Grower Summary

SF 132

Progressive die-back symptoms in blueberry: Identification and control

Final 2014

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GROWER SUMMARY

Headline

- Three species of Phomopsis and two from the Botryosphaeriaceae family were most associated with severe blueberry die-back.

Background and expected deliverables

The UK blueberry growing industry has expanded rapidly over recent years. Home grown production increased by approximately 50% between 2011 and 2012 as young plantations approached maturity. Sales of fresh blueberries now rival that of raspberries but UK production still accounts for only around 20% of total Summer/Autumn sales and almost none during other periods. Blueberries represent a clear opportunity for increased substitution of UK produce for imports.

When separately recorded, yields per bush are known to reach 6 – 10 kg which would amount to 18 – 30 t/ha if multiplied by standard numbers of plants per hectare. Actual yields per hectare being picked are lower. National output was less than 7 t/ha in 2012/13. Many plantations have yet to reach maturity but pest and disease problems have also been an important cause of lower yields.

There have been an increasing number of reports of growth decline in bushes due to die-back and crown rot type symptoms leading to further investigation by diagnostic laboratories. From 2009-2011, such decline led to severe losses in the west of England where the symptoms led to the grubbing of a young plantation at a farm in Herefordshire.

Affected bushes typically display severe nutrient deficiency symptoms in leaves and premature leaf drop, accompanied or followed by browning or blackening of shoots or whole branches. Symptoms are often limited to one or more branches while other parts of the bush continue to grow almost normally for a while. Affected branches may show signs of limited recovery, with new shoots breaking from previously dormant buds as a growing season progresses, only to fail completely the following spring. In 2010, intensive investigations of the problem on two sites were conducted by FAST LLP using the diagnostic services of Fera. A species of *Phomopsis* was identified in a majority of samples.

The type and progression of symptoms bore much in common with those seen in North American plantations known to be infected with *Phomopsis vaccinii*, a pathogen that is listed as an EU quarantine organism. Using DNA analysis, Fera were able to show that the pathogen was not the same as *Phomopsis vaccinii* but the precise identity of the species was not confirmed.

There were similarities between symptoms reported by blackcurrant growers and studied as part of GSK Project no. 223 (SF12) and those observed in blueberries. SF12 found that *Diaporthe strumella* syn. *Phomopsis ribicola* was consistently associated with blackcurrant die-back.

Die-back problems in gooseberries, redcurrants and grape-vines have also been the focus of recent HDC supported work (SF 131 and two 'expert mini-summit' meetings held at Fera, Sand Hutton). Precise diagnosis has been difficult but *Phomopsis*, *Botrytis, Botryosphaeria*, and *Phytophthora* have been variously implicated.

Diagnosis is made complicated by the knowledge that the presence of a particular fungus on, or within affected shoots, does not mean that it was responsible for causing the disease. Fungi can survive in association with woody plants in a variety of ways, not least as secondary infections of weakened tissue or growing as a saprophyte on already decaying tissue, persisting long after the first cause has perished.

Figure 1. Images of bushes from which *Phomopsis* was isolated (FAST LLP)

Even a cursory inspection of many blueberry plantations is likely to reveal a range of die-back type symptoms. Most common are symptoms associated with blossom, twig and shoot tip infections that progress for a few centimeters only. However it was known that, for example, *P vaccinii* causes such tip and blossom die-back symptoms in addition to the more severe and progressive die-back problems that are the subject of this project. If the same fungi were to be found consistently associated with both aerial symptoms and crown infections responsible for the death of whole branches or plants, this would have important implications for disease management.

The primary purpose of this project was to identify the cause of the aggressive type of dieback and crown rot symptom responsible for rapid decline or death in blueberry bushes. Armed with this information the project might then be able to shed some light on how the problem is spread, within and between sites, and consider methods to manage and control the problem.

Summary of the project and main conclusions

Survey and Sampling

Farms were visited in all the major UK cropping areas including the South East of England, Herefordshire, Devon, Dorset, Northamptonshire, Aberdeenshire and Perthshire. Four different symptom types were observed: tip dieback, tip dieback associated with dead flowers, progressive tip dieback and finally crown death leading to die up.

In the period March – December 2012, 72 plant tissue samples, collected by EMR and FAST, were submitted to Fera for diagnostic work. Sub-samples from some sites were also retained by EMR for separate testing. Samples ranged from whole plants, delivered directly to FERA in York by the researchers, through twigs, roots, fruits and leaves delivered by post or courier. The majority of samples were of plants displaying obvious dieback symptoms. Where fruit, leaves or root samples were submitted this was because symptoms evident on those organs appeared to bear some relation to the die-back symptoms within a plantation. Some additional samples were submitted during 2013.

Field Observations

Following damaging weather conditions during flowering in 2012, a great deal of 'blossom blight' was observed at two Herefordshire sites. *Phomopsis* was clearly identified as being associated with these symptoms, especially on the variety 'Darrow'.

On two sites visited in Scotland, frost damage to flowers was a common problem, with visible *Botrytis* sporing on the dead blossoms and associated shoot dieback. *Phomopsis* was not found in samples from these sites.

Bushes at the Herefordshire sites showed a remarkably high incidence of tip dieback. Most of the sites visited by EMR also showed a high incidence of tip dieback. Whereas this type of symptom is common in blueberry plantations, it is not universal. At various times before and during the period of the project, the authors have separately visited many blueberry plantations, in the UK and overseas, where bushes show few or no obvious tip die-back symptoms, especially of the type shown in Figure 2.

Figure 2. “Antler” symptom

With many fields showing significant shoot die-back but a smaller number suffering from the more serious branch dieback or even bush death, it was important to establish whether there is a link between aerial symptoms and decay symptoms affecting crowns or the base of canes. To that end, samples were distinguished according to whether necrotic symptoms appeared to be the result of basal, tip or side infections. Attempts were made to distinguish ‘die-back’ from ‘die-up’ symptoms by looking for necrotic staining within the otherwise healthy-looking wood below or above the area showing clearly visible external symptoms. Dan Chiuian marked and photographed diseased shoot tips and flowers during the Spring of 2013 and returned on several occasions during the following months to monitor the progress of any die-back symptoms. After a rapid early development, disease development slowed, failing to cause a serious and extensive ‘die-back’ symptom.

Figure 3. Blossom blight, May 2012

Table 1 (below) describes ways by which observed symptoms may develop. We do not know whether basal infections result from the systemic movement of propagules within plant tissues or infection by spores from an external source. Such information is of critical importance for the design of control strategies and the prognosis for plantations already showing significant dead arm like symptoms.

Table 1. Die-Up or dieback?

Tip infection	Lesion	Hyphae grow or other propagule move downwards	**Die-back** of tissue starting at tip and developing downwards
Side infection	Lesion	Girdling of stem by fungal decay or hypersensitive reactions in local tissues	Tissues above the lesion wilt and die due to starvation (**Die-up**)
		Hyphae grow, or other propagule move, downwards	Tissue above the infection point may die if original lesion girdles the stem (**Die-up**)
			Lesions may develop further down the stem or in the crown where opportunities arise (mechanical, chemical, freeze injury?) causing **dead arm** like symptoms
Basal infection	Lesion	Hyphae grow, or other propagule move, upwards, killing tissue	**Dead arm** like symptoms
		Hyphae kill a large enough volume of tissue at the base of shoots or cause a hypersensitive reaction (tyloses etc.)	Tissues above the lesion wilt and die due to starvation (**Die-up**)

Figure 4. Die-back, December 2012

Diagnosis

Ann Barnes and colleagues at Fera followed established protocols for identifying fungi present on the samples by visual diagnosis following dissection and, where appropriate setting up cultures to study them in more detail. The diagnostic focus based on previous experience, was centered on investigating potential fungal problems; previous analysis and initial analysis in the project had not identified any bacterial or viral pathogens.

Not surprisingly a very diverse range of fungi were identified as described in Table 2. *Phomopsis* was the most commonly isolated fungus, present in at least 32% of blueberry samples submitted. The incidence of *Phomopsis* may in fact be greater than this as a further 11% of samples submitted yielded a pathogen that could have been either *Phomopsis* or *Phoma.*

The taxonomy of fungi is complicated and subject to change; in particular both *Phomopsis* and *Botryosphaeria* are under major revision resulting in difficulties in both reliable identification to species and comparison with previously published work. Description of *Phomopsis* species is complicated by the fact that the same species may exist as *Phomopsis* (asexual state) or *Diaporthe* (sexual state). The two forms, although sharing the same DNA, are not morphologically similar. This is further complicated by the fact that the *Phomopsis* state is morphologically very similar to a similar fungus called *Phoma*.

Table 2. Type of fungi isolated from the collected samples (Year 1).

Fungi isolated:	**% of all blueberry samples**	**% of samples when leaf spot, fruit and root samples excluded**
Phomopsis/Diaporthe	32	30
Phoma	14	12
Phomopsis/Phoma	11	11
Botrytis	16	14
Fusarium	14	12
Cytospora	9	9
Botryosphaeria	7	7
Phytophthora	9	7
Coniothyrium	5	5
Cylindrocarpon	2	2
Ceratocystis	2	2
Ascochyta	2	2
None	4	2

At least three different species of *Phomopsis* were isolated from symptomatic blueberries based on molecular analysis *(P. viticola, P. eres/conorum and P. theicola).* Not all species satisfied Koch's postulates (a test to confirm pathogenicity) and could not therefore be considered to be primary fungal pathogens. Of the three species, *P.viticola* was the most damaging. Within the genus *Phomopsis* there is a wide range of pathogenicity ranging from aggressive primary pathogens, less aggressive wound pathogens to harmless saprophytes. There are also numerous reports in the literature from other similar woody plants of the ability of *Phomopsis* to live harmlessly and without causing any visual symptoms within woody tissue: A fungus surviving in this way is referred to as an endophyte. External biotic and abiotic factors may act as a trigger for these harmless endophytes to cause disease.

The project also investigated the potential significance of fungi belonging to the family Botryosphaeriaceae as they are potential pathogens of Vaccinium and other similar woody crops. Two species (*Botryosphaeria obtusa* and *Neofusicoccum australe*) were recovered from symptomatic tissue and both isolates satisfied Koch's postulates demonstrating their role as primary pathogens. Again these results are comparable to those found in other countries with evidence of interaction between *Phomopsis* and Botryosphaeriaceae as part of a disease complex.

The project has shown that progressive die-back of blueberries can be caused by more than one species of fungus including those from *Phomopsis/Diaporthe*, *Botryosphaeria/Neofusicoccum and Coniothyrium*. At least some of these species may be able to live within blueberry shoot tissue without causing harm. Even if not already living inside blueberry tissues these species are common inhabitants of plantation habitats, variously growing on such material as old tip and bud infections, decaying prunings and injured flowers. When inoculated directly into wounded tissues the isolates of *Phomopsis* and *Neofusicoccum* collected from sick bushes show only moderate of even weak pathogenicity in laboratory conditions. However, it is also clear from field observations that crown/basal infections may extend sufficiently to cause wilting and starvation of branches. There are an increasing number of studies showing that *Phomopsis/Diaporthe* and *Botryosphaeriaceae* can survive within plants without causing disease but may also cause disease when their host becomes stressed by other factors.

Interestingly, during pathogenicity testing, Fera discovered that symptomless plant material supplied for the harvesting of shoots used for pathogenicity tests was harbouring *Phomopsis,* apparently living as an endophyte within the plant tissues. Shoots wounded during the inoculation process used for pathogenicity testing developed lesions that were found to contain a species of *Phomopsis* that was not the one, for which a pure isolate had been prepared and inoculated into the wound. Some months after the experimental work was completed, three of the 20 stock plants that had been grown in isolation at Fera were found to have died. *Phomopsis* was isolated from one and *Coniothyrium* from another which again illustrates the potential of healthy looking young plants to be carrying potentially lethal pathogens.

Main conclusions

Throughout the UK, blueberry plantations contained plants with die-back symptoms. These ranged from blossom blight and tip dieback to a more serious and frequently lethal crown infection leading to branch death or plant loss.

- No evidence of any bacterial or viral pathogens was detected; no consistent fungus was isolated from the various symptoms, but a diverse range of potential fungal pathogens including *Phomopsis, Botryosphaeria, Botrytis* and *Coniothyrium* were isolated.

- *Phomopsis* was the most commonly isolated fungus from about a third of symptomatic plants; three different species were identified of which *P viticola* was the most damaging. Importantly, no evidence of the EC quarantine listed pathogen *P. vaccinii* was found.

- Two species of Botryosphaeriaceae were isolated and both also proven to be pathogenic.

- The species (*Phomopsis ribicola*) found by HDC Project SF12 to be most associated with severe dieback in blackcurrants, was not identified in blueberry samples for which diagnostic work was taken to the level of species identification.

- In-field symptoms did not provide a reliable indication of the species of fungal pathogen involved.

- UK field experience is that bushes exhibiting 'die up' symptoms due to crown death tend not to recover, becoming progressively weaker over a period of years.

- The speed with which bushes succumb to serious dieback symptoms appears to be controlled by factors other than the simple pathogenicity of the infecting fungi. There may be interaction with other pathogens in a disease complex or an over-riding influence of environmental and cultural factors. Work is needed to investigate the effect of chemicals on disease expression, in particular the plant strengtheners, elicitors and growth promoter products that are reported to increase resistance of plant to diseases.

- *Phomopsis*, perhaps living as an endophyte, was isolated from symptomless young plants used in the pathogenicity tests. Of the 20 symptomless plants provided for the pathogenicity tests, three died within six months from which *Phomopsis* was recovered on one occasion in addition to *Coniothyrium*.

- It is not known whether it is practically possible to eradicate these fungi from propagation sites, mother stock or cropping plantations nor whether eradication would make plants more or less susceptible to later disease infections. Further work on this subject is required.

- Confirmation that *Phomopsis* and species from the *Botryosphaeriaceae* family are important in the development of blueberry dieback symptoms should be used to inform fungicide choice.

Further work

More research is needed to investigate the relationship blueberry plants and *Phomopsis*, *Coniothyrium* and the *Botryosphaeriaceae*. Scientists need to find out how these fungi are able to survive within apparently disease free plant material, what stress factors are responsible for rapid and dangerous expansion of established basal lesions (causing progressive dieback) and by what means they might be eradicated from bushes and/or plantation or propagation environments. The findings of the blackcurrant project SF12 indicated that commonly available systemic fungicides may not be effective in this respect.

It is important to establish whether fresh plants may be planted into growing media from which infected bushes have been removed. If growers were simply able to rogue out plants showing symptoms of progressive dieback at any time during the development of plantations and to replace those plants with fresh material into the same growing media (pots or soil) with a minimum of additional inputs, this disease would immediately be made more manageable during the early years of plantation establishment.

It has become clear from contacts made and recent scientific reports, that the type of disease complex presented by progressive blueberry dieback has much in common with those observed in many other crops and caused by the same groups of fungi. We need to expand our knowledge of relevant work being carried out by pathologists working in other crop groups.

Information on the identity of the species of fungi associated with dieback may be used to inform decision making about fungicide selection.

Financial benefits

The establishment cost for a new blueberry plantation is particularly high. Fields are planted with at least 3,000 plants per hectare and often more when soil-less systems are used. Plants are supplied in pots at a cost of up to £3.00 per plant. Most soil grown plantations require expensive amendment of the soil using sulphur and organic mulches. Pot grown

blueberries incur the cost of pots, compost/growing media and supporting/drainage infrastructure. The final cost of establishment, may be as high as £6.00 per plant so it is of critical importance that they do not fail before achieving a return. The loss of plants to die-back disease can have a substantial impact on profitability. Sick plants demand extra husbandry work, produce small fruit and make no contribution to paying for sprays and other field costs. The situation is made harder still when the cause and source of infections are uncertain. The risk of cross infection from replanted soil, composts and other materials cannot be quantified or properly addressed.

Identification of the species most likely to cause progressive die-back of blueberries has already enabled agronomists and growers to focus on strategies known to reduce disease pressure (hygiene, fungicide selection, spray timing) from those species during fruit production and in propagation.

Action points for growers

Several fungi were found to be associated with blueberry dieback and death. The species most commonly found may be described as 'wound pathogens', tending to take advantage of weakened or damaged tissues including flower parts, frost damaged shoots and shoots damaged by such things as vine weevil larvae and pruning.

Blossom and shoot tip infections caused by *Botrytis cinerea* and several *Phomopsis* species frequently appear but it was found that they tend not to progress into more severe die-back symptoms. These species, together with *Botryosphaeria (Neofusicoccum)* and *Coniothyrium* species identified in some samples are all capable of infecting blueberry shoots. Although it appears that the relationship between these fungi and disease development is a complicated one, the following guidance can be given to growers:

- Prune or snap out twigs showing any kind of dieback symptom during the dormant season as it is possible these are a source of inoculum. Remove the prunings, ideally from the plantation but at least from around the base of bushes. If left on the ground they may still provide inoculum because the fungi are able survive successfully in dead material.

- During highest risk periods (eg. blossom, frost/hail events, freeze damage, planting/transplanting) select fungicides with a sufficiently broad spectrum of activity to

control all of *Botrytis*, *Phomopsis* and the *Botryosphaeriaceae*. It should be noted that, in America, the days between bud break and petal fall is regarded as the highest risk for infection by *Phomopsis* species.

- Avoid basal injuries to young plants during propagation and after planting out. Potential causes of basal injury include vine weevil larvae, pruning, rough handling, fertiliser scorch and freeze injury. High risk situations include permanently moist compost in contact with wounds, humidity (growing and storage) and harsh weather between bud break and flowering. Weak basal shoots that are typical of young plants during propagation and on arrival at farms for planting out are vulnerable to infection, often showing symptoms that are characteristic of the severe die-back symptoms studied for this project. They should be regarded with suspicion. All possible measures should be taken to prevent the infection of basal shoots by dieback fungi, both in nurseries and after planting out.