Grower Summary

PE 007

Sweet pepper: aspects of the biology and control of Fusarium fruit rot

Final 2014

Disclaimer

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

HDC
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

HDC is a division of the Agriculture and Horticulture Development Board.

Project Number:	PE 007
Project Title:	Sweet pepper: aspects of the biology and control of Fusarium fruit rot
Project Leader:	Dr Tim O'Neill
Contractor:	ADAS UK Ltd
Industry Representative:	Gill Wardell, Abbey View Nurseries
Report:	Final Report 2014
Publication Date:	April 2014
Previous report/(s):	Annual Report 2013
Start Date:	01 April 2011
End Date:	31 March 2014

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).

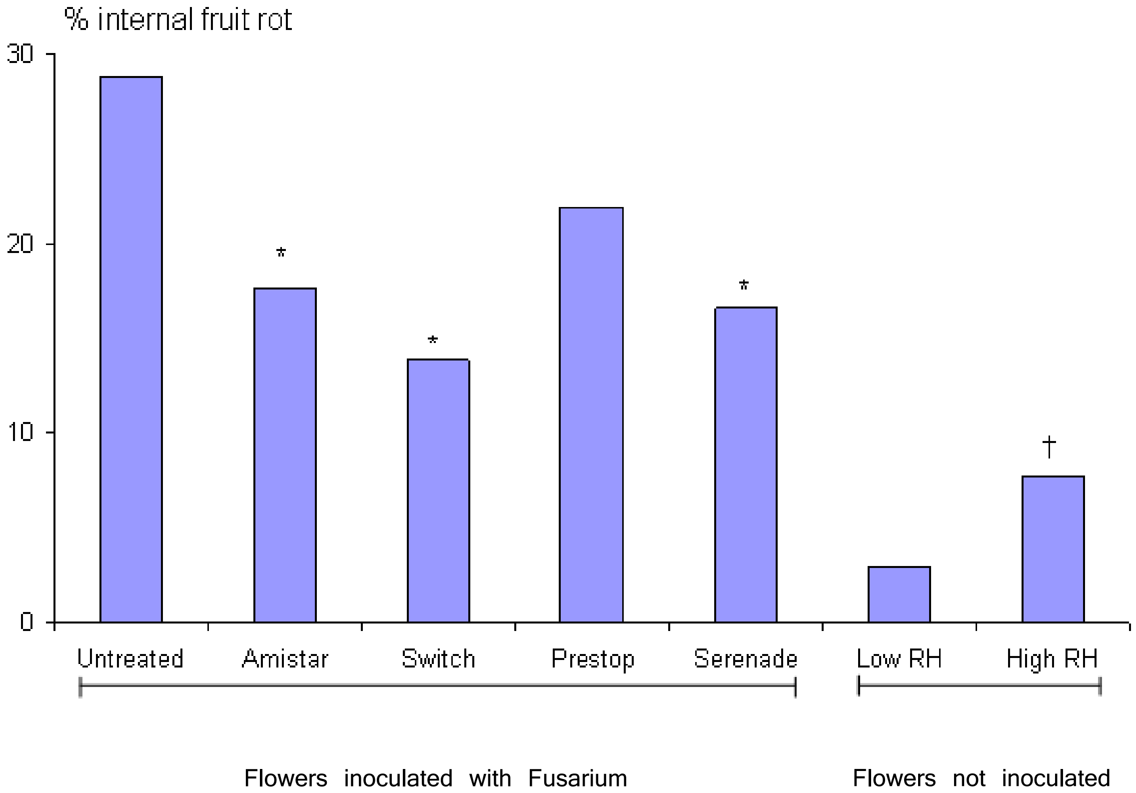

Figure 1. Effect of fungicides, biofungicides and imposed high humidity around flowers on Fusarium internal fruit rot of pepper, cv. Cupra – Lee Valley, 2012.

*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.