incubation at ambient temperature for 5 days

Financial Benefits

An initial simple estimate of the financial benefit of spraying with Serenade ASO is given below. A more detailed estimate will be made when on-going experimental work with Serenade ASO in this project and in the Netherlands is concluded.

A worse-case scenario is considered in which mature fruit are kept at ambient temperature for five days after harvest; all fruits developing either external and/or internal symptoms of Fusarium rot are deemed unmarketable. For harvest five only, one and three sprays of Serenade ASO appeared to increase the proportion of marketable fruit from 85.7% to 92.8 and 94%. The mean total of marketable fruits harvested from each untreated plot (a single row of 72 m^2) was 69. These % increases from one and three sprays of Serenade ASO equate to six and seven additional fruit/row. Assuming a net price of 35p/fruit, the increased production from one row equates to £2.10 and £2.45 for one and three sprays respectively. Assuming Serenade ASO is applied at 10 L/ha and the product is £14.80/L, the cost of product to treat one 50 m length of double sided crop (trial rows were 46 m long) row is approximately £1.48. In this instance the value of additional harvested fruit outweighs the cost of product.

The cost of spray application also needs to be considered for a more accurate estimate. It should also be noted that this example considered the fruit harvest where incidence of Fusarium in untreated fruit was greatest. No benefit would have been gained at harvests 1-4.

The potential financial benefits to be gained from application of Serenade ASO would be greater if a) product is effective when applied by LVM in order to facilitate timely treatment and reduce application costs; b) a reliable method to quantify Fusarium inoculum at the flowering stage is available so that sprays could be targeted for use during periods only when the infection risk is high. Both of these aspects are being investigated in the Netherlands in 2016.

Action Points

- Consider treatment of flowers with Serenade ASO in pepper crops where the risk of Fusarium internal fruit rot may be high (e.g. based on history of the disease in particular houses; the incidence of Fusarium internal fruit rot in fruit recently harvested from the crop; occurrence of persistent wet weather or persistent high humidities/condensation events in a crop). If possible, leave an untreated area and compare the incidence of Fusarium internal fruit rot in fruit harvested from treated and untreated crop.
- Where Fusarium internal fruit rot is known to be present in a crop at more than incidental level, seek to market visibly healthy fruit as soon as possible rather than store them. It is likely that some visibly healthy fruit from such crops will be infected internally, and this infection will likely continue to develop when fruit are in cold store or are marketed.

