Grower Summary

SF 142

Phomopsis dieback of blackcurrants: methodology development and control

Final 2014

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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.

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Project Number:	SF 142
Project Title:	Phomopsis dieback of blackcurrants: methodology development and control
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Headline

- Detection of stem dieback using a monoclonal antibody-based test was unreliable. Development of a DNA based detection method is recommended.
- Results indicate spread of stem dieback can be reduced by lowering levels of residual inoculum by removal of previous crop debris
- Reduction in damage to stems reduces risks of infection
- Risks of infection and rate of lesion development increases with temperature reaching an optimum at 20ºC.
- Several of the fungicides known to be effective against stem dieback did not affect growth and sporulation of the potential biocontrol agent *B. leucophthalma*
- Serenade was highly effective against growth and sporulation of Phomopsis ribicola

Background

In recent years dieback caused by *Phomopsis ribicola*, particularly of the varieties Ben Avon and Ben Tirran, has been a significant problem in commercial blackcurrant plantations. The severity of the problem and its speed of spread through a plantation showed significant variation between sites.

Typical symptoms in the spring/early summer include the production of leaves of reduced size or a failure of branches to leaf out. One or both of these symptoms can often be present, along with healthy-looking branches on the same bush. As the summer progresses wilting of leaves may develop and these then generally turn yellow and then brown. Cutting open of symptomatic tissue reveals a firm, brown decay of the wood, with an associated brown decay of the pith at the base of the branch. Tiny, black fungal fruiting bodies may be found on branches that have been dead for some time .In extreme cases, all of the branches on an affected plant may fail to produce leaves.

HDC project SF 12-223 showed that *Diaporthe strumella*, (asexual state *Phomopsis ribicola*), was the only pathogen isolated consistently from blackcurrant plants showing symptoms of dieback that was capable of reproducing dieback symptoms when inoculated into healthy plants. As part of project SF 12-223, visits were also made to stoolbed sites which supplied cutting material for use on fruiting plantations. These visits revealed low levels of *D. strumella* at the majority of stoolbed sites. In some cases the stoolbed stems

from which the fungus was recovered showed internal symptoms of wood and pith decay identical to those found in the affected plantations, but in others the fungus was found on stems that had appeared healthy at the time of sampling.

At present the sources of the fungus and the route by which it spreads through a plantation are poorly understood. This one year project aimed to develop routine methodologies for the detection of *P. ribicola* in substrates such as plant material, soil and water, develop a better understanding of the infection process and explore potential control strategies in more detail. In the longer term the development of these methodologies would aid the establishment of inoculum sources, routes of infection/spread, and methods of management/control of the disease.

Summary

Evaluation of an antibody-based test for detection of P. ribicola

Initial tests on the Phomopsis genus specific antiserum, Phomopsis-IgG 59/II, showed that extraction from woody stem material was possible, particularly when coating buffer was used as the buffer in the extraction process. The optimal dilution rate for the antiserum was 1:1000. However, use of the optimised test for detection of *P. ribicola* from artificially infected stem material showed results from the test to be inconsistent. It was concluded that the production of PCR primers to detect *P. ribicola* was likely to provide more consistent results.

Development of baiting techniques for improved detection

Three bait types, leaf, green stem and woody stem, were tested for the detection of *P. ribicola* in soil and water. Initial tests carried out in sterile soil and water, primed with differing levels of *P. ribicola* spores, showed that all three bait types detected *P. ribicola* although it was more readily detected in woody stem material. Use of this bait had a lower detection limit of 100 spores/ 75 g soil and 10 spores/ 200 ml water.

When the test was repeated using non-sterile soil or rainwater, the presence of faster growing fungal species, such as fusaria, over-grew *P. ribicola;* as a result the presence or absence of *P. ribicola* in the bait could not be confirmed. Further work would be needed to develop a selective medium for isolation from baits used in field (non-sterile) conditions.

Understanding the infection process in blackcurrant woody stem sections

Stem sections of the blackcurrant variety Ben Avon were tested to determine methods for producing stem infection. Stems were used either wounded or unwounded and inoculated with either a spore suspension or mycelial plug taken from an agar plate of *P. ribicola*. Both methods of inoculation produced symptoms typical of those associated with Phomopsis stem dieback on wounded material. No symptoms were produced on any unwounded material. Lesions produced following inoculation with a spore suspension were larger than those produced following inoculation with a mycelial plug. Observation of the spores after application revealed that spore suspension had been absorbed into the stems and potentially distributed internally within a few hours of inoculation. This is in contrast to the mycelium inoculation would need time to grow into the wound before infection could occur. Continued incubation of the infected stems led to the production of the long-necked fruiting bodies associated with *Diaporthe strumella,* the sexual state of *P. ribicola.* This may indicate that crop debris on the soil surface of blackcurrant plantations could become colonised by *P. ribicola* present in the soil and provide a source of inoculum for subsequent infection of a crop.

The effect of temperature on infection was established using mycelial plug inoculum of *P. ribicola* and wounded woody stem sections of Ben Avon. Seven temperatures (0, 5, 10, 15, 20, 25 and 30°C) and seven exposure times were tested (2 hours, 1, 2, 3, 4, 5 and 6 days). No infection occurred following a 2 hour exposure to *P. ribicola* at any of the temperatures tested. Equally there was no consistent infection at 0 and 5ºC at any of the exposures times tested. Infection rates were also inconsistent for all temperatures where the exposure to inoculum was less than 4 days. At temperatures of 20ºC or higher infections occurred after a 1 day exposure to inoculum; however the level of infection was sporadic until an exposure time of 5 days.

The effect of temperature on symptom extension was tested at temperatures between 0 and 30ºC. Minimal lesion extension was recorded for temperatures 10ºC and below over the 10 day incubation period. For temperatures of 15ºC to 30ºC there was no significant difference in lesion size up to 5 days incubation, however by 7 days incubation the lesion size was significantly smaller at 15 and 30ºC compared to those at 20 and 25ºC. The optimum incubation temperature for lesion extension in woody stem tissue was 20ºC.

These data suggest that where infections occur in blackcurrant stem tissue then lesion expansion will be relatively rapid at temperatures typical of late spring, summer and early autumn in the UK. The expansion is likely to continue, although at a much reduced rate at temperatures more representative of early spring and late autumn. These laboratory tests were carried out on woody stem tissue and will be typical of the host tissue on individual

blackcurrant stems. However, it is likely that where infection occurs at the crown, lesion expansion will be slower due to the denser nature of the tissue.

Effect of fungicide treatments for control of dieback on beneficials (Bloxamia leucophthalma)

B. leucophthalma is a fungus which has been isolated from blackcurrant plantations and has been shown to have *in vitro* biological activity against *P. ribicola*. As a result it would be beneficial to try and ensure that where possible fungicide programmes used to control *P. ribicola* did not have an adverse effect on *B. leucophthalma.*

Tests were carried out to determine whether Signum (boscalid (267 g/kg) + pyraclostrobin (670 g/kg)), Scala (pyrimethanil (400 g/L)), Stroby (kresoxim-methyl (500 g/kg)), Switch (cyprodinil (375 g/kg) + fludioxonil (250 g/kg)), Systhane 20 EW (myclobutanil (200 g/L) or Teldor (fenhexamid (500 g/L)) had any detrimental effect on growth or spore germination of *B. leucophthalma*. EC_{50} values for the fungicides tested showed that Stroby, Teldor and Systhane 20 EW had the least effect on *B. leucophthalma*. Results indicate that there are several fungicides which could be used safely to control of botrytis and leaf spot whilst maintaining the beneficial effects of *B. leucophthalma*.

Efficacy of biocontrol agents (Serenade) against P. ribicola

In HDC project SF 12-223 it was shown that Serenade (a suspension concentrate containing 13.96 g/L *Bacillus subtilis* strain QST 713 at a minimum of $1x10^{12}$ cfu/L) inhibited the growth of *P. ribicola* isolates at the lowest concentration tested (10 ml/L). The work undertaken in SF 12-223 was continued in this project to establish the EC_{50} and minimum inhibitory concentration of Serenade towards *P. ribicola*. The results show that Serenade was highly effective at controlling mycelial growth of *P. ribicola* with EC_{50} values ranging from 0.92×10^{-9} to 8×10^{-9} ml Serenade per litre of agar. The lowest concentration of Serenade that resulted in no growth of the *P. ribicola* was 0.001 ml/L. These data suggest efficacy such that this biocontrol agent could form a key element in an integrated control strategy.

Financial Benefits

The severity of *Phomopsis* dieback varies between blackcurrant plantations, however on one plantation 80% of plants showed symptoms during the course of one year. The cost of losses has been estimated at £5,000 per hectare per year for this plantation alone. Severe attacks have also led to the premature grubbing of plantations.

Establishing an understanding of the pathogen responsible for blackcurrant dieback will ensure effective management strategies can be developed which will minimize future losses from the disease.

Action Points

The aim of this project was to develop methodologies which would allow for potential further work on disease monitoring and epidemiological studies to be carried out. However a number of effective management actions have already been identified from the project;

- Limit wounding to stem material as tissue damage was required for infection to occur.
- Remove crop debris from stoolbeds and the wider blackcurrant plantation as this can provide material on which inoculum can develop to reinfect plants in the following season.