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Project leader:	Dr Tim O'Neill, ADAS
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Date project completed:	31 March 2014

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

Fusarium lactis appears to be the main cause of internal fruit rot in peppers in the UK, as it was the predominant species in aborted immature fruit and mature rotting peppers. The application of Amistar, Switch and Serenade ASO at flowering significantly reduced disease incidence.

Background and expected deliverables

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide since around 2000. In the UK, surveys in 2007 showed infected fruits were present in many crops at levels from 1 to 37% (PC 260). The disease causes some losses on production nurseries but more importantly Fusarium continues to be a frequent cause of rejection by packers and complaints by supermarkets. Losses vary greatly between crops and seasons, and growers are generally unaware a problem may be present until harvest or postharvest. The fruit rot arises through infection of flowers by spores of Fusarium. Several Fusarium species have been associated with the disease, notably *F. lactis* and *F. oxysporum*. Observations in commercial crops indicate the disease is favoured by high humidity and fluctuating temperatures. At present there is no effective method of control. This project aimed to reduce losses to Fusarium internal fruit rot through increased knowledge of factors associated with a high incidence of the disease and use of biofungicides and fungicides to control flower infection.

Summary of the project and main conclusions

Objective 1 - Review of overseas research on pepper Fusarium internal fruit rot (Year 1)

Fusarium internal fruit rot of sweet peppers has also become a significant problem in recent years in Canada and mainland Europe. There were widespread losses in the Netherlands in 2012. The fungi associated with the disease are variously reported as *F. lactis*, *F. oxysporum. F. proliferatum* and *F. solani* in Canada; as *F. oxysporum, F. proliferatum, F. nygamai* and *F. lactis* in Belgium, and as *F. oxysporum*, *F. proliferatum, F. solani* and an unknown species related to *F. lactis* in the Netherlands.

Recent work in Canada confirmed the infection pathway of *F. lactis*. *Fusarium* spores (conidia) deposited on the stigma grew down the style and into the ovary within 5-6 days

after inoculation. At 45 days after inoculation, typical internal fruit rot symptoms were observed and *F. lactis* was recovered from fruit tissue and seeds within externally symptomless fruit. It was suggested that *Fusarium* conidia were deposited on the stigma by insect pollinators or from the air.

Further work in Canada with *F. subglutinans* (subsequently re-identified as *F. lactis*), identified treatments that significantly reduced Fusarium internal fruit rot when applied to flowers 1 day before inoculation. Effective treatments included preparations of *Bacillus subtilis, Gliocladium catenulatum* and *Trichoderma harzianum*-T22 and the fungicide Rovral (iprodione).

A research group in Belgium published results in 2012 on the genetic diversity of *Fusarium lactis* species complex isolates obtained from sweet pepper. Out of 98 isolates obtained from Belgium (82), Canada (1), the Netherlands (9) and the UK (6), 74 were identified by molecular tests as *F. lactis* or *F. lactis*-like, 13 as *F. oxysporum*, nine as *F. proliferatum* and two as *F. solani*. Members of the *F. lactis* species complex showed large genetic and phenotypic diversity. It was suggested that the emergence of Fusarium internal fruit rot over the last 10 years in major sweet pepper growing regions was due to the introduction of new varieties with reduced resistance, and possibly to changes in greenhouse climate control that allowed higher relative humidity.

Objective 2 - Molecular characterisation of Fusarium isolates associated with pepper Fusarium internal fruit rot in the UK (Year 1)

Out of six Fusarium isolates obtained from UK pepper crops and sent to Belgium for molecular characterisation, two were confirmed as *F. oxysporum,* three as *F. lactis*-like (but different from the type-strain), and one as *F. proliferatum*.

Nineteen isolates of Fusarium sp. were obtained from fruit on four UK pepper nurseries and compared with reference DNA sequences by molecular tests at the University of Warwick. Eight isolates were found to be closely related to *F. lactis*, six to *F. oxysporum*, three to *F. proliferatum* and two to *F. solani.* An isolate obtained from a pepper stem base lesion on a fifth nursery was identified as *F. solani*.

These results indicate Fusarium internal fruit rot in the UK is most commonly caused by *F. lactis* and less frequently by *F. oxysporum* and *F. proliferatum*.

Objective 3 - Effect of Fusarium inoculum and flower age on infection (Year 1)

Two fully replicated experiments were done in commercial crops of sweet pepper in Essex. Flowers were inoculated and the fruits developing from them examined for Fusarium internal fruit rot.

In May 2011, five methods of inoculation with an isolate of *F. proliferatum* were compared on fully open white flowers. Only 56 out of 280 inoculated flowers developed into mature fruit; 14 of these were infected with Fusarium internally. Inoculation of flowers with Fusarium spores by spraying in water, placing a small water droplet in the flower or dry spore transfer using a paintbrush, all resulted in infected fruit. Spray inoculation with a low *Fusarium* spore concentration was most successful. No fruit developing from uninoculated flowers were affected by Fusarium.

In August 2011, a further set of flower inoculation treatments was examined using the same isolate of *F. proliferatum*. Twice as many fruit developed from flowers left untreated, inoculated with water only, or mist-inoculated with Fusarium spores than the other treatments. Fusarium internal fruit rot only occurred in fruit that developed from inoculated flowers (Table 1). Inoculation of young white flowers (50%) resulted in more Fusarium fruit rot than old brown flowers (19%). The level of infected fruit at harvest (35-56%) varied little with spore concentration or method of applying the spores.

Eight isolates of Fusarium sp. recovered from affected fruit were characterised by molecular tests. Three isolates were identified as *F. proliferatum*, four as *F. lactis* and one as *F. lactis*-like. *F. lactis* probably occurred through natural infection; occurrence of Fusarium within small aborted fruit from uninoculated flowers supports this explanation.

Table 1. Effect of flower inoculation with *Fusarium proliferatum* and flower age on occurrence of Fusarium fruit rot in sweet pepper cv. Ferrari – November 2011 (Experiment 2)

Treatment	Total number fruit at harvest[a]	Occurrence of Fusarium (% of fruit harvested)			
		External rot	Internal rot	On seed	Any symptoms
1. Untreated	33	0	0	0	0
2. Water control	27	0	0	0	0
3. Mist of spores	26	12	31	19	35
4. Dry spore transfer	18	11	17	33	33
5. Spray – low concentration (5 x 10^3/ml)	16	44	56	38	56
6. Spray – medium concentration (5 x 10^5/ml)	14	29	43	36	50
7. Spray – old flowers, medium concentration (5 x 10^5/ml)	21	14	19	5	19

[a] Fruit were harvested on 19 October and 2 November, 10 weeks after flower inoculation; data shown are for the combined harvests.

Objective 4 – Relative susceptibility of different varieties (Year 2)

Visibly healthy peppers of six varieties collected from glasshouses on one day in May 2012 differed in their level of Fusarium internal fruit rot. After holding fruit at ambient temperature for 5 days, internal fruit rot ranged from 0.8% in Ferrari (green fruit) to 14.2% in Pele (yellow). Infection in Fiesta (8.3%), Spider (6.7%) and Boogie (5.8%) was also relatively high compared with Cupra (2.5%) and Ferrari. Two of the varieties that differed (Cupra and Spider) were from the same glasshouse. These results on varietal differences are supported by grower experience.

Peppers of the same six varieties were compared for their susceptibility to Fusarium fruit rot by inoculation of the inner wall with *F. lactis*. The diameter of rot lesions after 10 days was greater in Pele, Spider and Cupra than in Ferrari (green) or Boogie, and was intermediate in Fiesta.

The effect of fruit sugar content (% Brix) on the rate of Fusarium rot development was examined. Sugar content ranged from 4.0% (Ferrari green) to 7.2% (Cupra red). No relationship was found between sugar content and the rate of Fusarium fruit rot development, following inoculation.

Taken together, these results indicate:

- Pepper varieties differ in their susceptibility to Fusarium internal fruit rot, with Pele (yellow) very susceptible and Ferrari (green) less susceptible. The red variety Cupra is less susceptible than red Spider.
- Differences between varieties in the incidence of Fusarium internal fruit rot are not determined simply by fruit sugar content.
- The interval between fruit set and harvest may have some effect on incidence of Fusarium fruit rot, as green fruit, which show least infection, are harvested 10-14 days before coloured fruit. However, Pele showed the highest level of infection and yet is generally harvested 1 week earlier than other coloured fruit.
- Differences in varietal susceptibility appear to be determined by factors other than, or in addition to, those noted above and may include, for example, flower characteristics or fruit chemical constituents.

Objective 5a – Effect of high humidity on flower infection (Year 2)

A replicated experiment was done in a commercial crop of peppers, variety Cupra, to determine the effect of imposing high humidity at flowering on the incidence of Fusarium internal fruit rot. In May 2012, flowers were loosely enclosed in small polythene bags for periods of 3, 6, 15 or 24 hours after inoculation with *F. lactis*; moisture droplets on the inside of bags indicated very high humidity conditions were achieved. Only 10-18% of inoculated flowers developed to mature fruit. In this experiment, imposed high humidity for 3-24 hours did not significantly increase the incidence of internal fruit rot (43-65%) compared with flowers inoculated and not enclosed in a polythene bag (71% with internal fruit rot).

This lack of an increase in internal fruit rot with high humidity duration is not consistent with grower observations which suggest the disease is worse during periods of high humidity. It is possible that artificial inoculation of flowers with a spray of *F. lactis* spores overrode any humidity influence on infection success, or the experiment was insufficiently sensitive to detect humidity effects due to the relatively small number of fruit that developed to maturity in each treatment. Effect of humidity was further examined in Objective 6.

Objective 5b – Monitoring of condensation and humidity and in commercial pepper crops (Year 2)

Air relative humidity (RH) and temperature and stem temperature in a pepper crop canopy were measured at three positions on two nurseries in the Lee Valley from March to October 2012. Potential condensation events were determined by calculation of dew point. The frequency and duration of potential condensation events differed between nurseries and

monitoring points. At one nursery, one position had over 100 potential condensation events of greater than 3 hours. Prolonged periods of high RH (>85%) were also more common at this nursery, occurring on average every other day throughout cropping. Comparing the same variety across sites, Fiesta, incidence of Fusarium rot was greater at the nursery with prolonged RH and more condensation events, indicating that the disease is favoured by greater occurrence of high humidity and condensation.

Objective 6 – Evaluation of potential control treatments applied to flowers (Year 2 and 3)

In 2012 a replicated experiment was done in a commercial crop of peppers, variety Cupra, to determine the effect of four products approved for use on protected pepper on incidence of Fusarium internal fruit rot. Sprays of Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil), Serenade ASO (*Bacillus subtilis*) and Prestop (*Gliocladium catenulatum*) were applied to flowers in July and August one day before inoculation with *F. lactis*. The incidence of Fusarium internal fruit rot at harvest was significantly reduced by Switch, Amistar and Serenade ASO (Figure 2).

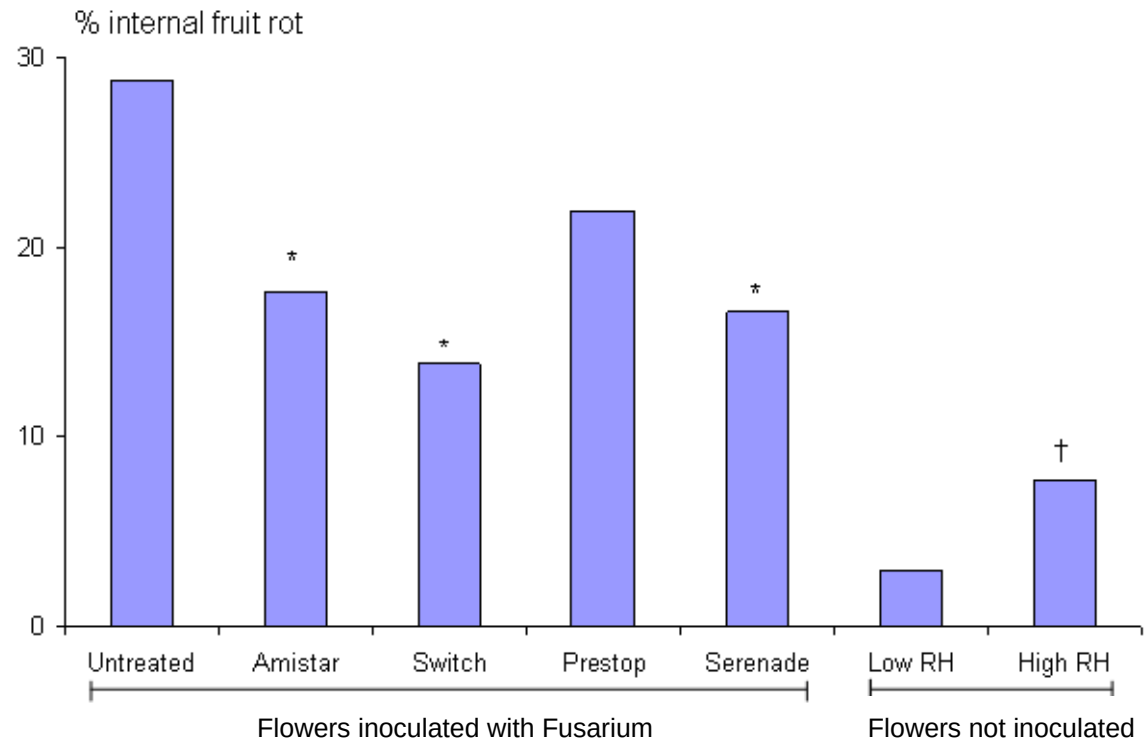


Figure 1. Effect of fungicides, biofungicides and imposed high humidity around flowers on Fusarium internal fruit rot of pepper, cv. Cupra – Lee Valley, 2012.

*significantly different from untreated; †significantly different from low RH treatment.

An extra treatment was included in this experiment to further investigate the effect of high humidity on occurrence of Fusarium internal fruit rot (see Objective 5a). High humidity was created by loosely enclosing flowers in a polythene bag. In contrast to the previous work, no *Fusarium* inoculum was applied. This treatment significantly increased the level of Fusarium internal fruit rot, from 2.9% to 7.7%, supporting the hypothesis that Fusarium development is favoured by high humidity.

In 2013 further experiments on disease control were done in a commercial crop of cv. Cupra in Essex. In the first experiment, the same techniques were used as in 2012. Incidence of Fusarium internal fruit rot was reduced from 39% (untreated) to 22.1% by Switch. Prestop and Amistar reduced levels to below 30%.

In the second experiment, the whole of one row was sprayed three times with Serenade ASO at 10 L/ha. Compared with fruit from an adjacent untreated row, the incidence of *Fusarium* internal fruit rot was reduced from 41% of fruit affected to 23.6% of fruit affected, across both harvests.

Objective 7 – Effect of season and fruit size on Fusarium species in pepper fruit (Year 2)

The identity of Fusarium species associated with pepper fruit at different times of the year and in fruit of different sizes was examined. Small brown aborted fruit and mature fruit with symptoms of Fusarium fruit rot were collected from a crop of variety Cupra at intervals between April and November 2012. The incidence of Fusarium was determined in 50 aborted fruit by culture on agar; identity of Fusarium species was determined by molecular tests.

The incidence of aborted fruit containing Fusarium spp. was 48%, 88%, 84% and 100% in April, June, August and November respectively. *Fusarium lactis* was the predominant species in all samples; *F. oxysporum* and *F. proliferatum* were each detected at a low incidence in both aborted fruit and mature fruit over most of the season.

The high incidence of Fusarium species capable of causing internal fruit rot found in aborted fruit from early in the year was surprising. The possible role of Fusarium spp. in causing fruit abortion may warrant investigation.

Objective 8 – Sensitivity of Fusarium lactis to selected fungicides (Year 3)

In 2013 the efficacies of six fungicides were tested against three isolates of *F. lactis*. Agar plates were amended with concentrations of 2, 20 and 100 ppm active ingredient, and

control plates remained unamended. Fungicide products tested were Amistar, Signum, Switch and three coded products (F160, F161, F162). For those products containing two active ingredients, the concentrations were based on the active ingredient with the greatest concentration.

Plates were inoculated with mycelial plugs of *F. lactis* and subsequent growth was recorded. By comparison with the growth observed on control plates, the level of inhibition was calculated. F161 and F162 were the most effective products, giving inhibitions of 93% and 92% respectively at 100 ppm. Even at 2 ppm, inhibition for both products remained above 89%. This gives valuable information on the potential efficacies of fungicides against Fusarium internal fruit rot, and promising products may warrant further testing.

Financial benefits

Fusarium internal fruit rot of sweet pepper occurs in many UK sweet pepper crops, the severity varying with variety, nursery, glasshouse and time of year. The disease is more common in the spring and autumn when fruit take longer to ripen. Growers have reported that occasionally up to 20% of a day's pick may be affected. Assuming a return to grower of 33p per fruit and a harvest of 10,000 fruit/ha on a single day (0.8 kg/m^2/week or 7 fruit/m^2/week), this represents a loss of £660/ha/day. Assuming a single spray application to flowers reduces Fusarium internal fruit rot by 50% as found in our work, and with an estimated treatment cost (product + spray application) of £320/ha, there would be a financial benefit of £340/ha in applying treatment. This depends on identifying the period at flowering when losses in resultant fruit are high (20% in the assumed case above). Another aspect which needs to be examined is whether a spray applied to flowers on one day results in a reduction in Fusarium internal rot of resultant fruit harvested over several days; as treated open flowers will set fruit and ripen at slightly different times, there may be a range of a few days in the period when treated flowers become mature fruit stage ready for picking. Assuming a single spray resulted in a mean 25% reduction in Fusarium internal rot over a period of 5 days' pick, when losses are running at a lower level of 10%, there would be a net financial benefit of £92.50/ha/week (0.25 x £330/ha/day x 5 days, less £320 application cost). These two scenarios indicate there could be a net financial benefit in treating flowers to reduce Fusarium internal fruit rot at periods of moderate to high risk; further work is needed to confirm if these levels of control can be achieved in commercial practice. It should be noted that additional losses arise when infected fruits are not detected at harvest or in the packhouse, but the rot develops subsequently causing supermarket rejection or customer complaint to the supermarket, both of which incur a cost for the grower. The potential financial benefits of applying this work are thus an increased

proportion of harvested fruit free from Fusarium internal infection and reduced risks of packhouse rejection, supermarket complaints and disruption to the supply chain.

Action points for growers

- The predominant cause of Fusarium internal fruit rot in the UK is *F. lactis*. The same fungus is commonly found in aborted fruit and may be a cause of fruit abortion.
- Note that varieties differ in susceptibility to Fusarium internal fruit rot. Red Cupra is generally less susceptible than red Spider; yellow Fiesta is generally less susceptible than yellow Pele; green fruit (e.g. Ferrari) are less susceptible than the above named coloured fruit. The actual level of Fusarium internal fruit rot in a particular variety will also be affected by glasshouse humidity and condensation and the level of inoculum in a house.
- Remove fallen aborted fruit trapped in the canopy and from the floor as much as reasonably practical in order to reduce inoculum levels of Fusarium.
- Grower experience, nursery monitoring and some experimental evidence indicate that Fusarium internal fruit rot is favoured by high humidity; control the glasshouse environment to minimise prolonged periods above 85% RH and the risk of condensation events.
- In houses and varieties where there is a history of Fusarium internal fruit rot, during periods of high relative humidity consider application of preventative sprays to flowers of Amistar, Switch or Serenade ASO.

SCIENCE SECTION

Internal fruit rot of sweet pepper grown in glasshouses has been an increasing problem worldwide since around 2000. In the UK, surveys in 2007 showed infected fruits were present in many crops at levels from 1 to 37% (PC 260). The disease causes some losses on production nurseries but more importantly Fusarium continues to be a frequent cause of rejection by packers and complaints by supermarkets. Losses vary greatly between crops and seasons, and growers are generally unaware a problem may be present until harvest or postharvest. The fruit rot arises through infection of flowers by spores of *Fusarium*. Several *Fusarium* species have been associated with the disease, notably *F. lactis* and *F. oxysporum*. Observations in commercial crops indicate the disease is favoured by high humidity and fluctuating temperatures. At present there is no effective method of control. This project aims to reduce losses to Fusarium internal fruit rot through increased knowledge of factors associated with a high incidence of the disease and use of biofungicides and fungicides to control flower infection.

The objectives of this project were:

1. To review and summarise overseas research on Fusarium internal fruit rot
2. To determine the identity of *Fusarium* isolates associated with pepper Fusarium internal fruit rot in the UK by molecular characterisation
3. To determine the effect of Fusarium inoculum and flower age on infection
4. To investigate the relative susceptibility of different varieties

5a. To determine the effect of high humidity on flower infection

5b. To monitor the occurrence of condensation and high humidity in two commercial crops

6. To evaluate fungicide and biofungicide treatments for control of Fusarium internal fruit rot
7. To investigate the effect of season and fruit size on the identity of Fusarium species occurring in a pepper crop
8. To determine the sensitivity of *Fusarium lactis* to selected fungicides

The results of Objectives 1-3 are presented in the Year 1 report (March 2012) and results for Objectives 4-7 are presented in the Year 2 report (March 2013). The three aims of the work this year were:

1. To conduct a second replicated experiment on control of Fusarium internal fruit rot in pepper (Objective 6);
2. To conduct a large plot comparison of Serenade ASO spray treatment for control of Fusarium internal fruit rot in pepper, under natural infection pressure (Objective 6).
3. To examine the sensitivity of *Fusarium lactis* to selected fungicides (Objective 8).

Objective 6 – Evaluation of potential control treatments applied to flowers

Introduction

Experimental work in Canada identified some chemical and biological treatments that significantly reduced Fusarium internal fruit rot in pepper when applied to flowers (see Year 1 report). Effective treatments included products based on *Bacillus subtilis*, *Gliocladium catenulatum* and iprodione (Rovral WG). In the Year 2 experiment in this project, treatment with Switch (cyprodinil + fludioxonil) was found to be most effective, reducing incidence of Fusarium internal fruit rot from 28.8% (untreated) to 13.8%. Effects of treatment with Amistar (azoxystrobin) and Serenade ASO (*Bacillus subtilis*) were also significant. The aim of this experiment in Year 3 was to further evaluate the chemical and biological products approved for use on protected pepper in the UK, and with known activity against Fusarium diseases, for control of Fusarium internal fruit rot. Following on from year 2, opportunity was taken to further investigate the effect of an imposed high humidity period at flowering on development of the disease.

Additionally, a large plot experiment was set up to test the effect of Serenade ASO on Fusarium internal fruit rot in a more realistic commercial situation with natural infection pressure.

Materials and methods

Site and crop details

A fully replicated experiment (Exp. 1) was done in a commercial crop of cv. Cupra in the same glasshouse as used in Year 2, starting on 23 July 2013. In another portion of the glasshouse a large plot trial compared treated and untreated rows separated by a row not included in the trial (Exp.2). Diaries for both of these experiments are given in Appendix 1.

Treatments – Experiment 1

Fungicide and biofungicide treatments aimed at control of Fusarium internal fruit rot were applied to flowers one day before inoculation with a spore suspension of *F. lactis* (Table 1). A single 24 h high humidity period was applied at the same time to uninoculated flowers (T2). Results from both treatments were compared with uninoculated, unbagged flowers (T1) and inoculated unbagged flowers sprayed with water (T3). Each treatment was applied on three dates (12 August, 19 August, and 18 September) at times when flower setting was considered by the grower to be good. Inoculum production and inoculation with *F. lactis* was as described in Year 2. Fungicide and biofungicides were applied using new plastic hand-held sprayers, as used for *Fusarium* inoculation. High humidity around flowers was created using small plastic bags as described in the Year 2 Annual Report.

Table 2. Detail of fungicide, biofungicide and high humidity treatments applied to flowers in a crop of cv. Cupra – July and August 2013

Treatment		Active ingredients	Rate of use	Approval status
Applied	Inoculation			
1. Untreated	-	-	-	-
2. High RH for 24 h	-	-	-	-
3. Water	✓	Sterile distilled water	-	-
4. Amistar	✓	25% azoxystrobin	1 ml/L	Label
5. Prestop	✓	32% *Gliocladium catenulatum*	5 g/L	EAMU*
6. Serenade ASO	✓	*Bacillus subtilis*	100 ml/L	EAMU
7. Switch	✓	37.5% cyprodinil + 25% fludioxonil	1 g/L	Label

*Extension of Authorisation for Minor Uses

Treatments – Experiment 2

Flowers, stems, rockwool cube surface and floor were sprayed with Serenade ASO at 100 ml product/L in a volume of 100 L water/ha using a backpack pressurized sprayer and lance fitted with Lurmark 30HCx4 hollow cone nozzles. Another whole row length close by remained untreated for comparison. Twenty treated flowers in one stanchion length of each row were tagged after each spray application so that at the time these flowers had matured into fruit they could be identified and picked. Sprays were applied on 12 August, 19 August and 18 September, 2013.

Experimental design and data analysis – Experiment 1

The experiment was done as a randomised block design with four replicates (rows) of the seven treatments. Methods were as described in the year 2 Annual Report except that 30 flowers per plot were inoculated on each of three occasions (or as close to 30 as could be achieved). Results were examined by Generalised linear modelling with logit transformation on combined data or by ANOVA as appropriate.

Experimental design and data analysis – Experiment 2

The experiment was carried out on two large plots (approx. 40 m length of crop wall). For each harvest, around 300 fruit from each row (one day's pick for the row) were examined and the proportion with any external defect was noted. These fruit were incubated for 5 days at ambient temperature and then examined for external Fusarium fruit rot, internal Fusarium fruit rot and Fusarium on seed as described previously. Results were examined by Generalised linear modelling with logit transformation on combined data.

Results and discussion

Experiment 1

This experiment was less successful than the previous one in 2012 in that a relatively low proportion of flowers developed into mature fruit (Table 3).

There was no significant difference between treatments in the total number of flowers that developed into fruit ($p > 0.05$), although there were significant differences in fruit development following individual inoculations (Table 3). The proportion of flowers that developed into mature fruit was similar for both inoculation 1, 13th August (27.7%), and inoculation 2, 20th August (23.2%). No fruit were harvested from inoculation 3 due to early removal of the crop.

There were observable trends in treatment effect on the incidence of *Fusarium* internal rot in fruit that developed after inoculation 1 (Table 4) and 2 (Table 5), however when data were analysed no differences were significant ($p < 0.05$). Additionally, the trends were not conserved between inoculation timings. There appeared to be a treatment effect after inoculation 2 but this was not statistically significant ($p > 0.05$) (Table 4). With the combined data (Table 6), although no treatment effects were found to be significant, Switch appeared to be the best treatment as in 2012 and consistently reduced Fusarium internal fruit rot when compared to the untreated. Overall levels in fruit treated with Switch were 22%, compared to 39% in fruit inoculated but treated with only water.

The data on the fungicide and biofungicides treatments was re-examined excluding the uninoculated treatments (Table 7) to reduce variability introduced through the different inoculation methods (natural and artificial). However, there were still no significant differences ($p>0.05$) between treatments. The mean results for treatments 3-7 in Tables 6 and 7 are marginally different as a result of back transformation in the statistical analyses.

Although Fusarium internal fruit rot was detected in fruit developing from uninoculated flowers in the second set of fruit, none was found in the first set (Tables 4 and 5). Untreated flowers were uninoculated and it is possible that natural Fusarium internal fruit rot inoculum had not had sufficient time to build up in the glasshouse to infect at a level comparable to that achieved with the use of artificial inoculum at the first inoculation timing. By the second inoculation timing natural levels of Fusarium in the glasshouse were likely high enough to show up higher levels of infection in the untreated fruit at harvest than in treated fruit.

Although there appeared to be an effect of high humidity after inoculation 1, imposition of a 24 h high humidity period on uninoculated fully open flowers did not significantly ($p>0.05$) increase the incidence of Fusarium internal fruit rot when compared to untreated fruit. The occurrence of Fusarium internal fruit rot in these two treatments indicates that there was natural dispersal of *Fusarium* sp. spores in the glasshouse, probably via air currents and/or on insects. One of two experiments in 2012 also failed to show significant effects of humidity on Fusarium rot and it was thought the sensitivity of the experiment may not be high enough. Additionally, placing the bags over the open flowers may have influenced the likelihood of a good fruit set in this treatment.

The temperature and humidity recorded in the crop at and immediately after inoculation on 13 August (10:30 am), 20 August (10:45 am) and 19 September (10:30 am) are shown in Figures 1-3 respectively. The minimum night temperature was around 16ºC and the maximum day temperature was around 30ºC around the time of all three inoculations. For inoculation 1, RH was above 80% for around 7.5 hours the following night; for inoculation 2 it was above 80% for around 8 hours and for inoculation 3 for over 2 hours. The RH peaked at around 85% for a short period on each occasion. Given that a low incidence of Fusarium fruit rot developed in the uninoculated plants (T1) on each occasion (i.e. on plants where no water was applied and flowers were not enclosed in bags), it is possible that the temperature and humidity conditions shown are not highly conducive to the establishment of Fusarium infection in flowers that leads to Fusarium internal fruit rot. Unfortunately the variability in the level of internal fruit rot between replicate plots on the same inoculation date is too large to attempt further interpretation of the effect of environmental conditions on infection by comparison of the results of the three inoculations; also, the time at which

flowers became naturally inoculated by *Fusarium* sp. is unknown, so it is not possible to identify the critical period when spore germination occurred.

Table 3. Number of mature fruit harvested following inoculation of pepper flowers on two occasions – summer 2013

Treatment	Inoculation 1 (13th Aug)	Inoculation 2 (20th Aug)	Total
1. Untreated	2.3	5.0	7.3
2. High RH	6.5	1.8	8.3
3. Water	7.5	1.3	8.8
4. Amistar	5.3	2.3	7.5
5. Prestop	5.3	2.5	7.8
6. Serenade ASO	7.0	1.8	8.8
7. Switch	5.0	1.8	6.8
Grand Mean	5.5	2.3	7.8
Significance (18 df)	0.003	0.017	NS

*Number of flowers per plot was 20 for inoculation 1 and 10 for inoculations 2 and 3.

Inoculation was done on tagged flowers on 13 and 20 August and 19 September. There is no data for the harvest of inoculation 3 as the crop was pulled out before this could occur due to unfavourable growing conditions. Note that though the two harvested inoculation timings both show significant differences in harvested fruit numbers, the combined data does not. This is due to the difference in the levels of fruit harvested from untreated plots.

Table 4. Effect of fungicides, biofungicides and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2013 (inoculation 1)

Treatment	Inoculation	Total no. fruit	Occurrence of Fusarium (% fruit)			
			External rot	Internal rot	On seed	Any symptom
1. Untreated	-	9	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)
2. High RH for 24 h	-	26	11.6 (6.4)	23.3 (8.2)	19.8 (8.8)	23.4 (9.4)
3. Water	✓	30	12.7 (6.1)	30.3 (8.4)	25.8 (8.9)	40.1 (10.2)
4. Amistar	✓	21	14.2 (7.8)	24.3 (9.3)	24.5 (10.6)	24.3 (10.6)
5. Prestop	✓	21	4.7 (4.7)	27.4 (9.5)	18.3 (9.3)	36.8 (11.8)
6. Serenade ASO	✓	28	7.2 (5.1)	28.6 (8.4)	39.3 (10.3)	42.8 (10.5)
7. Switch	✓	20	0.0 (0.0)	19.4 (8.7)	9.7 (7.3)	19.5 (9.9)
Significance (18 df)		-	NS	NS	NS	NS

( ) – standard error.

Table 5. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2013 (inoculation 2)

Treatment	Inoculation	Total no. fruit	Occurrence of Fusarium (% fruit)			
			External rot	Internal rot	On seed	Any symptom
1. Untreated	-	20	8.3 (4.5)	19.2 (8.7)	19.3 (5.4)	24.9 (7.4)
2. High RH for 24 h	-	7	12.9 (9.5)	14.9 (13.5)	0.0 (0.0)	13.3 (9.5)
3. Water	✓	5	38.3 (19.7)	30.4 (23.8)	61.5 (12.1)	55.9 (16.8)
4. Amistar	✓	9	0.0 (0.0)	23.2 (14.1)	9.9 (5.9)	32.0 (11.5)
5. Prestop	✓	10	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)
6. Serenade ASO	✓	7	25.3 (14.9)	14.7 (13.9)	16.9 (9.4)	15.0 (10.7)
7. Switch	✓	7	12.9 (9.5)	14.9 (13.5)	28.0 (10.0)	28.7 (12.8)
Significance (18 df)		-	NS	NS	NS	NS

( ) – standard error. NS – no significant differences.

Table 6. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – July to November 2013 (combined results of inoculation 1 and 2)

Treatment	Inoculation	Total no. fruit	Occurrence of Fusarium (% fruit)			
			External rot	Internal rot	On seed	Any symptom
1. Untreated	-	29	6.1 (4.3)	16.0 (7.6)	15.3 (7.9)	18.9 (8.5)
2. High RH for 24 h	-	33	12.4 (5.6)	20.6 (7.0)	14.8 (6.7)	20.8 (7.6)
3. Water	✓	35	14.9 (6.1)	27.3 (7.5)	27.7 (8.2)	39.0 (9.0)
4. Amistar	✓	30	10.0 (5.3)	23.4 (7.8)	20.0 (7.9)	26.7 (8.7)
5. Prestop	✓	31	3.2 (3.1)	19.5 (7.2)	13.0 (6.6)	26.0 (8.5)
6. Serenade ASO	✓	35	8.8 (4.8)	25.0 (7.3)	33.7 (8.7)	36.5 (8.8)
7. Switch	✓	27	3.7 (3.6)	18.3 (7.5)	14.7 (7.4)	22.1 (8.6)
Significance (18 df)		-	NS	NS	NS	NS

( ) – standard error.

Table 7. Effect of fungicide, biofungicide and imposed high humidity around flowers on Fusarium internal fruit rot in pepper, cv. Cupra – August to November 2013 (combined results of inoculation 1 and 2; excluding treatments 1 and 2)

Treatment	Inoculation	Occurrence of Fusarium (% fruit)			
		External rot	Internal rot	On seed	Any symptom
1. Untreated	-	-	-	-	-
2. High RH for 24 h	-	-	-	-	-
3. Water	✓	14.4 (5.6)	27.2 (6.3)	27.7 (7.0)	38.7 (8.1)
4. Amistar	✓	10.0 (5.0)	24.1 (6.8)	20.4 (7.0)	27.3 (8.1)
5. Prestop	✓	3.2 (2.9)	20.3 (6.3)	13.3 (5.8)	26.7 (8.0)
6. Serenade ASO	✓	8.6 (4.4)	25.2 (6.2)	33.9 (7.5)	36.6 (8.0)
7. Switch	✓	3.7 (3.3)	18.7 (6.5)	14.9 (6.4)	22.4 (8.0)
Significance (12 df)		NS	NS	NS	NS

() standard error

Figure 1. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 1 (13th August)

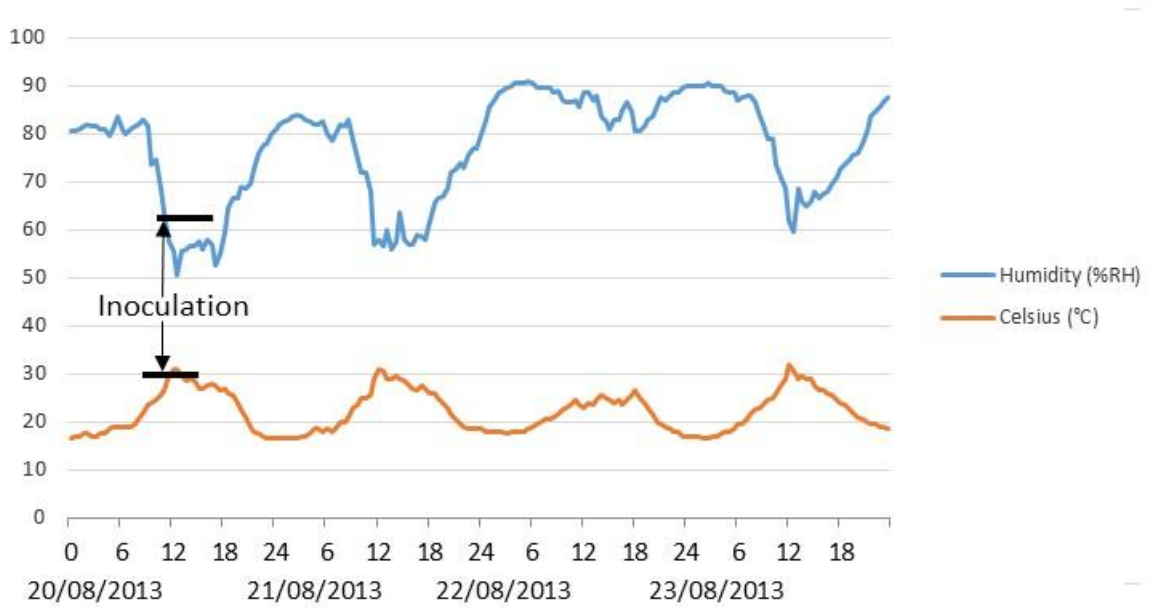


Figure 2. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 2 (20th August)

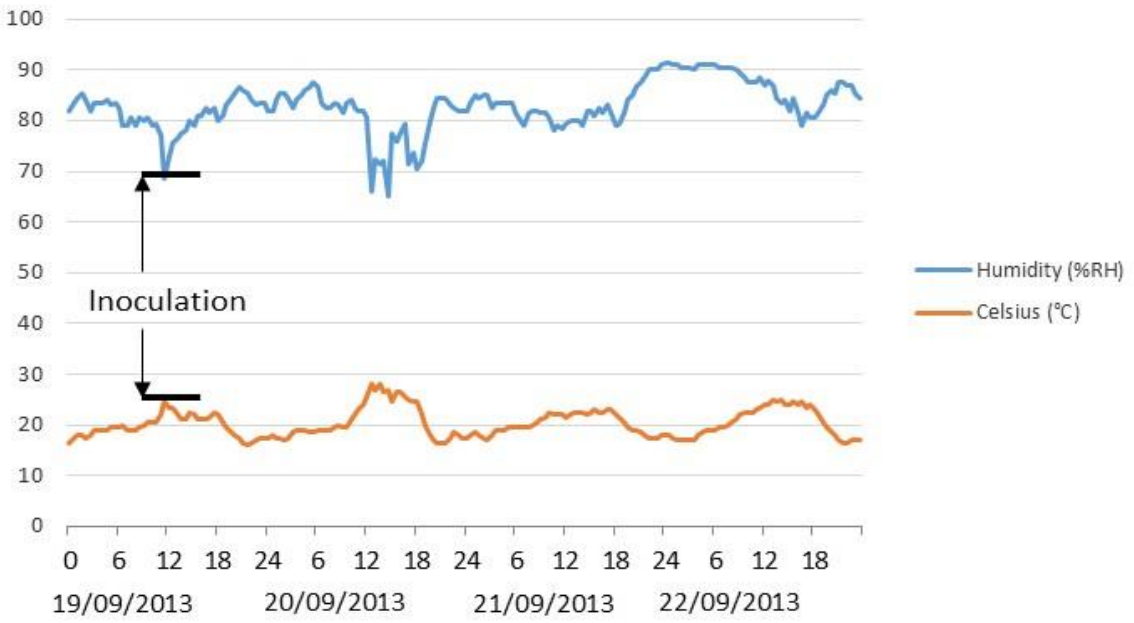


Figure 3. Relative humidity and temperature at time of flower inoculation with *Fusarium* and the following 3 days: Inoculation 3 (19th September)

Experiment 2

The fruit with symptoms of external damage were harvested then incubated and assessed in the laboratory. Around 340 fruit were examined per treated row following the first spray treatment and around 240 following the second (Table 8). Around 40% of the external

damage symptoms were indicative of Fusarium internal fruit rot in the untreated row. Overall the incidence of external damage was reduced from 14% (81/575) on untreated plants to 8% (46/591) on Serenade treated plants (Table 7). The incidence of Fusarium internal fruit rot in damaged fruit was reduced from 41.0% to 23.6% (Table 7).

Table 8. Effect of whole row Serenade ASO spray on the proportion of fruit with external symptoms of damage (physical, blossom end rot or suspect Fusarium internal fruit rot) at harvest - 2013

Treatment	Total no. fruit harvested	No. fruit with external damage	% fruit with external damage	% of damaged fruit with Fusarium
Spray 1				
Untreated	335	52	15.5	46.2
Serenade ASO	353	36	10.2	19.4
Spray 2				
Untreated	240	29	12.1	31.0
Serenade ASO	238	10	4.2	40.0
Combined				
Untreated	575	81	14.1	41.0
Serenade ASO	591	46	7.1	23.6

Full details of the fruit assessments are shown in Tables 9 – 11.

The results varied considerably with timing. For the first spray (12th August) significant effects on the level of Fusarium infection were observed between the Serenade ASO treated and untreated crop rows for both external and internal symptoms of Fusarium fruit rots ($p<0.001$) (Table 8). This data set illustrates that in a situation with natural levels of infection in a commercial glasshouse environment, using a Serenade ASO spray may be an effective control measure. With the smaller fruit number at the second harvest, no significant differences were observed (Table 9). Possibly prevailing environmental conditions were more favourable to efficacy of Serenade ASO on 12 August than on 19 August. However, the temperature and humidity conditions recorded in the 24 hours after application of Serenade ASO on these dates were very similar. When data from both spray timings were combined (Table 10), the mean occurrence of fruit with Fusarium internal fruit rot was reduced from 41.0 to 23.6% ($p<0.05$).

Table 9. Effect of whole row Serenade ASO spray on Fusarium internal fruit rot in pepper, cv. Cupra with external physical damage – August to November 2013 (spray 1)

Treatment	No. fruit assessed	Occurrence of Fusarium (% fruit)			
		External rot	Internal rot	On seed	Any symptom
1. Untreated	52	44.2 (6.9)	42.3 (6.9)	17.3 (5.2)	46.2 (6.9)
2. Serenade ASO	36	5.6 (3.8)	11.1 (5.2)	8.3 (4.6)	19.4 (6.5)
Significance (86 df)		<0.001	<0.001	NS	0.008

() standard error

Table 10. Effect of whole row Serenade ASO spray on Fusarium internal fruit rot in pepper, cv. Cupra with external physical damage – August to November 2013 (Spray 2)

Treatment	No. fruit assessed	Occurrence of Fusarium (% fruit)			
		External rot	Internal rot	On seed	Any symptom
1. Untreated	29	27.6 (8.3)	24.1 (7.9)	31.0 (8.6)	31.0 (8.6)
2. Serenade ASO	10	40.0 (15.5)	40.0 (15.5)	40.0 (15.5)	40.0 (15.5)
Significance (86 df)		NS	NS	NS	NS

() standard error

Table 11. Effect of whole row Serenade ASO spray on Fusarium internal fruit rot in pepper, cv. Cupra – August to November 2013 (combined results of sprays 1 and 2)

Treatment	No. fruit assessed	Occurrence of Fusarium (% fruit)			
		External rot	Internal rot	On seed	Any symptom
1. Untreated	81	38.4 (5.4)	36.1 (5.4)	17.1 (4.2)	41.0 (5.5)
2. Serenade ASO	46	13.0 (5.0)	17.1 (5.5)	11.1 (4.7)	23.6 (6.2)
Significance (86 df)		0.002	0.021	NS	0.046

() standard error

Objective 8 - Sensitivity of F. lactis to selected fungicides

Introduction

A replicated experiment was set up in the ADAS Boxworth pathology laboratory to investigate the efficacy of six fungicides against mycelial growth of *F. lactis*. Products

known to have efficacy against other Fusarium diseases and two products already approved for use on pepper were evaluated. These products were Amistar (azoxystrobin), F160, F161, F162, Signum (pyraclostrobin & boscalid) and Switch (cyprodinil & fludioxonil); Amistar and Switch are approved for use on protected pepper.

Materials and Methods

Fungicides were tested against mycelial growth of *F. lactis* using agar plates amended with each fungicide at three concentrations (2, 20 and 100 ppm active ingredient). This involved autoclaving Potato Dextrose Agar (PDA) and transferring the bottles to a water bath held at 50 degrees Celsius. In a laminar flow cabinet, each bottle was amended with a calculated amount of fungicide stock solution so that the plates poured would be at the appropriate concentration. Control plates remained free of fungicide. Agar plates were allowed to set and were stored in a dark refrigerator until required.

Plates were inoculated in a laminar flow cabinet using a 0.5 mm (No.2) cork borer, taking *F. lactis* from the edge of actively growing colonies also grown on PDA. Three isolates were used (OB3, OB4 and OB5) which each represented a replicate. Inoculated plates were placed in an incubator at 20 °C on a 12 hour light-dark cycle. Assessments were carried out 4 and 8 days later and involved measuring colony diameter twice at right angles. The degree of inhibition for a fungicide concentration was calculated by comparing growth of an isolate on a fungicide amended plate with that on unamended control plates, using the formula below.

$$\% \text{ inhibition} = \frac{(\text{growth on control agar} - \text{growth on fungicide agar})}{\text{growth on control agar}} \times 100$$

Results and Discussion

All products tested had some inhibitory effect on the growth of *F. lactis* in culture at all concentrations. F161 and F162 were most effective at inhibiting growth at all concentrations, and were over 90% inhibitory at 20 ppm. Of the approved products, Switch was most effective (Table 10 and Fig 4).

Table 12. Percentage inhibition of *Fusarium lactis* by six fungicide products

Treatment	a.i (%)	Concentration ppm a.i.	% Inhibition
1. Amistar	Azoxystrobin (23.1)	2	30.2
		20	56.4
		100	56.7
2. F160	-	2	53.0
		20	59.0
		100	73.4
3. F161	-	2	89.1
		20	90.4
		100	92.5
4. F162	-	2	89.3
		20	90.5
		100	91.8
5. Signum	Pyraclostrobin + boscalid (6.7+26.7)	2	41.5
		20	55.3
		100	47.4
6. Switch	Cyprodinil + fludioxonil (37.5+25)	2	74.8
		20	76.4
		100	75.2

Increasing efficacy with concentration of fungicide product can be seen in Figure 4 and this effect is more pronounced for some products than others.

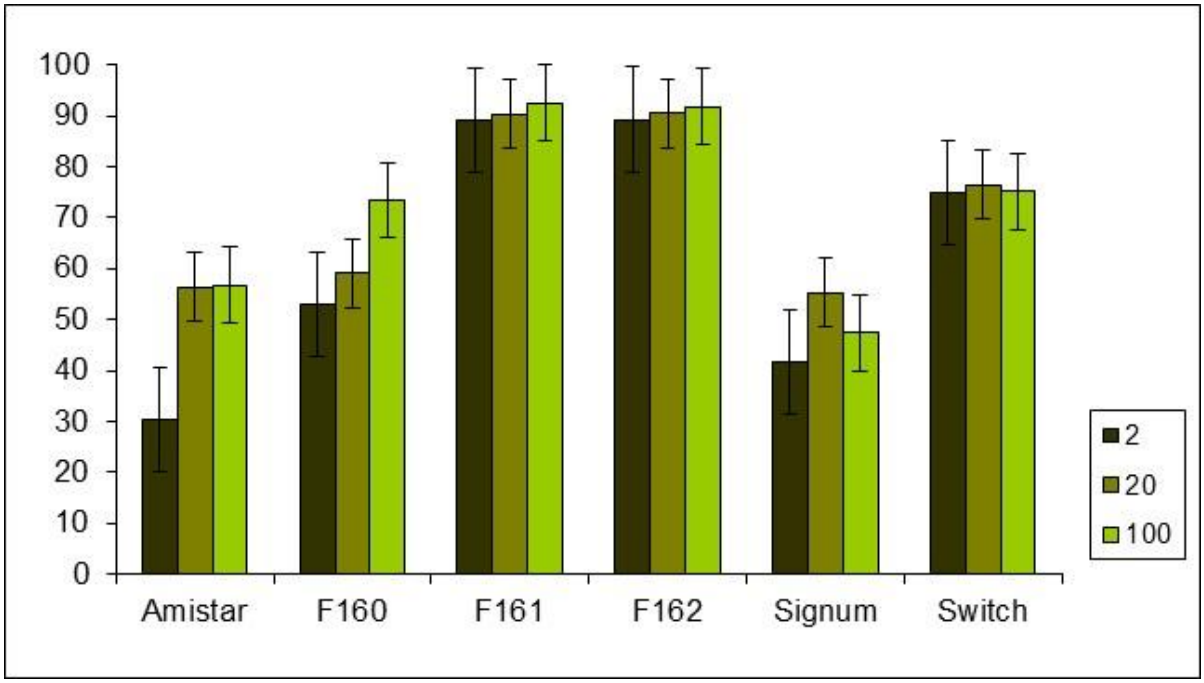


Figure 4. Effect of fungicides on growth of *F. lactis* cultures at three concentrations in 2013 laboratory tests

It is important to note that laboratory tests may not translate to commercial glasshouse situations perfectly, but those products that look promising warrant further testing in commercial situations. Further laboratory work to look at the effect of fungicides on the sporulation of *F. lactis* would also be useful. Some fungicides may be more effective against spore production / germination (high energy requirement, cytochrome inhibition etc.) than against mycelial growth.

Conclusions

Causes of Fusarium internal fruit rot

1. Fusarium internal fruit rot is most commonly caused by *F. lactis* and less frequently by *F. oxysporum* and *F. proliferatum*.
2. Fruit on a particular nursery may be affected by one of these species or variously by all three species.
3. *Fusarium lactis* is frequently present in fallen aborted fruit.
4. *Fusarium oxysporum* and *F. proliferatum* are closely related to each other and more distantly related to *F. lactis*; *F solani*, which causes external fruit rot and/or stem lesions is only very distantly related to these three species.
5. Colony colour on potato dextrose agar, although sometimes consistent for a particular species, is not a reliable feature for identification of the three *Fusarium* species that cause pepper internal fruit rot.

Infection

6. Inoculation of pepper flowers with spores of *F. proliferatum* (2011) and *F. lactis* (2012 and 2013) resulted in mature fruit with Fusarium internal fruit rot.
7. There is evidence that *Fusarium* species can cause fruit abortion: inoculation of pepper flowers by spray inoculation with *F. proliferatum* reduced the numbers that developed into mature fruit; small brown hard aborted fruit often contain Fusarium rot within them.
8. Pepper flowers are more susceptible to development of Fusarium internal fruit rot if inoculated when young (fresh white and fully open) than when old (off-white and flaccid).

Influence of humidity on infection

9. In one out of three experiments, an imposed high humidity of approximately 100% RH around flowers for 24 h increased the proportion of fruit that developed Fusarium internal fruit rot from natural inoculum (i.e. flowers that were not artificially inoculated with *Fusarium*).
10. High relative humidity (>85%) periods greater than 6 hours and potential condensation events greater than 15 minutes were commonly recorded in a crop on

two nurseries in 2012; these conditions appear to be conducive to development of Fusarium internal fruit rot. There were large differences in those conditions between different monitoring points in the same house and between different houses.

Type of fruit and susceptibility to Fusarium

11. Fusarium internal fruit rot is more likely to be found in more mature fruit (red stage) than less mature fruit (green stage) of the same variety.
12. No evidence was found to support the hypothesis that fruit sugar content (% Brix) alone determines the differing susceptibility of different coloured fruit.
13. The pepper varieties Cupra, Ferrari, Fiesta, Kelly, Pele, Special and Spider are all susceptible to Fusarium internal fruit rot. There is evidence that red Cupra is less susceptible than red Spider and that green Ferrari is less susceptible than yellow, orange or red fruited varieties.

Control

14. In fully replicated trials, Amistar (azoxystrobin), Switch (cyprodinil + fludioxonil) and Serenade ASO (*Bacillus subtilis*) reduced Fusarium internal fruit rot caused by *F. lactis* when applied directly as a spray to open flowers (2012); or appeared to reduce the disease (2013).
15. In a two-row comparison study, the incidence of Fusarium internal fruit rot was reduced from 41% to 23% following application of Serenade ASO to the whole row face and floor.

From recent overseas research

16. Pepper Fusarium internal fruit rot is an emerging disease that has occurred simultaneously in major sweet pepper growing regions (northern Europe and Canada) over the last 10 years.
17. The causes of pepper Fusarium internal fruit rot are the same in Belgium, Canada, the Netherlands and the UK.
18. *F. lactis* is a weak pathogen that grows down the style and develops slowly on the placenta and seeds as fruit swell.
19. Symptomless internal seed infection is one pathway by which *Fusarium lactis* can be introduced into a glasshouse; limited spore trapping in a glasshouse tomato crop

showed that *F. lactis* and *F. proliferatum* can occur in the air and may be introduced into a crop this way.

Technology transfer

Articles

O'Neill T M and Barbara D (2012). Fusarium sneaks in from flower to fruit *HDC News* **182,** 18-19.

O'Neill T M (2013). The route to infection, the route to control. *HDC News* **191**, 24-25.

O'Neill, T M & Mayne S (2014). Latest results on Fusarium. *HDC News* (submitted).

Presentation

UK experience of pepper Fusarium internal fruit rot. Glasshouse Research Station, Bleiswijk, The Netherlands, 4 July 2013 (Tim O'Neill).

Project review meetings

14 March 2013, Stoneleigh

22 February 2012, Abbey View Nursery

8 November 2011, Abbey View Nursery

Appendix 1 – Experiment diaries

Experiment 1

Date	Comment	Initial
23.07.13	Trial marked out	JK RD SM
12.08.13	Logger placed in crop. First treatments applied. Flowers tagged. Difficult getting bags over flowers but managed most. Some older flowers were treated to maintain numbers.	SM AW
13.08.13	Flowers inoculated and inoculated plants labeled with electrical tape. Taking the bags off was very difficult, but moisture could be seen to have built up in many of the bags. Mostly the older flowers inoculated caused problems and a few petals fell off.	SM AW
19.08.13	Second treatment applied to flowers on the main stem or ones at first branch of second stem. Flowers tagged.	AW RD
20.08.13	Flowers inoculated and bags removed from treatment 2 plots.	AW RD
18.9.2013	Third treatment applied to flowers on main stem or ones at first branch of second stem. Flowers tagged.	AW RD
19.9.2013	Flowers inoculated and bags removed from treatment 2 plots.	AW RD
22.10.13	First Harvest.	AW RD
30.10.13	Final Harvest of 2nd inoculation fruit. All plants removed from the glasshouse the following week due to a poor crop, so unable to do 3 separate harvests.	RD AW CW

Experiment 2

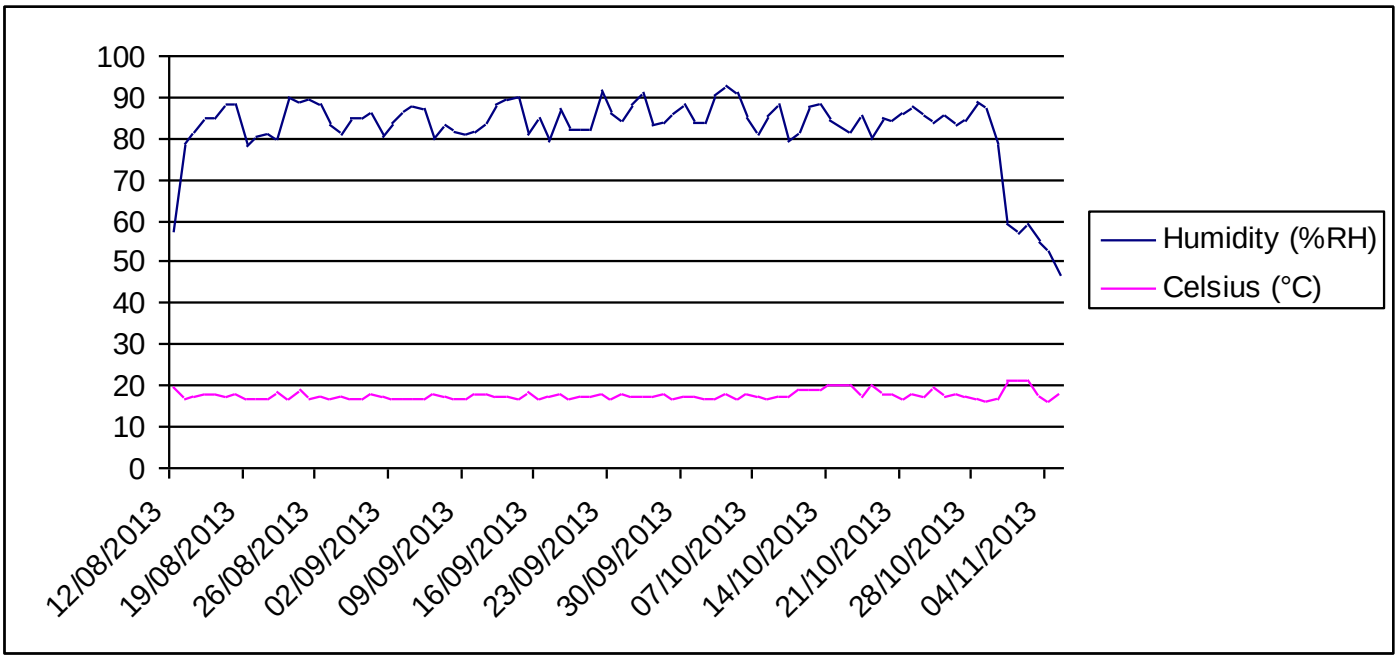
Date	Comment	Initial
23.07.13	Trial marked out	JK RD SM
13.08.13	First spray applied to marked out row nearest glasshouse door. 20 flowers tagged.	SM AW
20.08.13	Second spray applied. 20 flowers tagged on treated row and 20 tagged on untreated row (on main stem).	AW RD

19.9.13	Third spray applied. Again, had slight difficulties with the top nozzle so as before, boom lifted higher. 20 flowers tagged on treated row and 20 tagged on untreated row (on main stem).	AW RD
22.10.13	First Harvest. JK bought along to use experience of identifying symptoms. Fruit bought back and left in lab to assess 5 days later by RD and JK.	RD AW JK
30.10.13	Final Harvest, fruit was provided by pickers, some severe aphid symptoms. As before, fruit bought back and assessed by AW 5 days later.	RD AW JK

Experiment 3

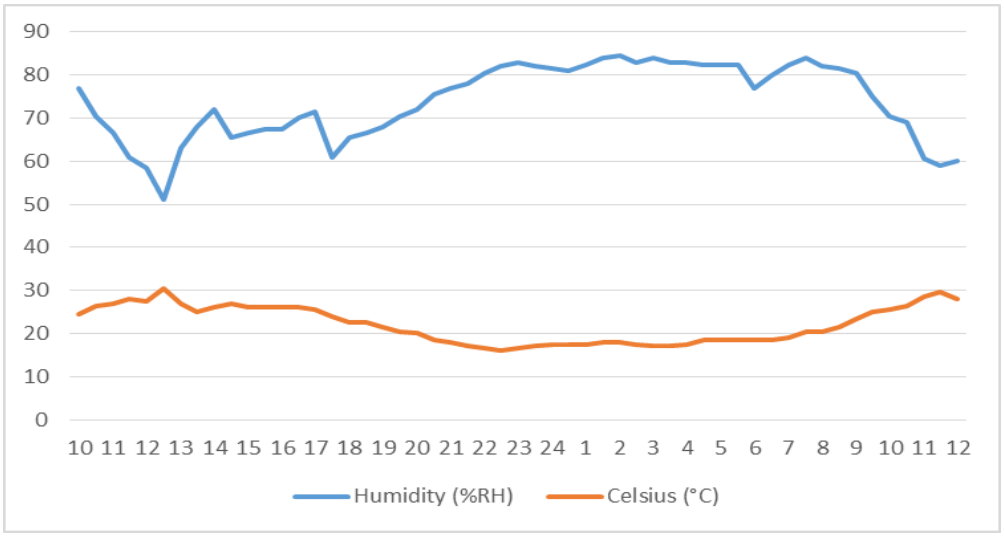
Date	**Comment**	**Initial**
17.07.13	Preparations for experiment carried out. *F. lactis* isolates subbed, fungicides booked out, data sheet updated.	SM
22.07.13	Fungicide amended agar prepared. Placed in fridge.	SM
25.07.13	Agar inoculated at end of day.	SM
30.07.13	Assessed first thing.	SM
02.08.13	Carried out second assessment first thing.	SM

Appendix 2 – Nursery monitoring (temperature and RH) – 2013

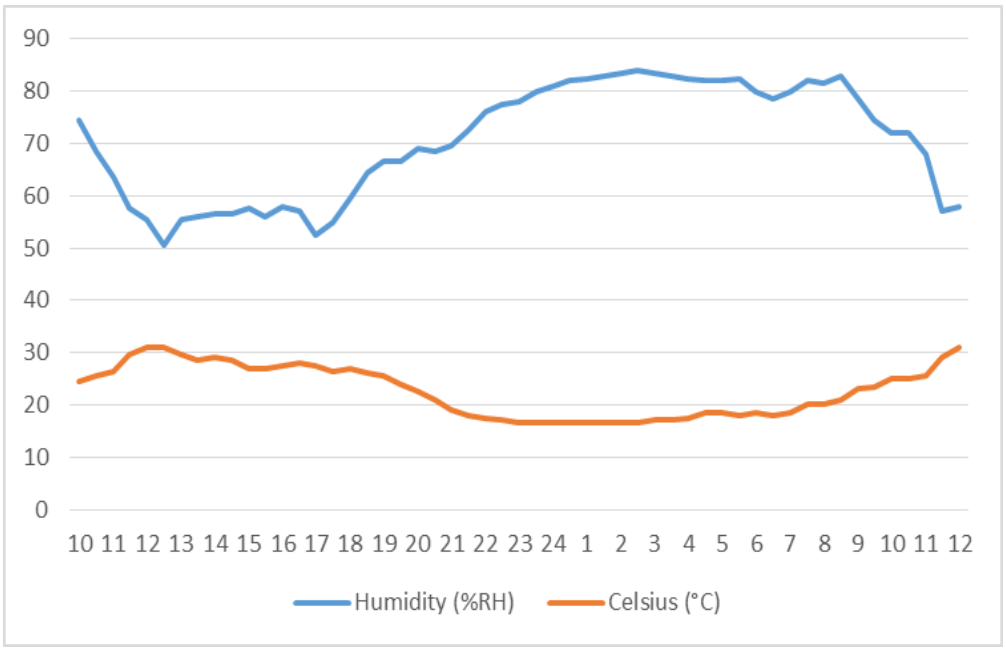


Appendix 3 –Temperature and RH after inoculation (Experiment 1) – 2013

Inoculation 1



Inoculation 2



Inoculation 3