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

Final Report 2017

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
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Report:	Final report, March 2017
Previous report:	Annual report, March 2016
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Date project commenced:	1st July 2015
Date project completed	31st March 2017

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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.

Project Leader

Tim O'Neill

Plant Pathologist

RSK ADAS Ltd

Signature ..... Date .....

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GROWER SUMMARY

Headline

- Nursery trials confirm two biofungicides reduce Fusarium internal fruit rot; one of them (Serenade ASO) is approved for use on protected pepper.
- Two biological treatments applied during propagation slightly reduced Fusarium infection of fruit.
- Fusarium internal fruit rot was confirmed in UK organic crops.

Background

For over 15 years sweet pepper internal fruit rot has been a growing problem within the UK and worldwide. Fusarium internal fruit rot losses are seen at production nurseries, pack houses and supermarkets through rejections and product returns. Loss levels vary greatly depending on crops and seasons. In the UK weakly pathogenic species of Fusarium including *F. lactis* and *F. oxysporum* have been shown to be associated with the disease. Fusarium spores deposited on the stigma during flowering grow through the style into the developing fruit. In recent studies we found: 1) *F. lactis* growing on glasshouse rockwool propagation blocks, a previously unidentified source of fungus; 2) although a large proportion of flowers and young fruits may be infected with *F. lactis*, generally only a small quantity of fruit develop internal fruit rot; 3) a single application of Serenade ASO during flowering can reduce fruit infection by 50%. Results were shared between ADAS and the pathology team at Bleiswijk Research Station (Wageningen University).

The aim of this project was to reduce the losses to Fusarium internal fruit rot. Specific objectives were:

1. Continued information exchange on the disease with Dutch researchers;
2. Examination if pepper seeds can be a source of *F. lactis* and *F. oxysporum* leading to growth of the fungi on rockwool propagation cubes;
3. To determine the reduction in fruit infection provided by several applications of Serenade ASO to a crop;
4. To determine if the use of biopesticides/plant resistance inducers applied preventatively provide protection to flowers against infection and fruit rot development;
5. To monitor occurrence of *F. lactis* in flowers and fruit in an organic pepper crop compared to a conventional crop.

Summary

Objective 1. Information exchange with Dutch researchers

Information was exchanged with Jantineke Hofland-Zijlstra, Plant Pathologist at Wageningen UR Greenhouse Horticulture in Bleiswijk, as well as hosting a visit to ADAS Boxworth and pepper growers in the Lee Valley for discussion on the disease (2015); and by a reciprocal visit by Sarah Mayne to Bleiswijk Research Station and a Dutch pepper nursery (2016).

In the Netherlands Fusarium internal fruit rot remains a major concern. It was found in 2014 that incidence of the disease varies greatly between individual crops, including between crops of the same variety on different nurseries. Detailed monitoring of growers' crops and practices revealed some factors that appear to reduce the disease including better 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 and 2016, levels of Fusarium internal fruit rot in Holland and in the UK were generally lower than in previous years. Experimental work at Bleiswijk showed a reduction in Fusarium infection in young fruit with Serenade ASO and antagonism of *F. lactis* by an experimental product. Twice daily change of slab EC from low to high, and vice-versa had no effect on the susceptibility of fruit to Fusarium internal fruit rot.

Risk assessment of fruit rot through measurement of Fusarium spore levels is of interest to growers in the Netherlands. In 2017 work will commence to quantify 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 are treatment of slabs with biological products to reduce sporulation by *F. lactis,* further experiments on antagonists and use of Serenade ASO, and the effect of climate change strategy (new generation growing with greater use of screens) on incidence of Fusarium fruit rot.

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

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

In 2015 five varieties of commercial seed lots were plated onto a fusarium selective agar growth medium. Many seeds of all varieties were found to be contaminated with saprophytic

fungi, especially species of *Aspergillus* and *Penicillium*. *Fusarium* species grew out from seeds of two of the varieties, at a low incidence ($^{5}/_{300}$ and $^{1}/_{300}$) but neither was typical of *F. lactis*.

In order to examine potential transfer of *F. lactis* from seeds to rockwool, a batch of seeds was artificially contaminated with the fungus and sown in rockwool plugs. The resultant seedlings were transferred to rockwool cubes. Five weeks later, samples of the rockwool were tested for *F. lactis*. A high level of *F. lactis* was recovered from the edge of propagation plugs even though no visible growth was evident. *F. lactis* was also isolated from the edges of rockwool cubes, though at lower incidence. These results indicate *F. lactis* is able to persist in rockwool in glasshouse conditions though spread across a cube may be slow.

To further examine the possible introduction of *F. lactis* into glasshouses at planting, two nurseries were visited within 24 h of plant delivery in January 2016 and samples of rockwool were collected from the edge of cubes from six crops. Additionally, at a propagation nursery, plants of cvs 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. No colonies were obtained in clean culture so it was not possible to determine if any were *F. lactis* by PCR test. From the propagation nursery, *Fusarium* sp. was isolated from 2/36 rockwool cubes growing cv. Ferrari and nil from cv. Fiesta. None appeared typical of *F. lactis*.

Objective 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 in a glasshouse with a history of the disease to determine the effectiveness of Serenade ASO applied as one or three sprays at weekly intervals, in reducing Fusarium infection in fruit. Serenade ASO mixed with Codacide oil was applied to the crop face as a fine spray in a single pass with a boom sprayer and to the pathway and slab surface using a lance. No inoculation with *F. lactis* was done. 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. Fifty 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.

In small green fruit, the incidence of Fusarium infection in the fourth and fifth samples was significantly reduced ($p<0.05$) by three sprays of Serenade ASO (Table 1). A single spray of Serenade ASO appeared to give a slight reduction. Levels of Fusarium were nil or virtually nil in harvests one to three.

In mature fruit, for the five harvests combined, there was a trend for a reduced incidence of Fusarium infection in the incubated marketable fruit (7.9%, 5.8% and 4.6% infection in untreated, Serenade ASO (one spray) and Serenade ASO (three sprays) respectively). Reductions in level of Fusarium infection in mature fruit were statistically significant ($p <0.05$) at harvest five (Table 1). The proportion of fruit with external symptoms of Fusarium internal fruit visible at harvest was low (<0.1%). This reflects the difficulty for growers given that fruit appearing healthy at harvest can progress to show internal rots.

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

	% fruit infected with Fusarium			
	External symptoms at picking	Incubated small green fruit	Incubated mature fruit	
			External symptoms	Internal symptoms
Harvest 4				
1. Untreated	0	21.5	4.0	3.9
2. Serenade ASO x 1	0	20.4	2.4	4.6
3. Serenade ASO x 3	0.2	**8.1**	1.3	2.9
Harvest 5				
1. Untreated	0	14.4	8.8	11.0
2. Serenade ASO x 1	0	11.7	4.0	7.2
3. Serenade ASO x 3	0	**3.9**	**0**	**0.6**

Values in bold are significantly different from untreated.

Objective 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 T34 Biocontrol) applied as protectant sprays to flowers and five biological treatments applied in propagation as potential resistance inducers/plant strengtheners (sodium chloride, Serenade ASO, T34 Biocontrol, Trianum P and F222), for their effect on Fusarium internal fruit rot. T34 Biocontrol and Trainum P were used under an experimental permit and cannot legally be applied as a spray to a commercial sweet pepper crop.

Plants were inoculated with *F. lactis* by spraying a spore suspension into flowers (direct inoculation) and into the air above plants, allowing the spores to drift down (indirect inoculation). The incidence of Fusarium fruit infection was greater where flowers were inoculated directly (97%) than indirectly (78%). Combining the data for both inoculation methods, Fusarium fruit infection at harvest 1 was significantly reduced by four treatments. Infection was reduced from 100% in the control plants, treated with water, to 65.6% by T34 Biocontrol applied as a biofungicide to protect flowers and to 74.1% by Serenade ASO applied the same way. Fusarium fruit infection was slightly but significantly reduced by T34 Biocontrol and F222 applied as inducers, to 81.1% and 81.5% respectively (Fig 1). There were no significant differences between treatments at harvests two and three.

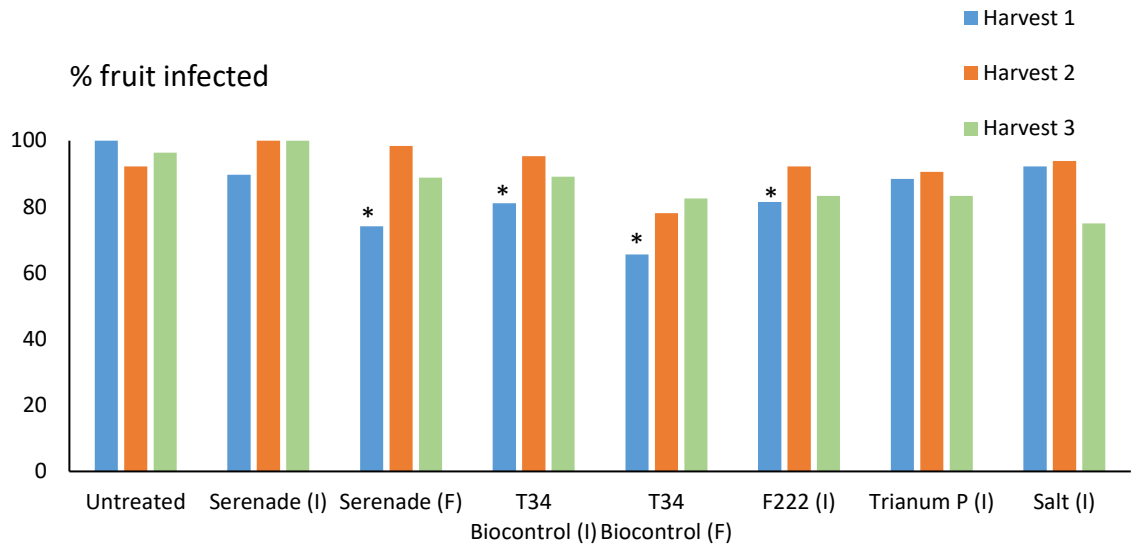

Figure 1. Effect of some biofungicide (F) and inducer (I) treatments on infection of pepper fruit by *F. lactis* – ADAS Boxworth, 2016. Treatments marked * are significantly reduced compared with untreated plants.

Serenade ASO and T34 Biocontrol flower protectant spray treatments were further examined in autumn 2016 in a replicated trial in a commercial crop of cv. Cupra. The trial was established in a glasshouse area with a history of Fusarium internal fruit rot. No inoculation with *F. lactis* was done. The biofungicides were sprayed in water to both sides of the crop rows on three occasions at weekly intervals from 29 July 2016. Open flowers were tagged at the time of spray treatment so that the corresponding cohorts of fruit could be harvested. Fusarium fruit infection was determined in four harvests of small green fruit, collected at weekly intervals after spray one and in three harvests of mature fruit collected on 20 and 26 September and 3 October. In small green fruit collected from untreated plots, Fusarium was detected at three harvests with incidences of 1.4, 11.4 and 4.3%. The mean level of infection

(5.7%) was reduced by Serenade ASO to 0% and by T34 Biocontrol to 0.4%. In mature red fruit the mean incidence of Fusarium internal fruit rot at 5 days after harvest was reduced from 2.2% (untreated) to 0.3% (Serenade ASO) and 0.5% (T34 Biocontrol). Results for individual harvests are shown in Table 2.

Table 2. Effect of two biofungicides applied as sprays on occurrence of Fusarium internal fruit rot in pepper fruit, cv. Cupra, at and after harvest – commercial crop, 2016

Harvest and treatment	% fruit with Fusarium symptoms at picking	% fruit visibly infected after 5 days ambient incubation
Harvest 1 (20 Sep)		
1. Untreated	0.7	3.6
2. Serenade ASO	0	0.7
3. T34 Biocontrol	0	0.7
Harvest 2 (26 Sep)		
1. Untreated	0.5	2.1
2. Serenade ASO	0	1.5
3. T34 Biocontrol	0	3.6
Harvest 3 (3 Oct)		
1. Untreated	0.9	7.9
2. Serenade ASO	0	0.7
3. T34 Biocontrol	0	0.7

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

Grower comments suggested 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 from July to September 2015 and from March to June in 2016 from two nurseries where crops were grown both to organic standards and conventionally. In 2015 both organic crops were cv. Artega while the conventional crop was cv. Sapporo at site one and cv. Falko at site two (no variety common to both organic and conventional production was available at either site). In 2015, *Fusarium* sp. was isolated and symptoms typical of Fusarium internal fruit rot were recorded at a low incidence in all crops at one or more of the sample dates. Generally, levels found in flowers and small green fruit were greater than those found in mature fruit, supporting previous observations. In 2016, both organic crops were cv. Artega and both conventional crops were cv. Falko. *Fusarium* sp. was detected less frequently than the previous summer, and only in flowers and mature fruit. This work confirms Fusarium internal fruit rot can occur in organic crops. It was not

possible to draw conclusions on the relative levels in organic and conventional crops due to no site using the same variety for both organic and conventional crops.

Financial Benefits

An initial simple estimate of the financial benefit of spraying with Serenade ASO is given below.

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 low volume machine (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.

One of the most important benefits of reducing Fusarium incidence in the crop would be a greatly improved customer supplier relationship due to less problems from cares complaints. Although this cost would be difficult to quantify in monetary terms it is very important to growers.

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.
- AHDB should continue liaison with the suppliers of T34 Biocontrol and explore the possibility of seeking a label or EAMU for its use as a foliar spray on protected pepper.
- The efficacy of Serenade ASO and T34 Biocontrol against Fusarium internal fruit rot when applied to crops via LVM warrants investigation.