Project title:	Pepper: Improved control of Fusarium internal fruit rot through increased knowledge exchange with the Netherlands and targeted application of plant protection products – phase 2
Project number:	PE 022a
Project leader:	Dr Tim O'Neill, ADAS, Cambs CB23 4NN
Report:	Annual report, March 2016
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Location of project:	ADAS Boxworth, commercial sites – Herts & Somerset
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The results and conclusions in this report are based on an investigation conducted over a nine month period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

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

Background

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide, including the UK, for the last 15 years. The disease causes some losses on production nurseries but more importantly Fusarium continues to be a frequent cause of rejection by packhouses and product returns from supermarkets. Losses vary greatly between crops and seasons. We have shown several weakly pathogenic *Fusarium* species are associated with the disease, notably *F. lactis* and *F. oxysporum* in the UK. Fusarium spores deposited on the stigma during flowering grow rapidly through the style resulting in infection of seeds and internal fruit wall. Work in PE 007 demonstrated that a single spray of Serenade ASO applied to a crop during flowering can reduce the incidence of infection in fruit developing from treated flowers by around 50%. In PE 022 (phase 1) we showed *F. lactis* commonly occurs on rockwool propagation blocks in production glasshouses, a previously unknown source of the fungus. We also found that a high proportion of flowers and young fruits (1-2 cm diameter) in commercial crops were infected with *F. lactis*, yet only a relatively small proportion of fruit develop internal fruit rot. Results from 2014 and plans for 2015 were shared between the pathology teams at Bleiswijk Research Station (Wageningen University) and ADAS.

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

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

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

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

Summary

1. Information exchange with Dutch researchers

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Financial Benefits

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

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

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

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

Action Points

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

SCIENCE SECTION

Introduction

Cause and occurrence

In the UK a Fusarium internal fruit rot of sweet pepper was first identified in 2005 when it affected up to 20% of a day's pick on one nursery; the cause was identified as *F. oxysporum* (O'Neill, 2005). Subsequently the disease was shown to be present in many glasshouse crops, the severity varying with variety, nursery, glasshouse and time of year (O'Neill, 2008; Gill Wardell, pers comm.). The disease is generally more of a problem in the spring and autumn when fruit take longer to ripen (56-60 days), than in the summer (38-40 days). Isolates of *F. oxysporum* from sweet pepper caused a rot when wound-inoculated onto pepper fruit, but did not cause a wilt in pepper, cucumber, tomato or lettuce. Many isolates from sweet pepper fruit showed a distinctive orange/peach colour in culture, different from the usual purple or white colour of *F. oxysporum* isolates that caused wilt in tomato and other crops. The same orange/peach-coloured Fusarium was isolated from pepper seeds in fruit affected with internal fruit rot. Subsequent work in Belgium identified the peach-coloured Fusarium as *F. lactis*. Both *F. oxysporum* and *F. lactis* were confirmed associated with the disease in UK crops.

Fusarium was found to be relatively common in UK pepper crops, especially on aborted fruit, crop debris on the floor and rotting mature fruit missed by pickers. Increased crop hygiene to remove sources of inoculum is practised on some nurseries and this appears to give some control. Occurrence of *Fusarium* sp. on commercial seed suggests that this is a possible means by which the disease is introduced to a nursery, although it is unknown how such seed infection might lead to fruit infection. Hypochlorite seed treatment was shown to reduce occurrence of *Fusarium* sp. on packeted seed, but an experiment comparing the incidence of Fusarium fruit and stem rot on plants grown from treated and untreated seed was inconclusive due to a low incidence of the disease (PC 260).

Limited observations in the UK and the Netherlands suggest that Fusarium internal fruit rot is less common in pepper crops grown to organic standards. If this is true, then possibly it may be associated with an increased diversity and/or quantity of competitive saprophytic microorganisms in the environment that reduce infection of flowers by *F. lactis*, or increase levels of host resistance.

Control treatments

In PE 007 (2013) we showed that *F. lactis* was the predominant species causing Fusarium internal fruit rot throughout the year. A small proportion of rots were caused by *F. oxysporum* and *F. proliferatum.* We found a significant reduction in the disease of up to 50%, when a single spray of Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil) or Serenade ASO (*Bacillus subtilis* strain QST 713) was applied to flowers one day before they were artificially inoculated with *F. lactis* spores, and flowers were tagged so that the associated fruit developing from them could be identified. Further, in a whole row comparison study, a single spray of Serenade ASO applied to the crop face (flowers, leaves and stems), to rockwool cubes and floor (to treat fallen debris) was associated with a 50% reduction in Fusarium internal fruit rot of fruit developing from open flowers at that time.

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

Initial source of *F. lactis* spores

In PE 022 (2014), following observations made in the Netherlands, we examined occurrence of *F. lactis* on rockwool propagation cubes in three commercial crops. *Fusarium* species, predominantly *F. lactis,* were commonly isolated from the surface of rockwool cubes in August, September and October. Potentially this may be an important source for introducing *F. lactis* spores into glasshouses; subsequent spore transfer from sporulating colonies on rockwool cube surfaces to pepper flowers may occur by insects and/or in air currents.

Latent infection

Also in PE 022 (2014) we examined occurrence of *Fusarium* spp. in flowers and in the associated cohort of young (1-2 cm diameter) green pepper fruit in three crops on three occasions. A high incidence of *Fusarium* spp., predominantly *F. lactis*, was found in most of the flowers and fruits sampled. There was no evidence of a relationship between incidence of flower infection and incidence of fruit infection in the associated cohort of fruit. The incidence of infection in small green fruit ranged from 0-100%, but was most usually around 30%. It seems highly unlikely that all infected fruit abort before maturity, or develop internal fruit rot, or there would be very few fruit left to market from the crops monitored. It is suggested that these results indicate that sometimes a high proportion of developing pepper fruit may be infected with *F. lactis* (or other *Fusarium* spp.), but in only a fraction of them does the infection progress to cause visible rot symptoms in harvested fruit. Possibly treatments that enhance a plant's general resistance to disease, either through cultural practices, or

through specific treatments to induce systemic acquired resistance, may reduce the proportion of fruit in which Fusarium internal fruit rot becomes visible.

Collaboration with Wageningen University

During 2014 a series of conference calls were held between ADAS and Wageningen University (Bleiswijk Glasshouse Crops Research Station) plant pathology teams to exchange and discuss recent results, and to discuss plans for future work. The 2014 results from the Netherlands are described in PE 022 report (March 2015). Funding for a new research programme in the Netherlands was recently agreed. The work programme described in this HDC project is designed to complement the Dutch work. The UK experiments are primarily nursery-based and examine treatment efficacy under commercial conditions; the Dutch experiments are primarily research-station based and examine effects of individual treatments under strictly controlled conditions. 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.

Materials and methods

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

Throughout 2015 contact was maintained 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 January 2016, researchers also shared the results of their work thus far.

Objective 2. Seed infection and transfer to rockwool propagation cubes

Seed testing

To establish what, if any, level of Fusarium infection was present on current batches of commercial seed, seeds of five varieties from two seed companies was sourced in 2015. Varieties are coded (A-E) to preserve supplier confidentiality. Four of the varieties were not coated in any pesticide treatments. One variety was commonly sold with a fungicide seed coating (thiram), and so both treated and untreated seed of this variety were included. All seeds were supplied in foil packets, and batch numbers were recorded. Of each variety, 300 seed were plated directly, whilst another 300 were surface disinfected in a sodium hypochlorite solution. None of the thiram treated seed were washed in sodium hypochlorite, as it was thought this would only serve to remove some of the thiram coating. 265 thiram treated seed were supplied, and rather than delay the experiment further, only 265 were plated for this treatment. A summary of treatments is below (Table 3).

Table 3. Detail of pepper seeds examined for Fusarium

Treatment	Variety	Seed treatment	Sodium chlorite treatment	Number plated
1	A	No	Yes	300
2	A	No	No	300
3	A	Thiram	No	265
4	B	No	Yes	300
5	B	No	No	300
6	C	No	Yes	300
7	C	No	No	300
8	D	No	Yes	300
9	D	No	No	300
10	E	No	Yes	300
11	E	No	No	300

On opening seed packets, a visual assessment of any fungal growth present was made (presence or absence of fungal mycelium visible by eye). After immersion in 1% sodium hypochlorite for 5 minutes, seed were rinsed in sterile distilled water (SDW) three times and allowed to dry in a laminar flow cabinet. Seeds from all treatments were then plated onto Fusarium Selective Agar (FSA, recipe given in Appendix 1.), with ten seeds plated per plate. Plates were then placed in an incubator at 24°C and on a 12 hour light/dark cycle. Plates were checked regularly for fungal growth, and assessed at 4, 8 and 13 days after plating. Plates were assessed for incidence of colonies typical of *F. lactis*, incidence of other *Fusarium* species, and for any seeds remaining free of fungal growth. Any suspect *F. lactis* cultures were retained for confirmation by PCR test.

Transfer to rockwool cubes

To establish if commercial peppers 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 PDA+S and used to infest seed. Fifty seeds were soaked in 200 mls of spore suspension in SDW at 1.2 x 10^6 spores per ml for 24 hours. A few drops of Tween 20 were 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 FSA and assessed for *F. lactis* outgrowth for up to 14 days after plating.

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. The treatments tested are summarised below (Table 4).

Table 4. Pepper seed (cv. Kelly) or plant treatments following artificial seed infestation with *F. lactis* and planting in new rockwool plugs – ADAS Boxworth, 2016.

Treatment	Rockwool	Seed treatment	Irrigation treatment
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

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 C, high 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.

Seed were sown on 21st January. 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, and resultant fungal and bacterial growth developing from them was recorded. Growth was assessed on the 21st and 23rd March.

Occurrence of Fusarium on rockwool at plant receipt

On 12 January 2016, two commercial nurseries were visited within 24 hours of receipt of new plants. Rockwool from cubes of varieties Doppio (from Amalaan), Falko (also Amalaan) and Cupra (from HollandPlant) were sampled from Site one and from varieties Falko, EZ0061 and RZ0189, all from propagator WPK, were sampled from Site 2. Fifty pieces of rockwool were sampled from each variety/propagator combination at each site and returned to ADAS Boxworth. Pieces of rockwool were not incubated on receipt at Boxworth to avoid encouraging the growth of other saprophytes such as *Mucor* spp., but instead were directly plated onto agar.

Occurrence of Fusarium on propagation nurseries

In late 2015/early 2016, no UK propagator with orders for supply of pepper plants to UK growers could be sourced. We therefore requested that a UK propagator grow plants of two commercial varieties specifically for this project using the same crop husbandry methods as they would for a commercial client. Thirty plants of cv. Ferrari and 30 of cv. Fiesta were grown from a late December 2015 sowing. On 22nd February, ADAS staff visited the propagation nursery to sample rockwool on-site. Fifty pieces of rockwool were sampled from each variety, returned to ADAS Boxworth and directly plated onto agar.

Throughout this objective, any Fusarium isolates recovered, especially those suspected of being *F. lactis*, were subbed onto fresh agar. A sub-set of isolates were sent to John Clarkson at Warwick Crop Centre for identification by PCR.

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

A fully randomised trial was set up in an established commercial crop of pepper cv. Cupra in July 2015. A whole crop row on one side of the central concrete pathway was a single plot (approx. 46 m x 2 m, with a 4.5 m discard at either end) and treatments were replicated three times. Treatments involved a single spray of Serenade ASO compared with three weekly sprays and untreated plots. Three temperature and relative humidity loggers were placed in the trial, one in each treatment. Plots were sprayed with a backpack sprayer fitted with a specialised boom, modified to best reflect commercial spray equipment. The boom was fitted with four low-drift hollow cone nozzles (Lurmark 30HCx4) and Serenade ASO was applied at the commercial rate of 10 L/ha and a water volume of 1000 L/ha. The spray equipment achieved a fine mist of Serenade ASO, which effectively coated leaves and flowers in fine droplets which did not run off. As well as applying the product to the crop, Serenade ASO was also applied with a lance to the floor of the rows, and to the surfaces of rockwool cubes. This application was difficult as the lower leaves of the pepper plants shielded some of the rockwool surface. As recommended, Serenade ASO was applied with an oil based wetter (Codacide at 0.83 L/ha). At each spray, 10 flowers per treatment were tagged in order to mark the cohort of flowers that received the sprays and follow them through to mature, ripe fruit. Following the first and third spray application, 50 pieces of rockwool per sprayed plot were sampled at each timing and returned to ADAS Boxworth. There they were damp incubated for 7 days, after which presence of *Fusarium* species was checked. Following this they were plated onto PDA+S and assessed for outgrowth of *Fusarium* species. Rockwool was sampled in the crop wearing gloves which were sterilised with alcohol between samples.

At one week after each spray application, 90 small green fruit (size gauged by the size of the fruit that had developed from tagged flowers) were sampled from each plot. Each plot was split into 3 sub-plots of equal size, and 30 small green fruit were sampled from each sub-plot. Green fruit were returned to ADAS Boxworth and laid out on tissue paper in trays, at least 2 cm apart to avoid them touching. After 4 weeks, fruit were cut open and examined for fungal growth, and specifically for *Fusarium* species. Any growth observed was checked under the microscope, and a subsample of 10 fruit were plated onto PDA+S. Green fruit were sampled three times, a week after each spray, and for an additional 2 weeks following this, giving five samples in total.

When fruit sprayed with the first (or only) Serenade ASO spray had reached full maturity (fully red; the point in growth they would usually be harvested) harvesting of the trial began. Fruit in each whole row plot were harvested by nursery staff into a crate left at the end of each row, in the central concrete pathway. Fruit in these crates were then assessed on-site by ADAS staff as marketable (no blemishes), unmarketable due to external symptoms of Fusarium, and unmarketable for other reasons (e.g. sunscorch, blossom end rot, poor size/shape). Fifty marketable fruit were then returned to ADAS Boxworth and incubated at ambient temperature in the Pathology laboratory for five days. After this incubation period, fruit were destructively assessed and incidence of Fusarium internal fruit rot, Fusarium growth on seed, and external Fusarium fruit rot were recorded. Additionally, all unmarketable fruits were assessed in the same way. The trial was harvested and assessed in this way five times, the first three times according to the size of tagged fruit from sprays 1-3 and also one and two weeks after the fruit from the final spray had been harvested. A crop diary showing dates of key events is given in Appendix 3.

Objective 4. Examination of a range of biopesticides and potential resistance inducers (growth stimulants) for control of Fusarium fruit rot

In January 2016, potential products to include in tests were investigated through liaison with the industry representative, AHDB Horticulture, and based on information from scientific literature. A set of five biological products were chosen for evaluation as potential resistance inducers (PRI), applied pre-flowering, and two as biofungicides (BioF) examined as protectants, applied to the flowers (Table 5). Products were applied on the propagation nursery from February 2016 until the point of first flowering and at ADAS Boxworth thereafter.

Table 5. Detail of resistance inducer and biofungicide treatments applied at a propagation nursery/ADAS Boxworth – spring 2016

Treatment	Product	a.i.	Application	
			PRI	BioF
1.	Untreated	-	-	-
2.	Serenade ASO	*Bacillus subtilis*	✓	-
3.	Serenade ASO	*Bacillus subtilis*	-	✓
4.	T 34 Biocontrol	*Trichoderma asperellum*	✓	-
5.	T 34 Biocontrol	*Trichoderma asperellum*	-	✓
6.	Trianum P	*Trichoderma harzianum*	✓	-
7.	Salt	sodium chloride	✓	-
8.	HDC F222	plant extract	✓	-

Further experimental detail and results will be given in the Final report.

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, and flowers, small green fruit and mature fruit sampled from conventional and organic crops. Crops at Site 1 were sampled on 21 July, 24 August and 21 September; crops at Site 2 were sampled on 28 July, 24 August and 28 September. Mature fruit sampled were Class 2 where possible to minimise impact on the growers. The crops sampled are summarised below in Table 6. Organic crops sampled were of the same variety, but conventional crops located close by to the organics were of different varieties at each site.

Table 6. Conventional and organic crops sampled for flowers, small green fruit and mature red fruit, 2015

Site	Growing system	Variety
1.	Organic	Artega
1.	Conventional	Sapporo
2.	Organic	Artega
2.	Conventional	Falko

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. On the second sampling occasion, sampled flower parts were plated onto both PDA+S and FSA in order to gauge if this was more effective at recovering *Fusarium* species. 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.

Sampling will continue at monthly intervals for 4 months in 2016 from the time first mature fruit are available.

Results and Discussion

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

In 2015, researchers in the Netherlands also further investigated the use of Serenade ASO for control of *F. lactis*. In laboratory tests 100% control was achieved with Serenade ASO at a low spore density of *F. lactis* (1 x 10^3 per ml). As in previous years, at a high spore density Serenade ASO had no effect. A glasshouse trial was established in September where Serenade ASO was applied with ultra-low volume misting equipment, one day before inoculation. Infection was reduced by 35%, which is promising as the glasshouse had a much higher density of *F. lactis* spores than usually found in commercial glasshouses.

Work will be continuing in the Netherlands in 2016, with focus moving towards testing more antagonists of *F. lactis*, and looking at the impact of water relations in the pepper plant influencing fruit firmness (and therefore, also Fusarium fruit rot). It has been postulated that if the plant has a high root pressure it will have a thinner fruit bottom, so be more susceptible to invasion.

Objective 2. Seed infection and transfer to rockwool propagation cubes

Seed testing

On opening the seed packets, no fungal growth was visible on any of the seeds. The fungi and bacteria that grew on commercial lots of pepper seed are shown in Table 7. Seven colonies were identified as possible *Fusarium* spp., the majority of which developed from seed of variety D that had been treated with hypochlorite. The most common fungi isolated from seed were *Aspergillus* spp. Varieties D and E both also had *Mucor* spp. present. In most cases, treatment with sodium hypochlorite reduced the number of fungal colonies which developed from plated seed. However, whether treated or not, no seed of variety D remained clean. The differences in microbes recovered from seed do not seem to be related to seed company.

Table 7. Fungi and bacteria recovered from pepper seed plated directly and after surface disinfection with sodium hypochlorite – ADAS Boxworth, 2015

Variety	SD	No. of seeds with probable *Fusarium*	No. of clean seeds	*Aspergillus*	*Penicillium*	Bacteria	*Mucor*
A	-	0	0	298	300	0	0
A	✓	0	254	33	46	0	0
B	-	0	0	289	300	0	0
B	✓	0	263	4	37	0	0
C	-	0	0	241	300	0	0
C	✓	5	231	46	69	0	9
D	-	0	0	293	300	9	10
D	✓	0	0	24	280	0	114
E	-	1	192	49	85	11	12
E	✓	1	95	93	187	0	10
E (thiram)	-	0	160	64	96	20	0

SD – surface disinfected in sodium hypochlorite.

Transfer to rockwool cubes

Following the low levels of *F. lactis* found naturally on commercial pepper seeds, artificially infestation of seed was deemed necessary. The infestation 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 8). 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 in a rockwool plug to the edge of a rockwool propagation cube, and the effect of hypochlorite and Jet 5 disinfectant treatments on the level of transfer. Visible *F. lactis* sporulation was evident on inoculated seeds that had failed to germinate, and on some seeds that had germinated. On plating rockwool pieces from the edge of cubes, and from the edge of propagation plugs, a considerable number of very probable *F. lactis* colonies had developed after 5 days incubation (Table 8). Colonies of a *Mucor* species were also commonly isolated. Treatment had a significant effect on the incidence of *Mucor* recovered when cubes and plugs were considered separately ($P<0.001$) but not when data was combined. There were also

significant differences in the incidence recovered from plugs and cubes (P<0.001). 100% of untreated cubes developed *Mucor* when plated, but 0% of plugs. Generally, a higher incidence of *Mucor* was associated with a lower incidence of *F. lactis*.

Table 8. 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

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

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

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 airbone route, when conditions are favourable.

Occurrence of Fusarium on rockwool at plant receipt

The fungi and bacteria isolated from rockwool sampled at two commercial sites within 24 hours of plant receipt are summarised (Table 9). Five combinations of variety/propagator were sampled across both sites.

Table 9. Recovery of *Fusarium* sp., other fungi and bacteria from rockwool cubes shortly after plant receipt on pepper production nurseries – January 2016

Site	Variety	*Fusarium*	*Penicillium*	Bacteria	*Pythium*	*Aspergillus*	*Mucor*
Site 1	EZ0061	1	41	9	23	18	0
	RZ0189	0	20	1	19	21	21
	Falko	0	22	2	2	47	0
Site 2	Falko	11	11	0	0	50	5
	Doppio	24	4	0	0	50	11
	Cupra	0	5	0	0	50	7

The appearance of the *Fusarium* sp. isolated was pink/purple rather than the peach colour usually found with *F. lactis*. Unfortunately it was not possible to identify isolates to species level by PCR due to the presence of contaminating microorganisms. Despite repeated attempts, no clean cultures were obtained.

Occurrence of Fusarium on propagation nurseries

When rockwool sampled from the cubes of pepper seedlings at a plant propagator, a number of fungal colonies developed. All rockwool sampled from both varieties Ferrari and Fiesta developed colonies of a *Mucor* species. No *Fusarium* species developed from plated rockwool taken from the cubes of cv. Fiesta, and two *Fusarium* species developed from plated rockwool from 36 cubes of cv. Ferrari, though based on colony morphology and colour these are not *F. lactis*. It is likely that a greater of number of propagators would have to be surveyed to rule out propagation as a potential source.

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

Small green fruit and mature red fruit were each first harvested when it was judged that the cohort of flowers treated with the first Serenade ASO spray had reached these growth stages, based on monitoring the development of a sample of tagged flowers. Thereafter fruits were sampled at weekly intervals, to match the application of Serenade ASO at weekly intervals,

for three weeks, and then weekly for two subsequent weeks. The experiment was located in a house and on a variety with a history of the disease and relied on natural infection pressure.

With small green fruit, very little *Fusarium* sp. was found at 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 (Table 10). 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 (Table 11). 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 (Table 11).

Table 10. Effect of Serenade ASO sprays on occurrence of *Fusarium* sp. in small green fruit at weekly intervals for five weeks after 1 or 3 sprays of the product to flowers – 2016

		% fruit affected				
Sample	Treatment	*Fusarium*	*Penicillium*	*Botrytis*	*Bacteria*	No growth
Harvest 1						
1.	Untreated	0.0	0.2	0.0	0.0	99.6
2.	One spray	0.1	0.3	0.0	0.0	99.0
3.	Three sprays	0.2	0.6	0.0	0.0	98.0
Significance		-	-	-	-	0.655
LSD		-	-	-	-	2.448
Harvest 2						
1.	Untreated	0.0	0.0	0.0	0.0	100.0
2.	One spray	0.0	0.1	0.0	0.0	99.6
3.	Three sprays	0.0	0.1	0.0	0.0	99.6
Significance		-	-	-	-	0.694
LSD		-	-	-	-	1.320
Harvest 3						
1.	Untreated	0.0	0.0	0.0	0.0	100
2.	One spray	0.0	0.2	0.2	0.0	99.6
3.	Three sprays	0.0	0.1	0.0	0.0	100
Significance		-	-	-	-	0.444
LSD		-	-	-	-	0.924
Harvest 4						
1.	Untreated	21.5	0.1	0.0	0.0	78.1
2.	One spray	20.4	0.0	0.0	0.0	80.0
3.	Three sprays	8.1	0.1	0.0	0.0	91.5
Significance		0.039	-	-	-	0.034
LSD		10.18	-	-	-	9.52
Harvest 5						
1.	Untreated	14.4	0.0	0.0	0.0	65.6
2.	One spray	11.7	0.0	0.2	0.4	88.3
3.	Three sprays	3.9	0.0	0.0	0.0	96.1
Significance		0.042	-	-	-	0.042
LSD		7.71	-	-	-	7.71

Results were analysed where sufficient non-zero values were present.

Table 11. Effect of Serenade ASO sprays on number of marketable fruit and occurrence of Fusarium internal fruit rot symptoms at harvest at weekly intervals for 5 weeks after 1 or 3 sprays of the product, plus % affected after 5 days incubation – 2015

Harvest and treatment	Mean number of harvested fruit in each category at harvest			% with external Fusarium symptoms after incubation for 5 days
	Marketable	Unmarketable (for reason other than Fusarium)	External Fusarium symptoms*	
Harvest 1				
1. Untreated	50.2	2.11	0	2.53
2. Serenade (x1)	39.6	2.44	0.11	0.95
3. Serenade (x3)	44.3	1.56	0	3.06
Significance	0.325	0.406		**0.011**
LSD	17.08	1.652		1.038
Harvest 2				
1. Untreated	73.8	2.44	0	3.09
2. Serenade (x1)	63.9	1.89	0.11	0.46
3. Serenade (x3)	76.2	1.89	0	2.05
Significance	0.51	0.733		0.074
LSD	28.65	2.174		2.24
Harvest 3				
1. Untreated	58.9	2.47	0	427
2. Serenade (x1)	70.4	1.89	0.11	4.74
3. Serenade (x3)	71.7	2.56	0	5.51
Significance	0.426	0.74		0.953
LSD	26.85	2.833		11.135
Harvest 4				
1. Untreated	88.2	1.89	0	3.99
2. Serenade (x1)	92.7	2.56	0	2.44
3. Serenade (x3)	97.3	3	0.22	1.34
Significance	0.625	0.191		0.414
LSD	24.59	1.368		4.906
Harvest 5				
1. Untreated	75	1.11	0	8.83
2. Serenade (x1)	71	1.78	0	3.99
3. Serenade (x3)	71.7	0.33	0	0
Significance	0.683	0.24		**0.042**
LSD	12.98	1.967		6.241

*numbers of fruit with Fusarium at this assessment were too low to carry out an ANOVA.

All assessment categories are summarised in Table 12 to show the proportion of marketable fruit (50 examined per plot) and the proportion of all fruit (the 50 marketable plus all unmarketable per plot) with external symptoms, internal symptoms, or any symptoms of Fusarium internal fruit rot after this incubation period.

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 (Table 12, right hand columns), there were significant reduction in Fusarium internal fruit rot following one Serenade ASO spray at harvest 1 and following three Serenade ASO sprays at harvest 5.

The detection of *Fusarium* sp. in mature fruit at harvests 1-3 and rarely in small green fruit at these timings may indicate that, in this experiment, incubation of mature fruit at ambient temperature for 1 week was a better method for assessing the true level of infection than incubation of small green fruit for 1 month. The intention of the experiment was that small green fruit and mature red fruit were both harvested at sample times such that they were fruit that had developed from the same cohort of flowers. Possibly this was not achieved, and this could also result in a discrepancy between small green fruit and mature red fruit results because of different infection pressure on flowers at different times in the season.

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.

In mature red fruit, the highest levels of Fusarium infection detected was at harvest 5, and for small green fruit at harvest 4 (Table 10 and 12). 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. A comparison of the RH and temperature for the 24 h periods before and after sprays 1-3 and the subsequent 2 weeks (weeks 4 and 5), shows that at week 5 flowering the RH had been greater than 90% for the 4 nights prior. Additionally, RH did not drop as low in the days as previously, reaching lows between 70 and 80%, whereas in the preceding period daytime humidities of around 60% were more usual. RH in the commercial glasshouse largely remained below 90% at night for the preceding period, though was also slightly above 90% on the night of spray 3 and increases again to higher humidities towards the end of September as mature fruit are being harvested. In terms of glasshouse temperature, temperatures remained between 20 and 30

around week 5, whereas in weeks 1-4, there were periods where temperatures above 30 were reached in the daytime.

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 of 5 developed from flowers that opened after spray 3. For example, some fruit might have developed from flowers open at spray 3 but which developed or ripened more slowly than the ones developing from tagged flowers. In future work, it may be useful to tag flowers opening at 1-3 weeks after the final Serenade ASO spray to determine if Fusarium internal fruit rot is reduced in fruit that develop from these flowers.

Table 12. Effect of Serenade ASO spray treatments on Fusarium internal fruit rot, assessed 5 days after harvest following ambient incubation – 2015

Harvest and treatment	% marketable fruit with:			% all fruit with		
	External symptoms	Internal symptoms	Any symptoms	External symptoms	Internal symptoms	Any symptoms
Harvest 1						
1. Untreated	1.1	6.1	7.2	2.5	6.5	9.1
2. Serenade(x1)	0	5.6	6.1	**1.0**	6.3	7.7
3. Serenade(x3)	2.2	5.6	5.6	3.1	6.6	6.6
Significance	0.250	0.969	0.744	0.011	0.991	0.653
LSD	3.08	7.01	5.90	1.04	7.08	7.19
Harvest 2						
1. Untreated	2.8	2.8	5.6	3.1	4.5	7.0
2. Serenade(x1)	0.6	2.8	4.4	0.5	3.1	4.6
3. Serenade(x3)	1.1	2.2	3.9	2.1	3.1	5.1
Significance	0.145	0.790	0.881	0.074	0.198	0.753

LSD	2.51	2.51	9.21	2.24	1.98	9.152
Harvest 3						
1. Untreated	4.4	7.8	10.0	4.3	7.4	9.5
2. Serenade(x1)	3.9	5.0	6.7	4.7	5.8	7.3
3. Serenade(x3)	3.9	6.1	8.3	5.5	7.6	10.1
Significance	0.991	0.881	0.808	0.953	0.911	0.785
LSD	13.32	15.16	13.80	11.14	12.51	11.59
Harvest 4						
1. Untreated	4.4	3.9	6.7	4.0	3.9	7.0
2. Serenade(x1)	2.2	4.4	6.7	2.4	4.6	6.6
3. Serenade(x3)	0	1.1	2.8	1.3	2.9	4.8
Significance	0.148	0.069	0.298	0.414	0.539	0.700
LSD	4.87	2.95	6.84	4.96	4.00	7.42
Harvest 5						
1. Untreated	7.8	9.4	12.8	8.8	11.0	14.3
2. Serenade(x1)	3.3	6.1	7.2	4.0	7.2	8.3
3. Serenade(x3)	0	0.6	5.6	**0**	**0.6**	7.1
Significance	0.070	0.116	0.110	0.042	0.045	0.051
LSD	6.48	8.95	11.41	6.24	7.59	9.95
All harvests						
1. Untreated	5.1	5.4	6.9	5.4	6.1	7.9
2. Serenade(x1)	3.1	4.6	5.0	3.6	5.2	5.8
3. Serenade(x3)	2.1	2.4	3.2	4.7	3.6	4.6
Significance	0.183	0.295	0.307	0.260	0.340	0.339
LSD	3.66	4.66	5.67	3.55	4.29	5.54

Values in bold are significantly different ($p < 0.05$) from the untreated.

Colonies suggestive of *F. lactis* and other *Fusarium* species were recovered from the rockwool cubes sampled one week after the first and third Serenade ASO sprays (Table 13). Levels of recovery were relatively low (0-10% of pieces for *F. lactis*) and there was no consistent trend in reduction of recovery with increase in number of Serenade ASO sprays. In practice, it was difficult to apply the Serenade ASO spray to the cube surface due to the

shading effect of lower leaves and the occurrence of ferns growing in the cubes. This may account for the lack of a treatment effect in this work.

Rockwool pieces were also sampled on two occasions (7 days after the first and third sprays had been applied). The results of isolations carried out on these pieces can be seen in Table 13. The effect of Serenade ASO treatment on recovery of Fusarium from rockwool cubes was unclear. This may have been due to the difficulty in applying product to adequately cover the surface.

Table 13. Recovery of *F. lactis* and other fungi from rockwool cube surface after treatment with Serenade ASO - 2015

Rockwool sampled	Treatment	% of colonies developed				
		Suspect *F. lactis*	Other *Fusarium*	*Mucor*	*Penicillium*	Bacteria
18th Aug	Untreated	0	4	100	20	32
	Serenade (x 1)	4	6	100	42	46
1st Sep	Untreated	10	18	100	2	58
	Serenade (x 1)	10	26	100	4	72
	Serenade (x 3)	0	18	100	4	32

Objective 4. Examination of a range of biopesticides and potential resistance inducers (growth stimulants) for control of Fusarium fruit rot

This experiment is currently (February 2016) in progress. Results will be reported at the end of this project in 2017.

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 summer 2015 are summarised in Tables 14-16 below. It should be noted that whilst the varieties sampled from organic crops were the same for both sites, the varieties sampled from conventional crops were different.

Probable *Fusarium* species were recovered from flowers, small green fruit and mature red fruit and from all four crops. *Fusarium* was present at levels ranging from 0-30% in flowers, 0-24% in small green fruit and 0-14% in mature red fruit after incubation. The greatest level of infection in mature red fruit (14%) was recorded in the organic crop at site 1 (cv. Artega) in September. Levels in organic and conventional crops were broadly similar. In September, levels were consistently greater in both organic and conventional crops at site 1 than at site

2; this was true for flowers, small green fruit and mature red fruit. The certainty of identification of *Fusarium* infection is greatest for mature red fruit (Table 16), with near 100% confidence that the probable Fusarium in these fruit is pathogenic because of the occurrence of typical Fusarium fruit rot symptoms. Based on previous results, we consider it likely that the probable *Fusarium* sp. reported in flowers and small green fruit is also a *Fusarium* sp. pathogenic to pepper. Two *Fusarium* species isolated from Site 1 were confirmed as *F. avenacearum* by DNA identification test at Warwick Crop Centre.

In addition to *Fusarium*, fungi found associated with sampled tissues, at relatively high levels, were *Cladosporium, Penicillium* and *Mucor* in flowers and *Penicillium* and *Botrytis* in small green fruit. Fungi other than *Fusarium* were rarely found in the sampled mature red fruit. The incidence of *Mucor* sp. was consistently greater on flowers from conventional crops than from organic crops (Table 17). Results do not support the hypothesis that organic pepper flowers or small green fruit were colonised by a greater incidence or diversity of non-pathogenic fungi than the same tissues sampled from conventional crops.

Table 14. Recovery of probable *Fusarium* sp. and other microorganisms from flowers of organic and conventional pepper crops: July – Sep 2015

Sample		Isolation Agar	% flowers				
Site	Crop		Probable *Fusarium*	*Cladosporium*	*Penicillium*	Bacteria	*Mucor*
July							
Site 1	Organic	PDA+S	6	100	96	0	0
	Conventional	PDA+S	0	2	72	0	70
Site 2	Organic	PDA+S	6	12	94	6	6
	Conventional	PDA+S	12	18	80	22	0
August							
Site 1	Organic	PDA+S	8	100	0	0	0
		FSA	0	98	5	3	3
	Conventional	PDA+S	30	26	35	0	94
		FSA	0	29	23	0	94
Site 2	Organic	PDA+S	12	0	98	42	22
	Conventional	PDA+S	2	0	94	18	26
September							
Site 1	Organic	PDA+S	12	0	98	42	10
	Conventional	PDA+S	2	0	88	14	16
Site 2	Organic	PDA+S	0	0	100	0	0
	Conventional	PDA+S	2	4	54	6	30

Table 15. Recovery of probable *Fusarium* sp. and other microorganisms from small green fruit of organic and conventional pepper crops: July – Sep 2015

Sample		% fruit affected						
Site	Crop	Browned	No growth	Probable Fusarium	Clad	Peni	Botrytis	Mucor
July								
Site 1	Organic	72	68	2	4	6	16	0
	Conventional	72	84	0	0	10	6	0
Site 2	Organic	92	68	0	0	20	0	2
	Conventional	82	78	2	2	16	2	0
August								
Site 1	Organic	86	86	0	0	14	0	12
	Conventional	40	86	2	0	0	0	0
Site 2	Organic	98	68	16	0	16	0	0
	Conventional	98	64	24	0	12	2	0
September								
Site 1	Organic	88	74	22	0	4	0	0
	Conventional	72	80	10	0	6	6	0
Site 2	Organic	90	64	2	0	8	24	0
	Conventional	70	52	6	0	22	16	2

Table 16. Occurrence of external and internal symptoms of Fusarium internal fruit rot in mature pepper fruit from organic and conventional crops: July – Sep 2015

Mature fruit			% of fruit			
Date	Site	Crop	External Fusarium	Internal Fusarium	Fusarium on seeds	Brown seeds
July	Site 1	Organic	2	0	0	16
		Conventional	2	2	2	22
	Site 2	Organic	0	2	0	30
		Conventional	2	0	0	28
August	Site 1	Organic	0	4	0	34
		Conventional	0	0	0	4
	Site 2	Organic	0	0	0	36
		Conventional	0	0	0	32
September	Site 1	Organic	14	14	4	24
		Conventional	2	6	0	18
	Site 2	Organic	2	4	2	6
		Conventional	0	2	2	8

Isolation of *Fusarium* colonies recorded on initial plates proved difficult due to the high levels of contamination present. It was therefore not possible to undertake PCR tests for identification to species level. There was a high level of *Mucor* in all tissues isolated, and this is recorded in Table 17 below.

Table 17. Recovery of *Mucor* sp. from flowers and small green fruit of organic and conventional pepper crops: July – Sep 2015

			% affected	
Date	Site	Crop	Flowers	Small green fruit
July	Site 1	Organic	0	0
		Conventional	70	0
	Site 2	Organic	6	2
		Conventional	0	0
August	Site 1	Organic	2	12
		Conventional	94	0
	Site 2	Organic	22	0
		Conventional	26	0
September	Site 1	Organic	10	0
		Conventional	16	0
	Site 2	Organic	0	0
		Conventional	30	2

Conclusions

Serenade ASO

- A nursery trial demonstrated that HV sprays of Serenade ASO + Codacide applied three times at weekly intervals to pepper cv. Cupra significantly reduced Fusarium internal fruit rot in fruit developing from the cohort of flowers that were open when sprays were applied.
- There was some evidence that the Serenade ASO also reduced Fusarium internal rot in fruit that develop from flowers which opened after application of sprays.
- 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 it is necessary to reduce Serenade ASO concentration by 50% if a silicon-based wetter is used.

Transfer of *F. lactis* from seed to rockwool

- A series of experiments confirmed that *F. lactis* can transfer from pepper seed to the edge of a rockwool propagation cube
- Seed treatment with sodium hypochlorite improved germination following inoculation with *F. lactis*, reduced sporulation on infested seed, and reduced spread to rockwool plugs
- Irrigation with Jet 5 appeared to control spread of *F. lactis* from infested plugs to the rockwool cube
- Some isolates of *Fusarium* spp. were recovered from 2015 batches of commercial pepper seed

Fusarium internal fruit rot in organic crops

- Symptoms typical of Fusarium internal fruit rot were found in three out of six samples (2 sites x 3 sample occasions) of mature pepper fruit taken from organic crops, at levels affecting 2-14% of fruit after incubation for 5 days. This was similar to incidence of the disease in conventional crops from the same sites.

Knowledge and Technology Transfer

Presentation

Pepper fruit rots examined. Presentation by Tim O'Neill and Sarah Mayne at AHDB Horticulture Cucumber and Pepper Conference, Waltham Abbey, 8 October 2015.

Article

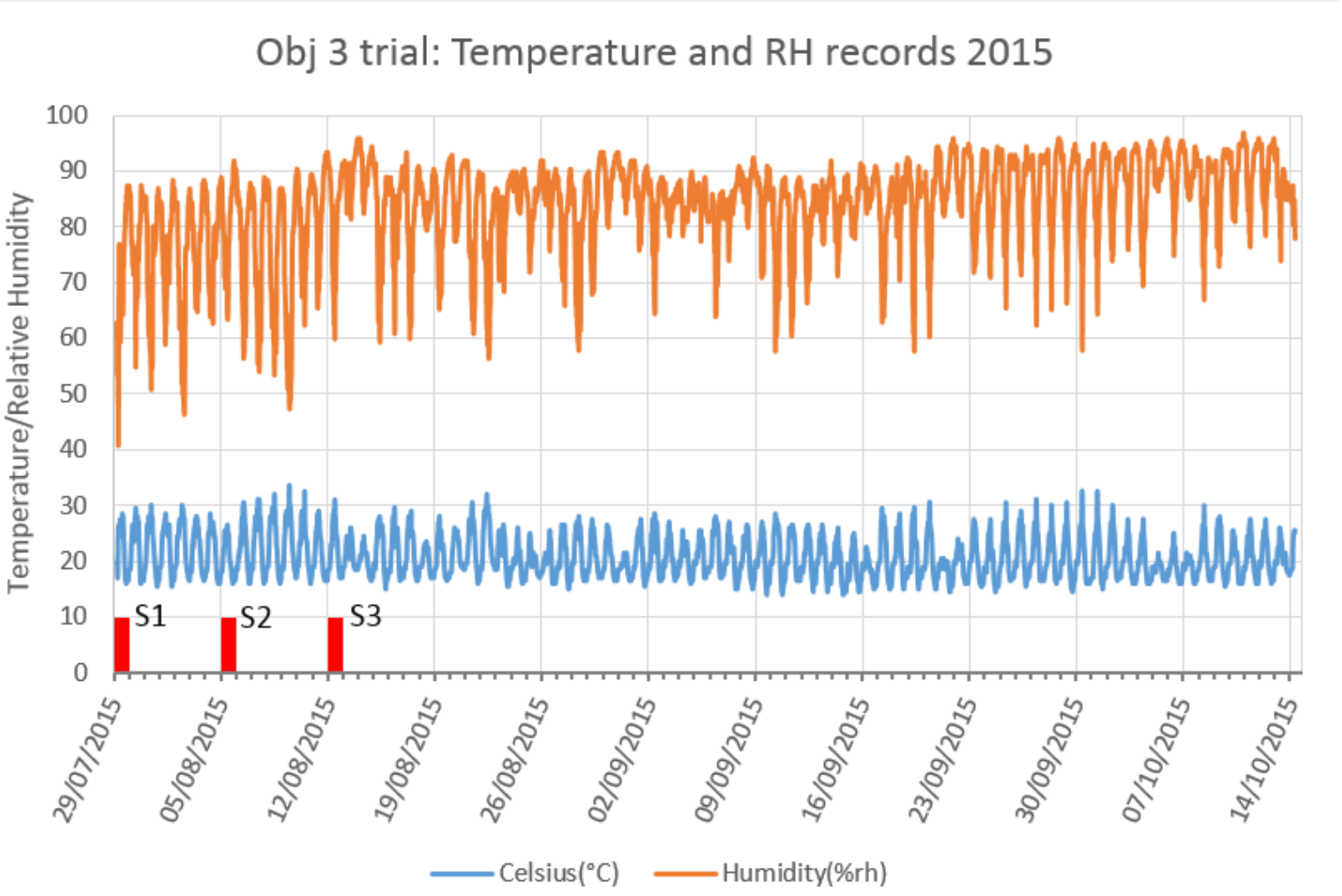
O'Neill TM & Mayne S (2016). Improved prospects for rot control. *AHDB Grower* (in press).

Appendices

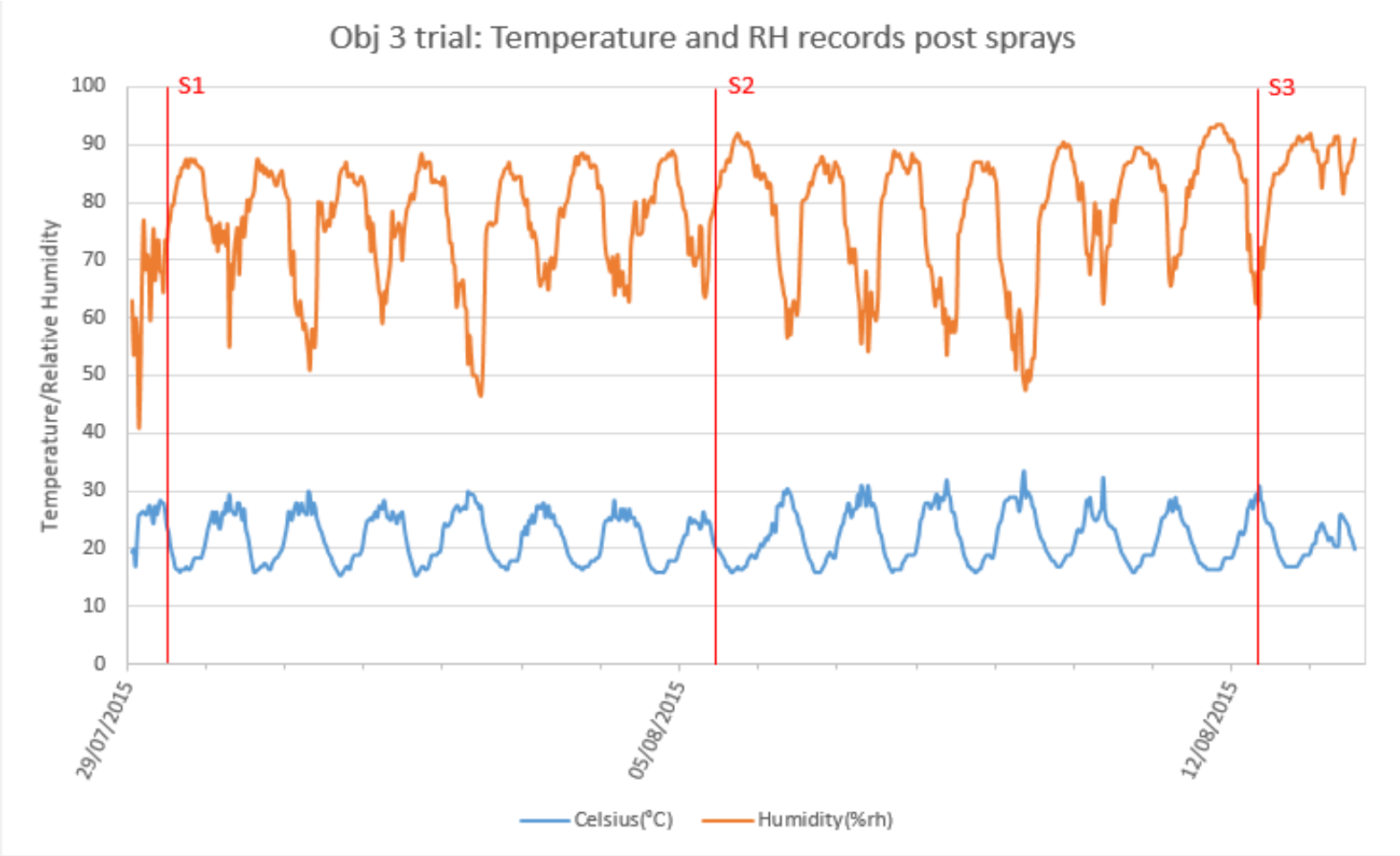
Appendix 1. Fusarium Selective Agar recipe

Dextrose	20 g
KH_2PO_4	500 mg
$NaNO^3$	2.0 g
$MgSO_4$.7H2O	500 mg
Yeast extract	1.0 g (marmite can substitute)
1% $FeSO_4$. 7H2O	1 ml solution
Agar	20.0 g
Water	1 L

Appendix 2. Objective 3 trial: Temperature and RH records – 2015 (S1 – S3 are dates of Serenade ASO spray application)



Appendix 3. Objective 3 trial. Temperature and RH records post sprays (S1 – S3 are spray dates)



Appendix 4. Objective 3 - Crop diary of key events (2015)

Date	Event
22/07/2015	Trial marked out
29/07/2015	Spray 1 applied to crop and walkway, loggers started
05/08/2015	Spray 2 applied. Rockwool and small green fruit sampled (SAMPLE 1)
12/08/2015	Spray 3 applied. Small green fruit sampled (SAMPLE 2)
19/08/2015	Second rockwool samples taken. Small green fruit sampled (SAMPLE 3)
26/08/2015	Small green fruit sampled (SAMPLE 4)
02/09/2015	Small green fruit sampled (SAMPLE 5)
16/09/2015	Mature fruit harvest 1
23/09/2015	Mature fruit harvest 2
30/09/2015	Mature fruit harvest 3
07/10/2015	Mature fruit harvest 4
14/10/2015	Mature fruit harvest 5. Trial cleared away

