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, RSK ADAS (July 2015 – March 2016) Sarah Mayne, RSK ADAS (April – December 2016) Dr Tim O'Neill and Dave Kaye, RSK ADAS (January – March 2017)
Report:	Final report, March 2017
Previous report:	Annual report, March 2016
Key staff:	Jonny Kerley and Chris Dyer, RSK ADAS
Location of project:	RSK ADAS Boxworth, commercial site – Herts, Essex & Somerset
Industry Representatives:	Alan Richardson, Abbey View Nursery, Galleyhill Road, Waltham Abbey, Essex, EN9 2AG Neal Ward, Cantelo Nurseries, Bradon Farm, Isle Abbots, Somerset, TA3 6RX
Date project commenced:	1st July 2015
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AUTHENTICATION

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

Project Leader

Tim O'Neill

Plant Pathologist

RSK ADAS Ltd

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

Report authorised by:

Barry Mulholland

Head of Horticulture

RSK ADAS Ltd

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

CONTENTS

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.

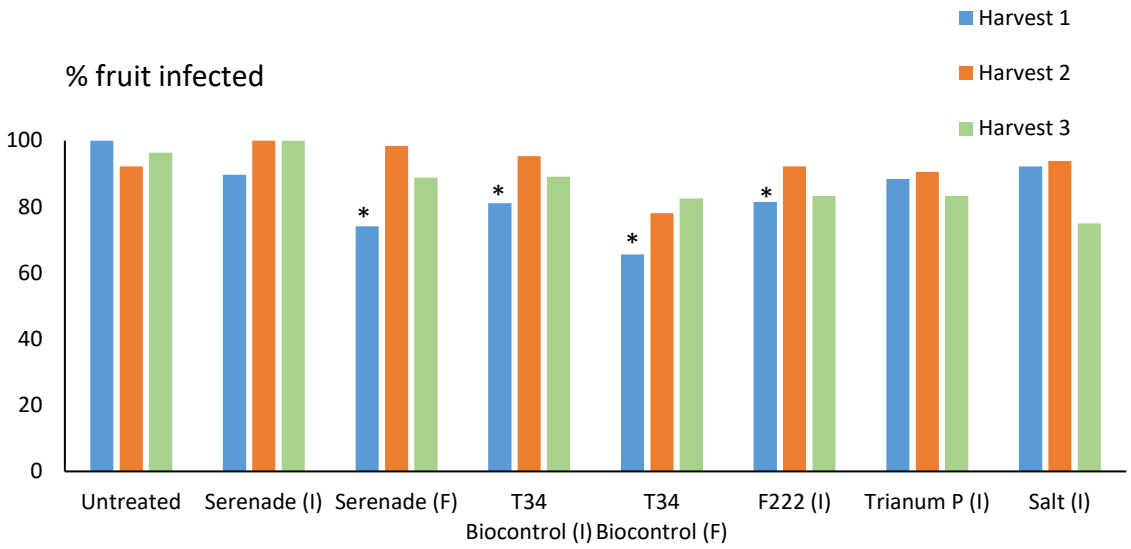


Figure 1. Effect of some biofungicide (F) and inducer (I) treatments on infection of pepper fruit by *F. lactis* – ADAS Boxworth, 2016. Treatments marked * are significantly reduced compared with untreated plants.

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.

SCIENCE SECTION

Introduction

Cause and occurrence

A Fusarium sweet pepper internal fruit rot was originally identified in 2005 affecting up to 20% of a day's pick at one nursery; identified as *F. oxysporum*. The disease has been shown to be present in multiple glasshouse crops with varying severity dependent upon variety, nursery, glasshouse and time of year (O'Neill, 2008; Gill Wardell, pers comm.). Generally, this is a greater issue in the spring and autumn where fruits take longer to ripen (56-60 days) rather than in the summer (38-40 days). When fruit including pepper, cucumber, tomato and lettuce were wound inoculated with isolates of *F. oxysporum* from sweet pepper wilt was not observed. Sweet pepper fruit isolates exhibited the distinctive orange/peach colour when grown in culture, different to the usual white or purple colour of *F. oxysporum* isolates producing wilt in other crops, including tomato. Orange/peach Fusarium was isolated from pepper seeds in fruit symptomatic of internal fruit rot. Work in Belgium identified a peach Fusarium as *F. lactis.* Both *F. lactis* and *F. oxysporum* are associated with sweet pepper internal fruit rot in UK crops (PC 022, 2015). .

Fusarium is relatively common in UK pepper crops, particularly on aborted fruit, rotting mature fruit and crop debris. Better crop hygiene, especially removing sources of inoculum practiced by some nurseries, appears to give some control. Presence of *Fusarium* sp. on commercial seed is a possible route for introduction of the disease into a nursery. Hyperchlorite seed treatment reduces *Fusarium* sp. incidence in packeted seed. An experiment comparing incidence of Fusarium fruit and stem rot on plants grown from treated and untreated seed was inconclusive due to low disease incidence (PC 260, 2007).

Limited observations in the Netherlands and the UK indicate Fusarium internal fruit rot is less common in organically grown pepper crops. If this is the case it may be associated with increased diversity and/or quantity of competitive saprophytic microorganisms in the environment which either increase levels of host resistance or reduce infection of flowers by *F. lactis.*

Control treatments

It was shown in PE 007 (2013) that the predominant species causing internal fruit rot in the UK was *F. lactis*. A smaller proportion of rots caused by *F. oxysporum* and *F. proliferatum* was also seen. In a replicated trial, single sprays of Amistar (azoxystrobin), Switch (cyprodinil + fluxioxonil) and Serenade ASO (*Bacillius subtilis* strain QST 713) resulted in significant

reductions in Fusarium internal fruit rot of up to 50% when applied to flowers one day before inoculation with *F. lactis* spores. Flowers were tagged so that the developing associated fruits could be identified. In another trial a whole row received a single spray of Serenade ASO to the crop face (flowers, leaves and stems), the rockwool cubes and the floor (treating fallen debris) as part of a whole row comparison study. This also showed a 50% reduction in Fusarium internal fruit rot in fruit developing from open flowers at the time of the spray application.

Fusarium internal fruit rot was discussed with the Pepper Technology Group and continues to be a frequent cause for rejection by packers and complaints from UK growers and supermarkets.

Initial source of *F. lactis* spores

In PE 022 (2014), following observations in the Netherlands, *F. lactis* was seen on rockwool propagation cubes in three commercial crops. *F. lactis* amongst other *Fusarium* species were isolated from the rockwool cubes from August through October. This may be an important source of inoculum for introduction of *F. lactis* into glasshouses. Air currents could facilitate spore transfer from sporulating colonies on the surface of rockwool to the surface of pepper flowers; transfer may also occur through insects.

Latent infection

PE 022 (2014) examined the occurrence of *Fusarium* spp. in both flowers and in the associated cohort of young (1-2 cm diameter) green pepper fruit in three crops on three occasions. High incidences of *Fusarium* spp., specifically *F. lactis*, were found in most of the flowers and fruits sampled. No relationship between incidence of flower infection and incidence of fruit infection in the associated fruit cohort was found. Infection incidence in small green fruit ranged from 0-100%, but was usually around 30%. It is very unlikely that all infected fruit are aborted before maturity, or develop internal fruit rot. If this was the case there would be few marketable fruit from those crops monitored. These results may indicate large proportions of developing pepper fruits may be infected with *F. lactis* but only a fraction of harvested fruit go on to develop visible rot symptoms. Treatments which enhance a plants natural resistance to disease, through cultural practices, or specific treatments inducing acquired resistance, may reduce the proportion of fruit in which Fusarium internal fruit rot becomes visible.

Collaboration with Wageningen University

A series of conference calls were made between ADAS and Wageningen University (Bleiswijk Glasshouse Crops Research Centre) plant pathology teams to discuss and exchange recent results and plan work for the future. The PE 022 report (March 2015) described the results from the Netherlands. Funding for a new research programme in the Netherlands was recently agreed. The work programme described in this ADHB project was designed to complement the Dutch work. The experiments carried out in the UK were predominantly nursery-based examining treatment efficacy in commercial conditions. Conversely, the Dutch experiments examined effects of individual treatments under strictly controlled conditions and were mainly research station based. The Dutch project is funded by the Dutch Production Association, Dutch Horticultural Production Board and Foundation TKI Horticulture.

Overall aim

This project aimed to build on knowledge of Fusarium internal fruit rot of pepper developed in earlier HDC projects, together with findings from the Netherlands, in order to devise improved control through knowledge exchange and timely application of plant protection products, and 'plant strengtheners'. Specific objectives were:

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

Work in this second year focussed on Objective 4; work on other objectives that was incomplete at March 2016 (first Annual report submitted) is also reported.

Materials and methods

Objective 1. Continue liaison with Dutch researchers working on pepper Fusarium internal fruit rot

Contact was maintained throughout 2015 and 2016 with Dutch researchers, with conference calls arranged at the project outset to compare work planned. Jantineke Hofland-Zilstra, Wageningen University, project leader of Dutch work on pepper diseases, visited ADAS Boxworth and two nurseries in the Lee Valley in April 2015 to discuss the disease and planned work. At the end of the 2015 season, and in 2016, researchers also shared the results of their work by conference call and email. In September 2016, Sarah Mayne visited Bleiswijk and presented a talk to Dutch pepper growers and visited a Dutch pepper nursery to discuss the disease.

Objective 2. Seed infection and transfer to rockwool propagation cubes

Transfer to rockwool cubes

To establish if commercial pepper seed could be effectively inoculated with *F. lactis*, a preliminary experiment was set up. A confirmed isolate of *F. lactis* from 2014 was grown on Potato Dextrose Agar + Streptomycin (PDA+S) media and used to inoculate seed. Fifty seeds were soaked in 200 mls of spore suspension in sterile distilled water (SDW) at 1.2 x 10^6 spores per ml for 24 hours. 100µl of Tween 20® was also added to prevent spores sticking together. After 24 hours, seeds were sieved out of the solution and allowed to dry in a laminar flow cabinet. Once dry, seed were plated onto Fusarium selective agar (FSA), grown at 20° in an incubator on a 16:8 light:dark cycle and assessed for *F. lactis* outgrowth for up to 14 days after plating. The recipe for Fusarium selective agar is located in Appendix 4.

In January 2016, three confirmed isolates of *F. lactis* were bulked up and pepper seed of a commercial variety, Kelly, were sown in rockwool plug trays after infestation with *F. lactis* as above. Hypochlorite seed treatment and Jet 5 irrigation of young plants were examined as potential methods to reduce fusarium transfer from seed to rockwool cubes. The treatments tested are summarised below (Table 3).

Table 3. Pepper seed (cv. Kelly) or plant treatments following artificial seed infection with *F. lactis* and planting in new rockwool plugs – ADAS Boxworth, 2016

Treatment	Rockwool	Seed treatment	Irrigation treatment to plants
1.	New rockwool plugs, seeded	Untreated	None

2.	New rockwool plugs, unseeded	N/A (negative control)	None
3.	New rockwool plugs, seeded	Hypochlorite treated pepper seed (1% available chlorine for 5 minutes)	None
4.	New rockwool plugs, seeded	Untreated	Seedling irrigated weekly with Jet 5 at 40 ppm H_2O_2

This experiment consisted of 6 replicates, with each plot containing 20 rockwool plugs. After sowing, rockwool plug trays were placed in Controlled Environment (CE) cabinets, set at a temperature and relative humidity favourable to pepper seed germination (25^0C, 70%RH). Seeds from each treatment were sown in separate trays to ensure no cross-contamination of treatments or inoculum. Irrigation with Jet 5 (Treatment 4) was carried out weekly once plants had reached 2 true leaves and there was no risk of phytotoxicity (first treatment administered 29th February). Other treatments were irrigated with mains water. In between the weekly applications of Jet 5, treatment 4 also received irrigation with mains water as and when required.

Seeds were sown on 21st January 2016. At 25 days after sowing, plugs containing seedlings and also plugs with ungerminated seed or no seedling were transferred to rockwool cubes (10 x 10 x 6.5 cm) laid out in a randomised block design in a glasshouse at ADAS Boxworth. A new set of gloves was used when transferring each treatment, to avoid cross-contamination. Cubes were placed, not touching one another, in trays of water and commercially available liquid feed which was adjusted as required based on pH and EC of solution and plant appearance. Plants were monitored for any differences in vigour or any phytotoxic symptoms as a result of treatments. At 5 weeks after transfer of plugs to cubes, on 18th March, rockwool pieces were sampled from the edges of each cube and the edge of each plug. These pieces of rockwool were directly plated onto FSA, incubated at 20°C at a 16:8 hour light:dark cycle, and resultant fungal and bacterial growth developing from them was recorded. Growth was assessed on the 21st and 23rd March. Samples of suspect *F. lactis* isolated from the rockwool were sent to John Clarkson at Warwick University for identification by PCR.

Objective 4. Examination of the efficacy of some biofungicides and resistance inducers applied preventatively for control of Fusarium internal fruit rot of pepper

In January 2016, potential products for inclusion were investigated through liaison with the industry representatives, AHDB Horticulture, and based on information from scientific literature.

ADAS glasshouse trial

A set of five biological products were chosen for evaluation as potential resistance inducers (I), applied pre-flowering, and two as biofungicides (F) examined as protectants, applied to the flowers (Table 4). The registration status (as of December 2016) of these products is given in Table 5. Products were applied on a propagation nursery from February 2016 until the point of first flowering and at ADAS Boxworth thereafter. T34 Biocontrol was used under an Experimental Permit and all fruit were destroyed. One product, not currently available in the UK, was coded (F222) and was also used experimentally and fruit destroyed. T34 Biocontrol has been authorised for use as a drench on sweet pepper as of June 2016.

Table 4. Detail of resistance inducer and biofungicide treatments, including timings, applied at a propagation nursery/ADAS Boxworth – Spring 2016

Treatment	Product	Timing	a.i.	Application	
				Inducer	Biofungicide
1.	Untreated	control	-	-	-
2.	Serenade ASO	from sowing to first flower (3 or 4 drenches)	*Bacillus subtilis*	✓	-
3.	Serenade ASO	from first flower every 2 weeks (3 sprays)	*Bacillus subtilis*	-	✓
4.	T 34 Biocontrol	from sowing to first flower (3 sprays)	*Trichoderma asperellum*	✓	-
5.	T 34 Biocontrol	from first flower every 2 weeks (3 sprays)	*Trichoderma asperellum*	-	✓
6.	Coded (F222)	from sowing to first flower (3 sprays)	-	✓	-
7.	Trianum P	from sowing to first flower (3 or 4 drenches)	*Trichoderma harzianum*	✓	-
8.	Salt	one drench treatment in propagation	Sodium chloride	✓	-

Table 5. UK registration status of biofungicides used on pepper in ADAS Boxworth and commercial trials – 2016

Product	MAPP number	EAMU number	Permitted for use on protected pepper as	
			Spray	Root drench
Serenade ASO	16139	0706/13	✓	X
T34 Biocontrol	17290	-	X	✓
Trianum P	16741	-	X	✓

At the time of work T34 biocontrol was used under an experimental permit. T34 Biocontrol was subsequently authorised for use as a drench on 6 June 2016.

Compact multi-branched pepper plants cv. Cupra (a known susceptible variety) in rockwool cubes were sown at the propagation nursery on 23rd February 2016. New untreated seed was used. Plants were pinched twice to produce four shoots. Twenty plants were prepared for each treatment. The initial sowing treatment was applied using a calibrated backpack pressurised sprayer with a single lance (02 F110 nozzle) to the surface of plugs applied at 100 ml/m^2 water volume (1000 L/ha as permitted by EAMU). Subsequent treatments were applied as drenches poured onto the rockwool cube surface around the base of seedlings (Table 6). At this stage, treatments 1, 3 and 5 were kept in one glasshouse with the other treatments kept separate in order to minimise risk of insect transfer of the biological fungicides between treatments. Preliminary work established that young pepper plants in rockwool blocks were tolerant of 30 and 60 mM salt solution, but not 120 mM; 60 mM was therefore used as the treatment concentration for the main experiment. Details for all treatments are shown in Table 6. Products were used at label recommended rates where available. In discussion with product suppliers, it was agreed not to add any wetters to Serenade ASO or T34 Biocontrol in order to allow a straight comparison between products. This differs from the 2015 commercial trial, where Codacide was used with Serenade ASO, the only product under test in that experiment.

Table 6. Details of treatments in ADAS Boxworth trial – 2016

Treatment	GS	Rate of use		Application volume	
		Inducers (Propagation nursery)	Bio-fungicides ADAS (Boxworth)	Inducers (Propagation nursery)	Bio-fungicides ADAS (Boxworth)
1. Control		-	-	-	-
2. Serenade ASO	Plugs	1 ml/100 ml	-	100 ml/m^2	-
	Cube	0.01 ml/cube	-	Apply in 65 ml water/cube	-

3. Serenade ASO	Flowers	-	100 ml/L	-	100 L/ha
4. T34 Biocontrol	Plugs	0.5 g/100 ml	-	100 ml/m^2	-
	Cube	0.1 g/L	-	65 ml/cube	-
5. T34 Biocontrol	Flowers	-	1 g/L	-	100 L/ha
6. Coded (F222)	Plugs	0.5 ml/100 ml	-	100 ml/m^2	-
	Cube	0.005 ml/L	-	65 ml/cube	-
7. Trianum P	Plugs	1.5 g/2.5 L	-	2.5 L/m^2	-
	Cube	0.3 g/1.3 L	-	65 ml/cube	-
8. Salt	Cube	60 mM	-	65 ml/cube	-

The trial was relocated to a glasshouse compartment at ADAS Boxworth on the 24th February 2016 and arranged in a split-plot randomised block design with four replicates of the eight treatments. Each plot contained four plants, two directly inoculated with *F. lactis* by spray application into the flowers and two indirectly inoculated (a more natural aerial inoculation) by a spray of spore suspension into the air above each plant. Two plants in a sub-plot were contained in one tray. The four replicates were divided between two glasshouse compartments (64 plants per compartment). A 1-2 m discard was placed between each row and at the plot ends to account for spray drift.

All flower spray inoculations took place at ADAS Boxworth. Treatment sprays were completed as described in the initial sowing treatment with artificial and natural inoculation completed 1 day after each treatment spray. All plants in the air-inoculated sub plots were kept separate from those which received treatment sprays in order to avoid cross contamination. The first flower emergence spray and the first flower inoculation with *F. lactis* took place on the 24th and 25th May 2016 respectively. A mixture of three isolates of *F. lactis* were inoculated at a spore concentration of $5x10^2$ spores/ml using a scent-mister in all inoculations. Flowers were not inoculated until a minimum of three fully-open flowers were present on each plant. A second series of treatment and inoculation sprays took place on the 6th and 7th June whilst the final flower treatment and inoculated sprays were completed on the 20th and 21st June. A full crop diary is given in Appendix 1.

All developing young fruit (approx. 1 cm diameter) were removed at 1-2 weeks after inoculation with *F. lactis*. All collapsed flowers and young fruit were removed before each subsequent treatment application spray.

Collected fruit, kept separated into sub-plots to avoid cross contamination, were surface disinfected with 1% sodium hypochlorite and left to dry in a laminar flow cabinet. Halved lengthwise the fruit was plated face down onto PDA+S. Up to five fruit, if from the same plant,

were placed on one agar plate. They were incubated at 24ºC and then assessed for Fusarium after 7 and 14 days; results of the final (14d) assessment are given.

Meteorological records including RH and temperature within the glasshouse were recorded every 30 minutes at flower height from the first spray until final harvest (Appendix 2).

Commercial trial

A commercial nursery in Essex hosted a trial to determine the efficacy of two biofungicides applied preventatively for control of Fusarium internal fruit rot of pepper. These were the best two treatments in the biofungicides and inducers trial conducted at ADAS Boxworth (see results section). Table 7 details the treatments. The fruit from all plants treated with T34 Biocontrol was harvested and destroyed as this product did not have approval for use on protected pepper crops in the UK.

Table 7. Commercial site biofungicide treatments – 2016

Treatment	Active ingredient	Rate of use
1. Control	-	Water
2. Serenade ASO	*Bacillus subtilis*	100 ml/L
3. T34 Biocontrol	*Trichoderma asperellum*	1 g/L

A fully randomised block design of the three treatments replicated seven times was set up, and the first spray applied on the 29th July 2016. Two further sprays of each treatment were applied at weekly intervals. Treatments were applied using a calibrated backpack pressurised sprayer and lance fitted with 4 hollow cone nozzles (Lurmark 30HCx4) to the flowers and crop face at 500 L/ha to each face. Untreated plots were sprayed with the same volume of water only. Plots were one third of a commercial row long (10 m) and the plots splits were marked. A 1-2 m discard was placed at the ends of each row and between plots to allow for any spray drift. Flowers were tagged in each treatment to mark the cohort of fully open flowers that had received the sprays and follow them through development. The grower treated the crop as normal avoiding pesticides in the trial area where possible; no fungicides or biofungicides were applied by the grower to the trial area. A full crop diary is given in Appendix 3.

Meteorological data was recorded throughout the duration of the trial. A RH logger close to the flowers in each treatment recorded the temperature and RH every hour from 1 day prior to spray to final harvest (Appendix 4).

Tagging of flowers at each treatment timing allowed the correct fruit to be harvested, corresponding to those developing from 7 days after spray 2 (Harvest 1) and 7, 14 and 21 days after spray 3 (Harvests 2, 3 and 4). No fruit had developed at 7d after spray 1 due to cold weather and so no sampling was possible then. Ten small green fruits were sampled. Fruits were separated and incubated at an ambient temperature. After 4 weeks fruits were cut open and the occurrence of Fusarium white/pink fungal growth recorded. Identification of *Fusarium* was confirmed microscopically. Samples from 10 fruit were then cultured on PDA + S and samples were sent to Warwick Crop Centre for identification by PCR.

At each harvest of all mature red fruit (3 in total) ready for harvest were collected from both sides of the row in each treated plot and assessed by ADAS into three categories: marketable, unmarketable due to Fusarium and those unmarketable due to non-Fusarium symptoms. Twenty marketable fruit per plot were transferred to ADAS Boxworth and incubated for 5 days before being destructively assessed for Fusarium internal fruit rot.

Objective 5. Monitoring occurrence of *Fusarium* sp. on flowers, young fruit and mature fruit in organic and conventional crops

Two commercial sites were identified in July 2015 that grew both conventional and organic crops of pepper. Each of these sites was visited on three occasions in 2015 and four occasions in 2016, and flowers, small green fruit and mature fruit sampled from conventional and organic crops. Mature fruit sampled were Class 2 where possible to minimise impact on the growers. The crops sampled are summarised below in Table 8.

Table 8. Conventional and organic crops sampled for flowers, small green fruit and mature red fruit – 2016

Site	Growing system	Variety	Sample dates			
1.	Organic	Artega	30 Mar	22 Apr	25 May	-
1.	Conventional	Falko	30 Mar	22 Apr	25 May	-
2.	Organic	Artega	29 Mar	28 Apr	31 May	28 Jun
2.	Conventional	Falko	29 Mar	28 Apr	31 May	28 Jun

In each crop at each site, 50 flowers, 50 small fruit (approx. 2-5 mm in diameter where available, depending on fruit set) and 50 mature fruit were sampled. Samples were spread over five rows of commercial crop, and presence of any other diseases in the area of the crop sampled was also noted.

On arrival at ADAS Boxworth, flowers were incubated overnight at 24°C and then flower parts plated onto PDA+S. Small green fruit sampled were laid onto tissue paper in trays, not touching (at least 2 cm apart). The small fruit were cut open after 4 weeks and any visible fungal growth assessed and examined under a microscope. Any incidence of *Fusarium* species was recorded, and a sub-sample of fruit plated onto PDA+S for later identification by PCR. Class 2, mature fruit were assessed on arrival as marketable, unmarketable due to external symptoms of Fusarium, or unmarketable for another reason. Fruit were then incubated at ambient temperature for five days, following which they were destructively assessed. In the destructive assessment presence of Fusarium internal fruit rot, presence of Fusarium on seed, and presence of Fusarium externally were recorded.

Results

Objective 1. Continue liaison with Dutch researchers working on pepper Fusarium internal fruit rot

In 2016 experiments were undertaken to examine Serenade ASO and Asperello T34 Biocontrol (*Trichoderma asperellum*) as protectant sprays to flowers for control of Fusarium internal fruit rot. Asperello T34 Biocontrol was approved in late 2016 for use as a root treatment to control Pythium in aubergine, sweet pepper and tomato in Belgium, the Netherlands and France; it is not currently approved for use as a foliar spray in commercial crops. Both Serenade ASO and Asperello T34 Biocontrol reduced Fusarium infection in fruit. The same products will be further evaluated in glasshouse trials in 2017.

It has now been postulated in the Netherlands that high root pressure in sweet pepper may cause fruit cells to distort or burst, or fruit development to be altered with production of thinner walls, or a small hole to form at the base of the fruit, all of which might increase fruit susceptibility to Fusarium internal fruit rot. To test this hypothesis, pepper plants were grown on rockwool slabs using a range of EC values to influence root pressure. In addition, EC was changed from high to low, and vice-versa, twice daily, or left at a constant level. Fruit were examined at four stages during development. No differences were observed between treatments. Mature fruits were inoculated with *F. lactis* to compare their susceptibility and no differences were found. It was concluded that root pressure is unlikely to have a significant effect on the susceptibility of pepper fruit to Fusarium rot.

Work was initiated to examine the use of biological products for suppression of Fusarium sporulation on rockwool slabs. A molecular marker will be used to detect *F. lactis* in rockwool samples and monitor development of the fungus.

Work is planned to examine the potential for using introduced bees to measure Fusarium risk in a crop. Bees that have been active in a crop will be caught and washed to determine if they have collected *F. lactis* spores during foraging. This work was planned for 2016 but has been delayed.

The influence of different glasshouse climate strategies on Fusarium internal fruit rot will also be examined in glasshouse trials in 2017. In particular, the greater use of screens to crop will be examined.

Objective 2. Seed infection and transfer to rockwool propagation cubes

Seed testing; Occurrence on rockwool at planting; Occurrence of Fusarium on propagation nurseries

Three commercial pepper nurseries were visited three times during 2015 to establish the occurence of suspect fungal growth on rockwool slabs. Nurseries one and three showed levels of growth which remained stable over the assessment period, with the third showing higher levels of fungal growth. A high incidence of fusarium species was confirmed in rockwool pieces from all three nurseries. PCR tests revealed 13 isolates as *F. lactis*, two as *F. equiseti* and one as *F. culmorum.* Viable *F. lactis* was regularly recovered from samples of rockwool cubes indicating that *Fusarium* species occurring in rockwool represents a significant source of inoculum that may lead to Fusarium internal fruit rot.

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.

Transfer to rockwool cubes

Following the low levels of *F. lactis* found naturally on commercial pepper seeds, artificial inoculation of seed was deemed necessary. The inoculation method was successful and all seeds soaked in inoculum produced colonies of *F. lactis* when plated onto FSA.

Following inoculation with a high rate of *F. lactis* spores, some of the seed in inoculated treatments failed to germinate (Table 9). Seed treatment with hypochlorite appeared to improve germination following inoculation with *F. lactis*, and no hypochlorite treated seed or Jet 5 irrigated plants exhibited symptoms of phytotoxicity. All rockwool plugs were transferred into rockwool cubes, irrespective of whether or not a pepper seedling was present, as the purpose of the experiment was to examine transfer or growth of *F. lactis* from seed to the rockwool plug and cube.

Table 9. Effect of contaminating pepper seed with *F. lactis* on seedling emergence and occurrence of the fungus at the edge of rockwool cubes at 28 days after transfer of plugs to cubes – 2016

Treatment	Seed treatment	Irrigation treatment	% seedlings emerged (2nd Feb)	% seeds with *F. lactis* sporulation (29th Feb)	% propagation plugs with *F. lactis* at margin (23 Mar)	% rockwool cubes with *F. lactis* at margin (23 Mar)
1. Inoculated seed	-	-	22	100	80	0
2. No seed	-	-	-	-	41.6	18.4
3. Inoculated seed	NaOCl	-	95	25.6	56.8	20.8
4. Inoculated seed	-	Jet 5	69*	100*	80	2.4

*Note that at this time Jet 5 irrigations had yet to begin, first treatment 29th Feb

Five Fusarium isolates recovered from the rockwool (three from the edge of plugs and two from the edge of cubes) were all identified as *F. lactis* (John Clarkson, University of Warwick). On statistical analysis, no significant differences between treatments were reported for *% F. lactis* recovered from plated rockwool cube, but some trends were evident. Treatment 3, a seed treatment with hypochlorite, was most effective at reducing *F. lactis* recovered, and resulted in a lower level of sporulation visible on seed. Significantly more *F. lactis* was recovered from plugs than cubes ($P < 0.001$), but some *F. lactis* was recovered from the cube edge. *F. lactis* was also recovered from rockwool in treatment 2, which had not been seeded, suggesting an airborne route of spread. Efforts were made to separate treatment 2 from the other treatments once sporulation was noted in the trial, but this action may not have been significantly prompt to avoid spread. Treatment 3, the hypochlorite seed treatment, appears to have reduced *F. lactis* present on the plug, but relatively high levels were recovered from the cube, potentially due to airborne spread. Conversely, the Jet 5 irrigation did not prevent colonisation of the plug from infested seed, but appears to have effectively reduced colonisation of the irrigated cubes by *F. lactis*.

This experiment confirms that *F. lactis* present on seed has the potential to spread to rockwool on sowing, and that it can persist there for a considerable time under standard glasshouse conditions. It also appears to spread throughout the glasshouse, most likely via an airborne route, when conditions are favourable.

Objective 3. Examination of Serenade ASO crop treatments for reduction of Fusarium internal fruit rot

A single spray and three weekly sprays of Serenade ASO were compared to examine the ability of Serenade ASO to reduce Fusarium internal fruit in sweet pepper cv. Cupra. A fine mist was used to coat the leaves and flowers, whilst a lance treated the floor and surface of the rockwool cubes. Flowers were tagged and samples of rockwool collected and damp incubated to determine *Fusarium* presence. Infected samples were plated onto PDA+S plates and assessed for outgrowth of Fusarium species.

Small green fruit and mature red fruit were harvested when it was judged that the cohort of flowers treated with the first Serenade ASO spray had reached these growth stages with fruits sampled at weekly intervals. Little *Fusarium* sp. was found in small green fruit during harvests 1-3.

At harvest 4, *Fusarium* sp. was present in 21.5% of fruit from untreated plots, and significantly less ($p = 0.039$), at 8.1%, in fruit from plots sprayed three times with Serenade ASO. A similar pattern was evident at harvest 5, with infection reduced from 14.4 to 3.9% ($p = 0.042$). Combining results across both harvests resulted in a significant ($p=0.05$) reduction from 18.7% to 6.5% after 3 sprays. A single Serenade ASO spray had no significant effect on the infection level at either harvest. The occurrence of *Pencillium* sp., *B. cinerea*, and bacteria in small green fruit was very low at all harvests.

With mature red fruit, Serenade ASO treatment had no effect on the number of marketable or unmarketable fruit. The incidence of fruit with external symptoms of Fusarium fruit rot at this time was very low. A more accurate estimate of the incidence of Fusarium internal fruit rot in a batch of sweet peppers is gained by incubation at ambient temperature for 5-7 days to allow any *Fusarium* sp. present in the fruit, on seed or the internal wall, to grow and become visible. The infection can then be readily seen by a visual inspection for the whitish-pink fungal growth of *F. lactis* on seed, or of a brown rot on the fruit internal wall when fruit are cut open. After incubation at ambient temperature for 5 days, the proportion of fruit showing external symptoms

was significantly reduced ($p<0.05$) by the single Serenade ASO spray at harvest 1 and by the three spray programme at harvest 5.

No significant differences between treatments were found when just the marketable fruit were examined, although there was a consistent trend for fewer fruit with Fusarium symptoms (especially the 'any symptom' category) as the number of Serenade ASO sprays increased from zero to one and three. When the sample size per plot was increased by including assessment of all unmarketable fruits, there were significant reductions in Fusarium internal fruit rot following one Serenade ASO spray at harvest 1 and following three Serenade ASO sprays at harvest 5.

In mature red fruit, the highest levels of Fusarium infection detected was at harvest 5, and for small green fruit at harvest 4. Possibly this indicates a greater inoculum of *Fusarium* spores in the house when these fruit were at the flowering stage later in the season; or conditions were more conducive to infection at this time.

The fruit sampled at harvests 4 and 5 were considered to be ones which developed from flowers than opened after sprays of Serenade ASO had ceased. If the effect from Serenade ASO was only due to a direct protectant effect on open flowers, a treatment effect at harvests 4 and 5 would not be expected. The fact that a significant reduction in Fusarium internal fruit rot was found at harvest 5, following the three-spray Serenade ASO programme, suggests that Serenade ASO has a more persistent effect on reduction of Fusarium internal fruit rot. Potential methods by which this might occur are persistence of *Bacillus subtilis* strain QST 13 on crops and its movement in the environment, induced systemic resistance in plants following Serenade ASO spray treatment, or possibly other mechanisms. Further work is required to substantiate this interesting result of a possible persistent effect of Serenade ASO spray treatment as we cannot categorically state that all fruit at harvest 5 developed from flowers that opened after spray 3.

No artificial inoculation of flowers was undertaken in order to determine the efficacy of Serenade ASO sprays under natural infection pressure and not to swamp flowers with a high inoculum of spores by artificial inoculation. Results in the Netherlands indicate that Serenade ASO treatment is less effective when a high infection pressure is present.

Objective 4. Examination of the efficacy of some biofungicides and resistance inducers applied preventatively for control of Fusarium internal fruit rot of pepper

Glasshouse trial – ADAS Boxworth

All young fruit (approx. 1 cm diameter) were taken at 1-2 weeks after each spray-inoculation with *F. lactis*, with the fruit from each of the 2 sub-plots (inoculation method) kept separate. Cut disinfected fruit were plated face down on agar plates and the proportions infected with Fusarium determined (Fig. 2). The mean number of fruits harvested per plot was greater at harvests 1 (8.3 – 13.3) and 2 (7.0 – 11.8) than 3 (1.3 – 4.8). There was no evidence that treatment or inoculation method reduced fruit number (Table 10).

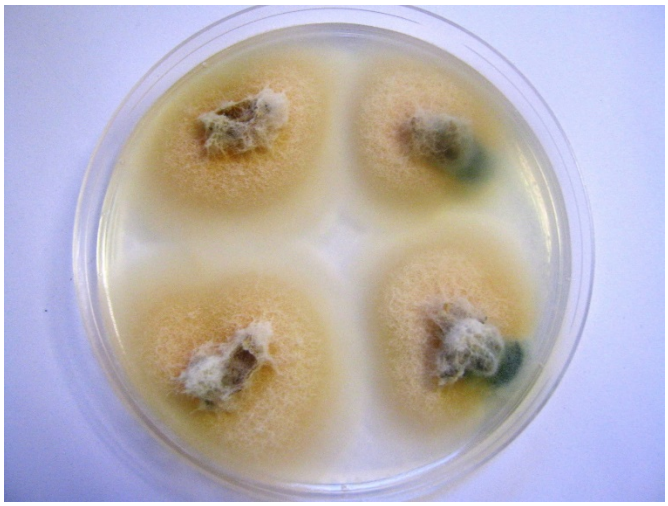


Figure 2. Outgrowth of *F. lactis* onto agar from halved young green fruit

Table 10. Mean number of small green fruit harvested from pepper plants treated with potential resistance inducers and biofungicides under two inoculation methods (direct and indirect) – ADAS Boxworth 2016

Treatment		Mean number of fruits					
		Harvest 1		Harvest 2		Harvest 3	
Product	Aim	Direct	Indirect	Direct	Indirect	Direct	Indirect
1. Untreated	Control	8.3	8.0	10.5	10.8	2.3	4.3
2. Serenade ASO	Inducer	10.5	9.5	11.8	11.0	2.0	2.3
3. Serenade ASO	Fungicide	8.5	8.0	7.0	9.3	7.3	4.5
4. T34 Biocontrol	Inducer	9.3	9.8	10.8	9.0	1.8	1.3

5. T34 Biocontrol	Fungicide	10.3	10.8	10.8	10.3	4.3	2.0
6. F222	Inducer	8.5	9.3	7.8	10.5	3.3	0.8
7. Trianum P	Inducer	11.5	13.3	11.0	11.3	2.8	2.5
8. Salt	Inducer	10.8	9.5	10.3	7.3	4.8	1.8
Probability (21 df)		0.361	0.017	0.405	0.736	0.025	0.171
LSD		3.325	2.746	4.710	5.001	3.187	3.040

Both inoculation methods resulted in a high incidence of infected fruit. When averaged across all treatments, direct inoculation resulted in significantly greater infection at harvests one (96.1% vs 72.0%, p <0.001) and three (99.7% vs 74.9%, p = 0.005), and there was a trend in the same direction at harvest two (96.1 vs 89%, p = 0.098). The efficacy of the indirect inoculation method indicates a high susceptibility of open flowers to spores of *F. lactis* in the air.

At harvest one, averaged across both inoculation methods, the incidence of infected fruit was significantly reduced by both Serenade ASO and T34 Biocontrol applied as protectant sprays to flowers (Table 11). T34 Biocontrol applied as an inducer (i.e. before flowers had fully developed) and F222 applied as an inducer also gave small but statistically significant reductions in fruit infection by *F. lactis* (Table 11). Serenade ASO, Trianum P and common salt were ineffective as disease resistance inducers when used as described in this work. None of the treatments reduced Fusarium infection at harvests 2 or 3, although T34 Biocontrol applied as a flower protectant spray at harvest 2 and common salt as an inducer at harvest 3 appeared to reduce infection (Table 11).

Table 11. Effect of some potential resistance inducers and biofungicides on infection of pepper fruit by *F. lactis* – 2016 (combined data for direct and indirect inoculation methods)

Treatment		Mean % fruit infected			
Product	Aim	Harvest 1	Harvest 2	Harvest 3	All harvests
1. Untreated	-	100.0	92.2	96.4	96.2
2. Serenade ASO	Inducer	89.7	100.0	100.0	97.2
3. Serenade ASO	Fungicide	**74.1**	98.4	88.8	87.1
4. T34 Biocontrol	Inducer	**81.1**	95.3	89.1	89.9
5. T34 Biocontrol	Fungicide	**65.6**	78.1	82.5	75.4
6. F222	Inducer	**81.5**	92.2	83.3	87.0
7. Trianum P	Inducer	88.4	90.6	83.3	87.8
8. Salt	Inducer	92.2	93.9	75.0	87.1

Probability (21 df)	0.011	0.098	0.727	0.454
LSD	16.98	13.75	30.04	18.34

Values in bold are significantly less (p <0.05) than the control.

Data were also examined with direct and indirect inoculation methods considered separately. The lower disease pressure of indirect inoculation was considered more likely to allow detection of any slight effects from treatments. In practice, indirect inoculation also proved to be a very efficient inoculation procedure. With indirect inoculation, the same four treatments appeared to reduce fruit infection at harvest one (Table 12); however, differences were not quite statistically significant at the 5% level. Although Serenade ASO, Trianum P and common salt appeared to result in slight reductions in infection compared with the water-treated control, none of the differences were statistically significant. Under direct inoculation, T34 Biocontrol applied as a protectant spray to flowers was again the best treatment, reducing infection from 100% to 84.7%. Serenade ASO also reduced fruit infection when used as a protectant spray to flowers. No statistically significant treatment differences were detected at harvest two or three.

Table 12. Effect of some potential resistance inducers and biofungicides on infection of pepper fruit by *F. lactis* under direct (into flowers) and indirect (over plants) inoculation – ADAS Boxworth, 2016

Treatment		% fruit infected					
		Harvest 1		Harvest 2		Harvest 3	
Product	Aim	Direct (into flowers)	Indirect (over plants)	Direct (into flowers)	Indirect (over plants)	Direct (into flowers)	Indirect (over plants)
1. Control	-	100	100	96.9	87.5	100	92.9
2. Serenade ASO	I	100	79.4	100	100	100	100
3. Serenade ASO	F	**87.6**	60.6	100	96.9	100	77.5
4. T34 Biocontrol	I	100	62.2	100	90.6	100	80.7
5. T34 Biocontrol	F	**84.7**	46.5	90.6	65.6	100	65.0
6. F222	I	100	62.9	93.8	90.6	100	66.7
7. Trianum P	I	96.7	80.1	87.5	93.8	100	66.7
8. Salt	I	100	84.3	100	87.9	100	50.0
Probability (21 df)		<0.001	0.061	0.749	0.128	-	0.742
LSD		5.25	32.53	18.76	22.46	-	65.20

I – inducer; F – biofungicide flower protectant.

Values in bold are significantly less than the untreated.

Further work is required to determine if the inducer treatments (T34 Biocontrol and F222) found effective at reducing incidence of Fusarium infection in small green fruit have an effect

that persists through to harvest of mature fruit. The efficacy of inducer treatments at lower inoculum pressures than used in this work, as might occur in commercial crops, also warrants consideration.

Commercial trial

Tagged flowers in each treatment at each application enabled fruit development to be monitored (Figure 3). These acted as a guide for the fruit to be harvested. Small green fruit were collected at one week after spray 2 (Harvest 1) and 1, 2 and 3 weeks after spray 3 (Harvests 2-4) and assessed. The experiment was located in a glasshouse on a susceptible variety, Cupra, and relied on natural infection pressure.

Figure 3. Flower tagged at TO (first spray application). The small green fruit in the foreground is typical of the size of those harvested at this stage.

Fusarium was detected in small green fruit at three of the four harvests (Table 13), at a relatively low incidence on all occasions. Nevertheless, the incidence of infection was consistently greatest in fruit from untreated plants. At the penultimate harvest, Fusarium infection was significantly reduced ($p = 0.005$) by Serenade ASO (nil) and T34 Biocontrol (1.4%) compared with the untreated (11.4%) (Table 13). *Penicillium* sp. were detected in fruit only at harvests 1 and 2, at levels from 2.9 – 8.6%, when nil or little Fusarium was found.

Possibly there is some interaction between *Fusarium* sp. and *Penicillium* sp. on flowers. *Botrytis cinerea* was detected only once, in fruit from untreated plants at harvest three.

Table 13. Occurrence of Fusarium in small green fruit at weekly intervals for four weeks after sprays of Serenade ASO and T23 Biocontrol – 2016

Harvest and treatment	% fruit affected		
	Fusarium	Penicillium	Botrytis
Harvest 1 (7 d after spray 2)			
1. Untreated	1.4	2.9	0
2. Serenade ASO	0	5.7	0
3. T34 Biocontrol	0	2.9	0
Harvest 2 (7 d after spray 3)			
1. Untreated	0	8.6	0
2. Serenade ASO	0	4.3	0
3. T34 Biocontrol	0	5.7	0
Harvest 3 (14 d after spray 3)			
1. Untreated	11.4	0	1.4
2. Serenade ASO	0	0	0
3. T34 Biocontrol	1.4	0	0
Probability (12 df)	0.005	-	-
LSD	0.664	-	-
Harvest 4 (21 d after spray 3)			
1. Untreated	4.3	0	0
2. Serenade ASO	0	0	0
3. T34 Biocontrol	1.4	0	0
Probability (12 df)	0.335	-	-
LSD	-	-	-

The mean numbers of mature red fruit harvested per plot was 112-114 at harvest one (20 September), 56-62 at harvest two (26 September) and 41-52 at harvest three (3 October), representing good sample sizes in which to assess Fusarium internal rot. The proportion of Class I marketable fruit averaged over the three harvests was 91-92% (Table 14). Treatment had no significant effect on Class I or Class II marketable fruit. Although relatively few fruits (0.5-0.9%) showed external symptoms of Fusarium internal fruit rot at harvest, they consistently and only occurred in untreated plots. Serenade ASO and T34 Biocontrol

significantly reduced visible Fusarium infection to zero (Table 14). The proportion of fruit deemed unmarketable for other reasons (e.g. mis-shape, blossom end rot) was unaffected by treatment.

Table 14. Effect of Serenade ASO and T34 Biocontrol on mean number and quality of fruit harvested and proportion with symptoms of Fusarium at harvest – commercial trial, 2016

Harvest and treatment	% fruit marketable		% unmarketable due to:	
	Class 1	Class 2	Fusarium	Other reason
Harvest 1				
1. Untreated	89.1	2.1	0.7	8.1
2. Serenade ASO	88.9	2.5	0	8.6
3. T34 Biocontrol	87.2	2.2	0	10.5
Probability (12 df)	0.802	0.965	<0.001	0.438
Harvest 2				
1. Untreated	93.2	5.3	0.5	5.7
2. Serenade ASO	95.4	8.6	0	3.7
3. T34 Biocontrol	95.9	9.4	0	3.2
Probability (12 df)	0.439	0.829	<0.001	0.429
Harvest 3				
1. Untreated	91.2	0.6	0.9	7.1
2. Serenade ASO	95.7	0	0	4.3
3. T34 Biocontrol	93.0	0.7	0	6.3
Probability (12 df)	0.341	0.083	<0.001	0.485
Total harvests				
1. Untreated	90.6	1.4	0.7	7.2
2. Serenade ASO	92.2	1.5	0	6.3
3. T34 Biocontrol	90.8	1.6	0	7.7
Probability (12 df)	0.704	0.975	<0.001	0.678

After incubation of mature fruit at ambient temperature for five days, to allow any internal Fusarium to further develop as it might under retail display and home storage, the incidence of internal Fusarium had increased by four-eight fold. The incidence of Fusarium infection detected, at any location within fruit, was 3.6%, 2.1% and 7.9% in samples from harvests one, two and three respectively (Table 15). Fusarium infection was significantly reduced ($p = 0.017$) by both Serenade ASO and T34 Biocontrol at harvest three; and appeared to be reduced at

harvest one (differences not quite statistically different at 5% level). All of five Fusarium isolates sampled from infected fruit were identified as *F. lactis* by PCR (John Clarkson, University of Warwick). Fusarium infection was primarily located on the internal walls of fruit and on seeds, often on both. When averaged across all three harvests, both Serenade ASO and T34 Biocontrol significantly reduced external Fusarium, internal Fusarium, Fusarium sporulation on seeds and Fusarium at any location in fruit (Table 15). Occasionally after the five day incubation period internal fruit rot had penetrated the fruit wall and showed as an external rot also (Fig. 4). It should be noted that where Fusarium infection is small and especially where it occurs only on seeds, it may be considered unimportant and overlooked by the end consumer; however, obvious rot of the internal wall or white/pink sporulation of Fusarium on the seeds could easily result in consumer complaints and returns.

Figure 4. Soft, sunken lesion of Fusarium at the tip of a fruit where an internal infection has rotted through the wall

Table 15. Effect of Serenade ASO and T34 Biocontrol on Fusarium internal fruit rot in Class I mature fruit – commercial trial, 2016

Harvest and treatment	% fruit affected (20 fruit/plot examined, incubated 5 days)			
	External Fusarium	Internal Fusarium	Fusarium on seeds	Any location on/in fruit
Harvest 1				
1. Untreated	0.7	2.9	2.1	3.6
2. Serenade ASO	0	0.7	0.7	0.7
3. T34 Biocontrol	0	0.7	0	0.7
Probability (12 df)	0.397	0.168	0.178	0.056
LSD	-	-	-	2.645
Harvest 2				

1. Untreated	0.7	2.1	1.4	2.1
2. Serenade ASO	0	0.7	1.4	1.5
3. T34 Biocontrol	0	2.1	1.4	3.6
Probability (12 df)	0.397	0.549	1.000	0.397
LSD				
Harvest 3				
1. Untreated	3.6	6.5	7.9	7.9
2. Serenade ASO	0	0	0.7	0.7
3. T34 Biocontrol	0	0	0.7	0.7
Probability (12 df)	0.095	0.015	0.017	0.017
LSD	-	4.640	5.265	3.690
Total harvest				
1. Untreated	1.7	3.8 (0.7)	3.8 (0.8)	4.5 (0.9)
2. Serenade ASO	0	0.4 (0.2)	1.0 (0.4)	1.0 (0.4)
3. T34 Biocontrol	0	1.0 (0.3)	0.7 (0.3)	1.7 (0.5
Probability (12 df)	<0.001	0.002	0.006	0.009

Objective 5. Monitoring occurrence of *Fusarium* sp. on flowers, young fruit and mature fruit in organic and conventional crops

The fungi and bacteria recovered from mature fruit, small green fruit and flowers sampled from both conventional and organic crops in 2016 are summarised in Tables 16-18 below. 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. Symptoms typical of Fusarium internal fruit rot were confirmed in mature fruit from both organic and conventional crops. In conclusion, and considering both 2015 and 2016 results, Fusarium internal fruit rot can occur in organic crops and, as in conventional crops, the level of infection can be very variable. It was not possible to draw conclusions on the relative levels of Fusarium internal fruit rot in organic and conventional crops due to no site using the same variety for both organic and conventional crops.

Table 16. Recovery of probable *Fusarium* sp. and other microorganisms from flowers of organic and conventional pepper crops: March – June 2016

Sample		% flowers				
Site	Crop	Probable *Fusarium*	*Cladosporium*	*Penicillium*	Bacteria	*Mucor*
March						

Site 1	Organic	0	0	8	0	
	Conventional	0	0	0	1	0
Site 2	Organic	0	24	46	6	1
	Conventional	0	6	49	3	1
April						
Site 1	Organic	0	0	40	0	0
	Conventional	0	0	36	0	0
Site 2	Organic	0	0	26	0	0
	Conventional	0	0	1	0	0
May						
Site 1	Organic	6	0	50	4	0
	Conventional	7	0	45	6	0
Site 2	Organic	0	0	50	5	0
	Conventional	17*	0	50	8	0
June						
Site 2	Organic	0	0	50	12	1
	Conventional	0	0	48	49	2

* confirmed as *F. lactis*. No samples taken at site 1 in June.

Table 17. Recovery of probable *Fusarium* sp. and other microorganisms from small green fruit of organic and conventional pepper crops: March – June 2016

Sample		% fruit affected						
Site	Crop	Browned	No growth	Probable Fusarium	Clad	Peni	Botrytis	Mucor
March								
Site 1	Organic	100	80	0	0	20	0	0
	Conventional	100	82	0	0	18	0	0
Site 2	Organic	100	84	0	0	16	0	0
	Conventional	100	80	0	0	18	0	2
April								
Site 1	Organic	100	64	0	0	34	0	0
	Conventional	100	86	0	0	14	0	0
Site 2	Organic	100	88	0	0	12	0	0
	Conventional	76	88	0	0	8	0	2
May								
Site 1	Organic	100	88	0	0	4	0	8
	Conventional	76	98	0	0	2	0	2
Site 2	Organic	100	78	0	0	22	0	0
	Conventional	100	90	0	0	10	0	0

June								
Site 2	Organic	100	70	0	10	14	0	6
	Conventional	100	82	0	2	10	0	6

Table 18. Occurrence of external and internal symptoms of Fusarium internal fruit rot in mature pepper fruit from organic and conventional crops: March – May 2016

Mature fruit			% of fruit			
Date	Site	Crop	External Fusarium	Internal Fusarium	Fusarium on seeds	Brown seeds
March	Site 1	Organic	-	-	-	-
		Conventional	-	-	-	-
	Site 2	Organic	-	-	-	-
		Conventional	36	27	0	0
April	Site 1	Organic	8	8	0	0
		Conventional	0	0	0	4
	Site 2	Organic	0	0	0	2
		Conventional	2	2	0	0
May	Site 1	Organic	0	0	0	0
		Conventional	0	0	0	0
	Site 2	Organic	0	0	0	0
		Conventional	0	0	0	0

Isolation of *Fusarium* colonies recorded on initial plates proved difficult due to the high levels of contamination present. Although *F. lactis* was confirmed at one site, in a conventional crop, no isolates from organic crops were obtained in clean culture to allow PCR analysis.

Conclusions

Objective 2 – Seed infection and transfer to rockwool

1. No evidence was found that commercial pepper seed in 2015 was infected with *F. lactis.*
2. A series of experiments confirmed that *F. lactis* can transfer from infested seed to the edge of a rockwool propagation cube.
3. Seed treatment with sodium hypochlorite improved germination following inoculation with *F. lactis*, reduced sporulation on infested seed, and reduced spread to rockwool cubes.

4. Irrigation with Jet 5 appeared to control spread of *F. lactis* from infested plugs to the rockwool cube.

Objective 3 – Efficacy of Serenade ASO sprays

5. A nursery trial in 2015 demonstrated the sprays of Serenade ASO + Codacide applied three times at weekly intervals to pepper cv. Cupra significantly reduced Fusarium internal rot in fruit that developed from the cohorts of flowers that were open when sprays were applied.
6. There was evidence that Serenade ASO also reduced Fusarium internal rot in fruit that developed from flowers which opened after application of sprays.
7. Work in the Netherlands indicates that Serenade ASO applied by ultra low volume misting equipment reduces Fusarium internal fruit rot; and that product efficacy is improved by use of a wetter (e.g. Silwet Gold). Note that in the UK application of Serenade ASO by ULV is not currently permitted; and it is necessary to reduce Serenade ASO concentration by 50% if a silicon-based wetter is used.
8. A nursery trial in 2016 demonstrated that HV sprays of Serenade ASO with no wetter applied three times at weekly intervals significantly reduced Fusarium internal fruit rot.

Objective 4 – Biofungicides and plant disease resistance inducers

9. A glasshouse experiment using inoculated plants and a nursery experiment reliant on natural infection by Fusarium both demonstrated that T34 Biocontrol applied as a foliar spray significantly reduced Fusarium internal fruit rot.
10. The level of control with T34 Biocontrol was equivalent to that of Serenade ASO. Note that T34 Biocontrol is not currently approved for use on protected pepper in the UK.
11. Two biological treatments (T34 Biocontrol and coded product F222) with potential as disease resistance inducers applied to pepper plants during propagation, from seed treatment to first appearance of flower buds, gave a small but significant reduction in Fusarium infection in small green fruit.

Objective 5 – Organic crops

12. Symptoms typical of Fusarium internal fruit rot were found in four out of 10 samples (2 crops x 3 sample occasions in 2015 + 2 crops x 2 sample occasions in 2016) of mature pepper fruit taken from organic crops at levels ranging from 2-14% of fruit after incubation for 5 days. There was no consistent difference between organic and conventional crops at the same sites.

General

13. Flowers of pepper are highly susceptible to infection by *F. lactis*. When a spore suspension of *F. lactis* in water was sprayed into the air above plants, 78% of small green fruit that developed were infected with the fungus.

14. The incidence of Fusarium infection in pepper flowers and small green fruit was generally found to be greater than in mature red fruit. This observation implies that some infected flowers and fruit abort and/or that Fusarium infection is restricted in some mature fruit.

Knowledge and Technology Transfer

Presentations

O'Neill TM & Mayne S (2015). Pepper fruit rots examined. AHDB Horticulture Cucumber and Pepper Conference, Waltham Abbey, 8 October 2015.

Mayne S (2016). Fusarium internal fruit rot of sweet pepper in UK crops. Bleiswijk Research Station, The Netherlands, 8 September 2016.

Articles

Mayne S & O'Neill TM (2015). Serenade takes on pepper rot. AHDB Grower, May 2016, pp 28-29.

O'Neill TM & Mayne S (2017). Latest results on pepper fruit rot. AHDB Grower, in press.

References

FV 402. Brassicas: pre-adaptation of seedlings for increased resistance to pest and pathogen attack.

Komada, H. (1976). A new selective medium for isolating Fusarium from natural soil. Proceedings of the American Phytopathological Society 3:221.

PC 260 (2008). Sweet pepper: aspects of the epidemiology of a stem and fruit rot caused by *Fusarium oxysporum*.

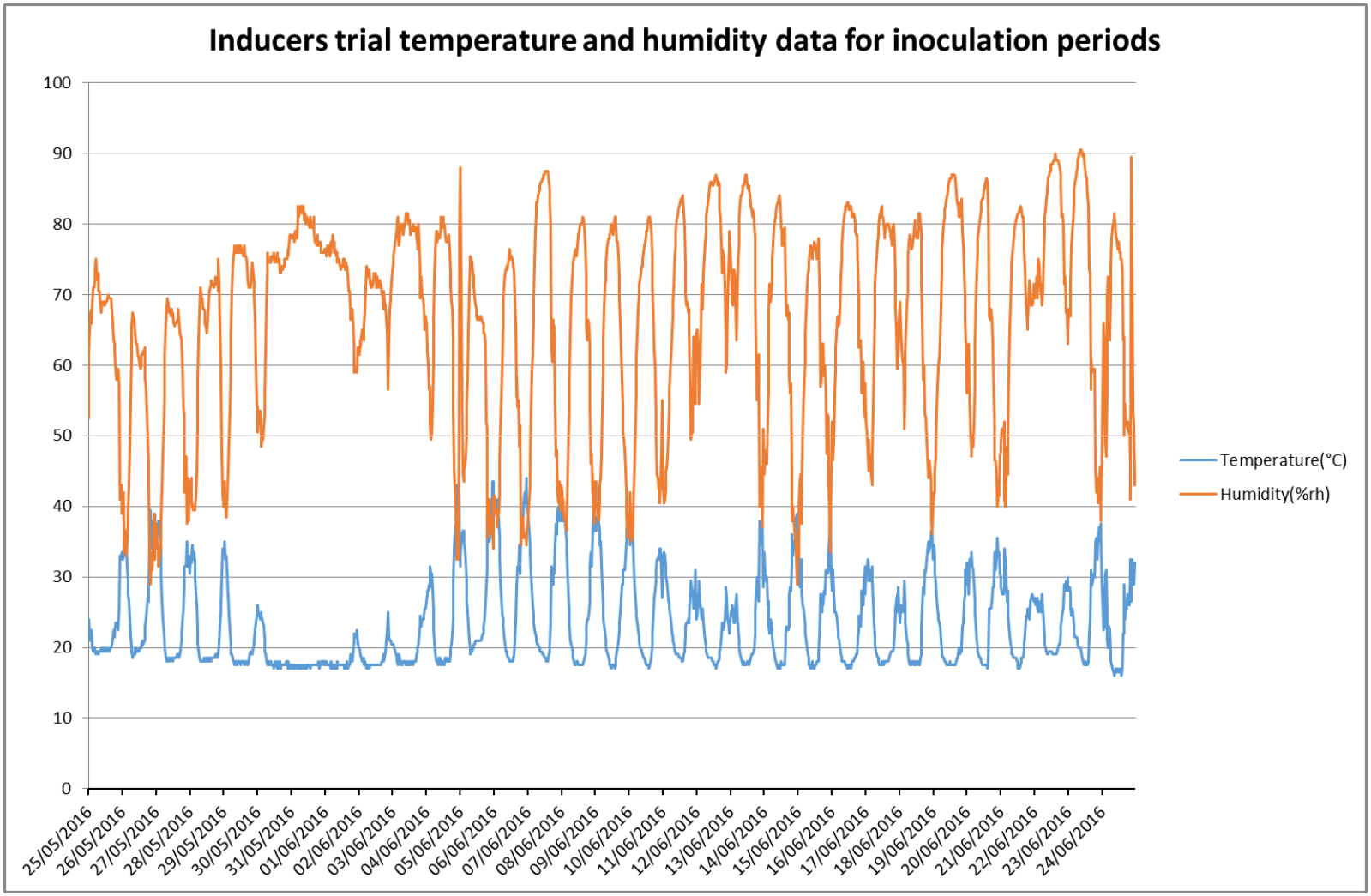
PC 007 (2014). Sweet pepper: aspects of the biology and control of Fusarium fruit rot.

PE 022 (2015). Pepper: improved control of Fusarium internal fruit rot through increased knowledge exchange with the Netherlands and Belgium and targeted application of plant protection products.

Appendix 1 Crop diary – Biological and inducer trial – ADAS Boxworth

Date	Biologicals and inducers trial
23/02/2016	Pepper seeds sown into plug trays and initial four inducer treatments applied
24/02/2016	Salinity stress preliminary experiment set up in glasshouse using salt concentrations of 30,60 and 120mM
01/03/2016	Salt stress preliminary treatments repeated
10/03/2016	Seedlings transferred to cubes
17/03/2016	Post transfer to cubes. First drenches applied
14/04/2016	Second drenches applied
13/05/2016	Third drenches applied
20/05/2016	Trial moved from propagation nursery to Glasshouse at Boxworth
24/05/2016	First flower sprays completed- Serenade and T34
25/05/2016	First inoculation completed with spore concentration of 5x10^2 F.Lactis spores/ml
02/06/2016	First fruit harvest
06/06/2016	Second flower sprays completed
07/06/2016	Second inoculation completed
14/06/2016	Second fruit harvest
20/06/2016	Third and final flower spray
21/06/2016	Third and final inoculation completed
27/06/2016	Third and final fruit harvest

Appendix 2 Inducers trial temperature and humidity data for inoculation periods



Appendix 3 Crop diary – Commercial trial – 2016

Date	Commercial trial
08/01/2016	Commercial pepper plants set up in glasshouse at Abbey View Nursery
29/07/2016	Trial set up and first sprays applied. Flowers marked with blue string to indicate first spray timing
05/08/2016	Second spray applied. White string used to mark flowers from the second spray timing. First small fruit harvest not possible due to commercial humidity issues affecting fruit set
12/08/2016	Third and final spray applied. Green string used to mark flowers from the third spray timing. Second green fruit harvest completed
19/08/2016	Third green fruit harvest completed
26/08/2016	Fourth green fruit harvest completed
02/09/2016	Fifth and final green fruit harvest completed
20/09/2016	First mature fruit harvest completed
26/09/2016	Second mature fruit harvest completed
03/10/2016	Third and final mature fruit harvest completed. All T34 treatments fruit picked and destroyed throughout trial

Appendix 4 Fusarium selective agar recipe (1 Litre),

(Komada, H. 1967)

- 1L Distilled water
- 1g $Na_2B_4O_7 \bullet 10H_2O$ (Borax)
- 1g K_2HPO_4 (DiPotassium hydrogen Phosphate)
- 0.5g KC1 (Potassium Chloride)
- 0.5g $MgSO_4 \bullet 7H_2O$ (Epsom salts)
- 0.01g Fe-Na- EDTA (Iron III Sodium salts)
- 20g D-Galactose
- 2g L-Asparagine
- 15g Agar technical number 3

Following Autoclave, at around 50-55°C, add:

- 1g PCNB
- 0.5g Bile Bovine
- 0.3g Streptomycin sulfate

Appendix 5 Commercial trial temperature and humidity data over spraying period