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Project number:	SF150
Project leader:	Graham Moore, Fruit Advisory Services Team LLP
Report:	Final Report (October 2015)
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Key staff:	Charles Lane (FERA) John Scrace (Freelance Plant Pathologist) Angela Berrie (EMR) Dan Chiuian (FAST)
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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.

Graham Moore

Senior Adviser and Designated Partner

Fruit Advisory Services Team LLP

Signature ..... Date .....

Report authorised by:

Timothy Colin Biddlecombe

Chairman and Chief Executive

Farm Advisory Services Team LLP

Signature ..... Date .....

CONTENTS

Appendices

GROWER SUMMARY

Headline

Blueberry dieback may result from infection by several different pathogens and there is a strong interaction between growing conditions and symptom development.

Background and expected deliverables

Since 2010 there has been an increasing concern about the extent of dieback symptoms in UK blueberry plantations. AHDB Horticulture project SF132 involved an extensive survey of affected plantations in an attempt to discover the causes of dieback. Fera, FAST and EMR collaborated to gather samples and experienced pathologists isolated the fungi associated with visible symptoms. The project showed that a surprisingly wide range of fungi may be found associated with dieback symptoms but it also established that certain species were more commonly associated with severe problems.

Laboratory tests were undertaken to prove that selected species of *Phomopsis* and those from the *Botryosphaeria* family were able to cause disease directly. During the work required to prove this, it was found that apparently symptomless plant material may harbour infection by these and other species within its tissues.

A lack of UK based knowledge about the occurrence, epidemiology and control of many of the species isolated from and associated with blueberries, prompted the funding of this project (SF150).

John Scrace, an experienced plant pathologist working under contract to Fera and who had also worked closely with blackcurrant growers affected by a similar dieback problem, was tasked with carrying out the literature survey, for which he lists more than 104 scientific papers as references from countries as diverse as USA, Canada, Chile, Italy, Spain, France, Poland and New Zealand.

Summary of the project and main conclusions

The results of SF132 were shown to be different from those of similar work in blackcurrants (SF012) in that instead of showing that a single species (*Phomopsis ribicola*) was largely responsible for the problem, a wide range of fungi were found to be associated with blueberry dieback.

The literature survey has established that in other countries this situation is normal for blueberries, rather than the exception. These pathogens may be present in the same plantation, on the same plant and even sometimes in the same lesion.

While some of the earlier books and papers on blueberry diseases might give the impression that there are a limited number of dieback, blight and canker pathogens with quite clearly defined symptoms, it has become obvious from more recent studies that the association of a complex of fungi with such symptoms is nothing new. Having said that, it is also obvious that the presence of the crop in many 'new' growing areas will have exposed it to a greater range of potential pathogens than might be found in their native North America.

There has also been a change in the techniques used in the diagnosis of plant diseases – particularly in the development and use of DNA analysis, which has changed our understanding of the true identity of some pathogens and the relationship between species. SF150 has confirmed the importance of several *Phomopsis* species and of species from the *Botryosphaeria* family.

The survey has also provided a helpful summary for all of the known disease causing agents which are conveniently listed in the contents pages of the Science Section of this report: Literature.

Results of the review

For each of the pathogens described information gleaned from the scientific papers is broken down into the following headings:

- Symptoms
- Epidemiology
- Control (cultivar selection and use of fungicides)

The impact of plant stress factors and the risk presented by apparently symptomless infections are discussed. The following are the main conclusions:

- Symptoms are often associated with the presence of a complex of fungi.
- Studies have shown that several different *Diaporthe* species (asexual states = *Phomopsis*) can cause very similar symptoms. A similar situation exists for some other pathogens, including those from the *Botryosphaeria* family.
- Symptoms may arise as a result of complicated interaction between more than one species of fungus and abiotic factors such as mechanical damage and drought stress. Growers should be aware of the likely importance of irrigation problems (pots and soil) and soil structural factors affecting root growth (organic matter, aeration) as factors in the development of symptoms.

- Species associated with dieback in UK blueberries, for which pathogenicity to blueberries was confirmed by SF132 and which are reported as being responsible for disease in other countries, include *Phomopsis eres / conorum* complex*, Phomopsis theicola*, *Neofusicoccum australe, Botryosphaeria obtusa*. A number of fungicide active ingredients and plant defence boosting materials (harpin, chitosan) are reported to contribute to disease reduction. Unfortunately, of the fungicides, several have been withdrawn from use in the UK or are not currently registered for use in any similar crop.
- Epidemiology studies show that the more commonly found species produce spores that survive on twigs and stem lesions and are readily dispersed in wet conditions. Many are more active at temperatures above the normal for UK but that does not preclude infection of material held under warm, moist conditions during propagation and early establishment. It is common practice in propagation to grow plants at very high densities, to trim the plants at least once during the growing season and to employ overhead sprinklers as the main source of irrigation. The use of clean stock and ultra-careful hygiene practices must therefore be given priority both in nurseries and during crop establishment when plants are grown in pots at high densities and under humid tunnel conditions.
- While fungicides may be useful for disease prevention (blossom, leaf/fruit scar and wound infections) they are not generally effective against established / deep-seated infections. Latent, symptomless / endophytic infections have been demonstrated or strongly suspected as a cause of later plant failure. A controversial subject but one that is no less important for study by blueberry scientists as by those concerned with other crops.

Financial benefits

The annual farm-gate value of blueberries produced in the UK is thought to be c.£20 million.

Before the start of Projects SF132 and SF150, growers were starting to see widespread and costly bush dieback. In one instance a young plantation had been grubbed one year after establishment using expensive potted plants. The authors are aware of several other plantations that have failed or are declining due dieback problems. Furthermore within most, if not all commercial plantations, growers are seeing an unacceptably high number of bushes affected by dieback symptoms – perhaps 1% or more.

The authors believe that identification of the pathogens involved, a better general understanding of their epidemiology and possible control methods gained from contact with scientists from other countries, has already improved the position of growers. Given improved understanding and communication of some of the findings about the way the disease may be spreading during propagation and early establishment, and knowledge of interactions between soil conditions and disease resistance, the cost of SF132 and SF150 will have been more than justified given that even a 1% increase in yield would generate c. £130,000 *per annum* in extra income net of picking and post harvest costs.

Action points for growers

- Seek ways to eradicate infection of plant pathogens, especially *Phomopsis* spp., fungi from the *Botryosphaeria* family, *Botrytis cinerea* and *Conithyrium* spp. in nurseries and during establishment of new plantations. Look to review overhead irrigation practices, plant density and options for the use of plant protection products pre- and post-trimming operations.
- Recognise that dead, twiggy shoots often found at the base of young plants and wounds caused by vine weevil grubs, may be infected with *Phomopsis* and/or other important dieback fungi. Implement thorough monitoring and quality control procedures.
- Respect the risk of infection via pruning and transplanting wounds.
- Recognise that the combination of moist substrate and raised temperature provided by closed tunnels, is likely to widen the range of species able to infect blueberries and shorten the infection time for all.
- Recognise that infections are most likely to spread within tissues and strangle branches or whole blueberry plants when they are not ‘happy’ for other reasons – perhaps especially when roots are struggling to adapt to drought, water-logging or other problematic soil/growing media conditions.
- Where possible, use available plant protection products or otherwise manage conditions to suppress disease pressure, especially during the periods of bud break - fruit set, immediately post harvest and at leaf fall.

SCIENCE SECTION

Introduction

The UK blueberry growing industry has expanded rapidly in recent years, but in some plantations pest and disease problems have been identified as an important cause of lower than optimal yields. There have been many cases of growth decline linked to dieback and crown rot type symptoms. Such decline has resulted in severe losses in the west of England and 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. The symptoms are often limited to one or more branches whilst other parts of the bush continue to grow normally for a while. Affected branches may show signs of limited recovery, with new shoots breaking from previously dormant buds as the growing season progresses, only to fail completely the following spring.

Surveys of affected plantations carried out as part of project SF 132 revealed four main symptom types:

A. A limited tip dieback (Figure 1)
B. A tip dieback associated with flower infection (Figure 2)
C. A progressive tip dieback (Figure 3)
D. A ‘die-up’ originating as a crown rot (Figure 4)

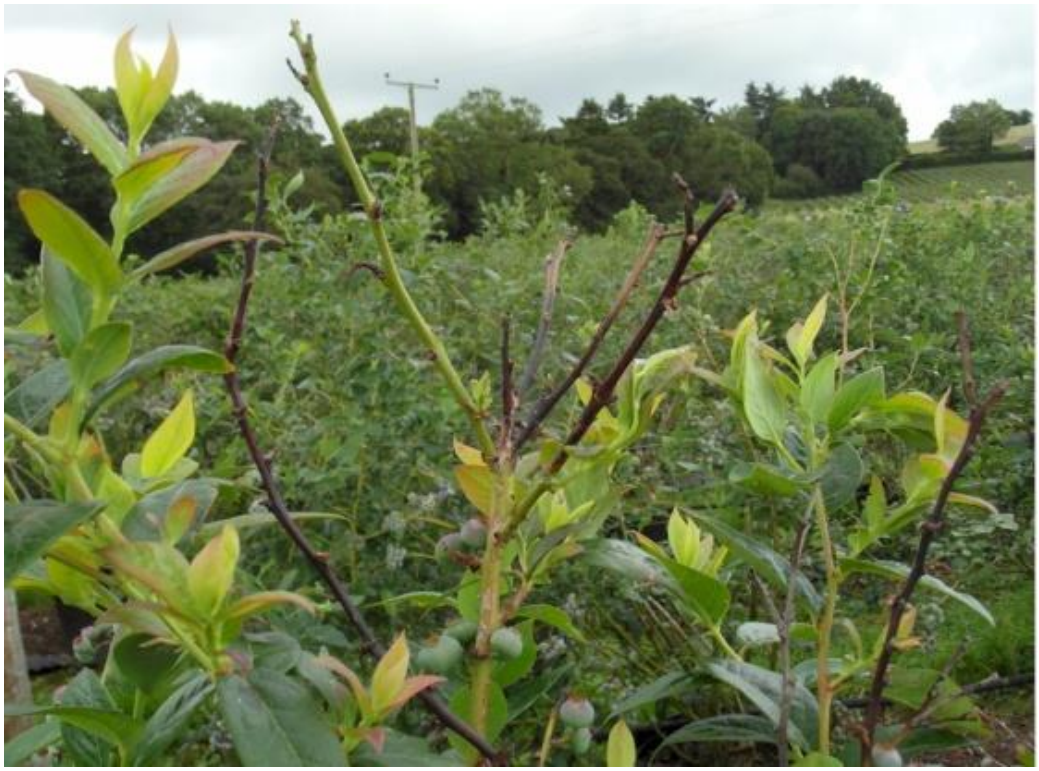

Figure 1. Limited tip dieback

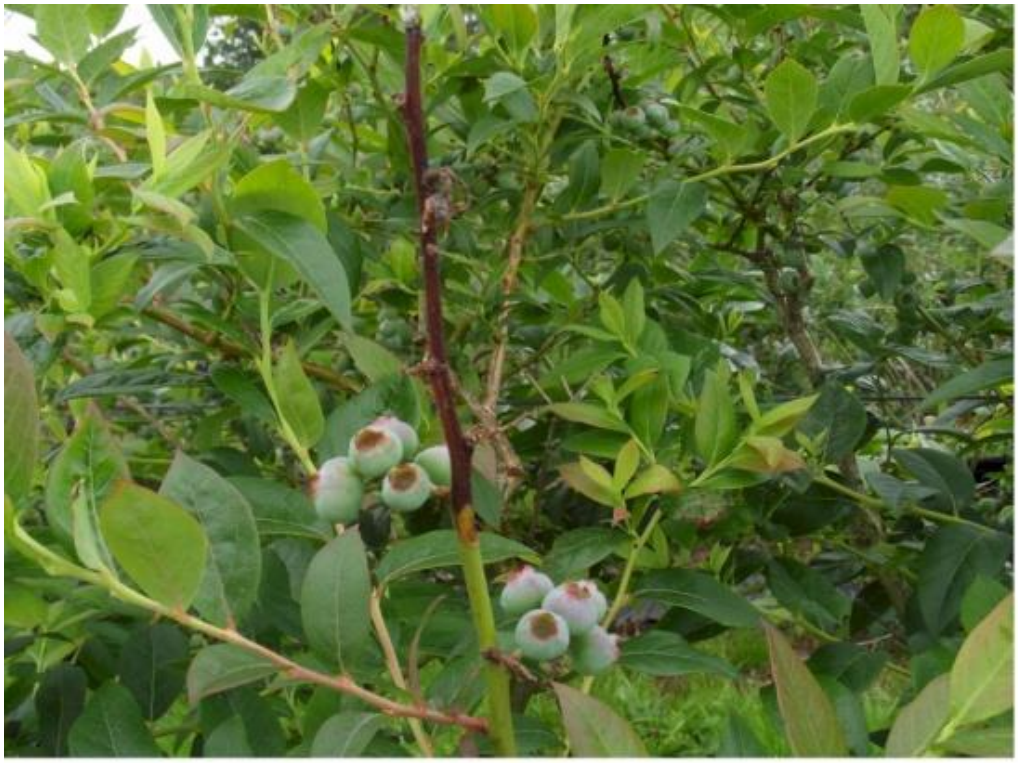

Figure 2. Tip dieback associated with flower infection

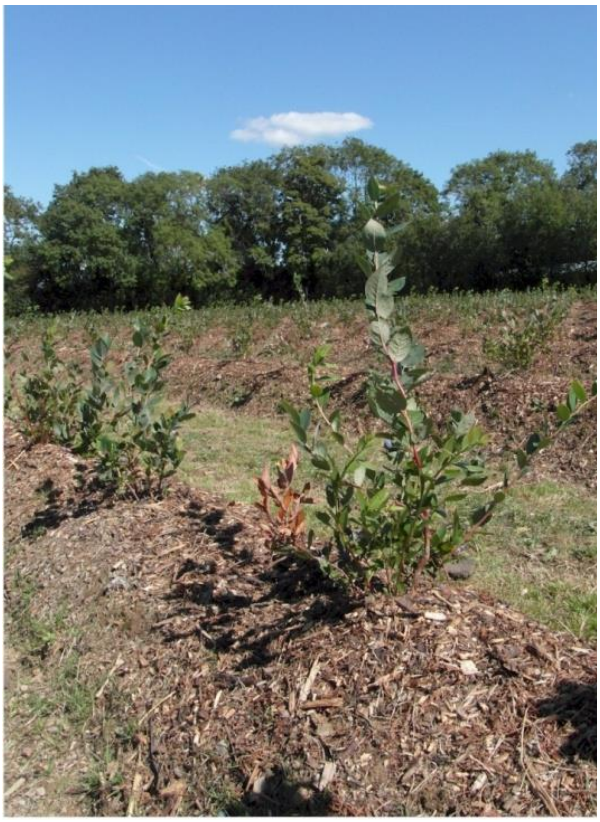

Figure 3. Progressive tip dieback

Figure 4. Die-up

The crown rot/decay appeared to originate as a lesion in the crown itself rather than from the progression of a dieback down the branches and stems and into the crown. This symptom was most likely to result in the death of whole branches or the entire plant. In many instances it has been our experience that symptoms similar to those shown in Figure 1 may be a precursor to more serious decline / die-up symptoms shown in Figure 4.

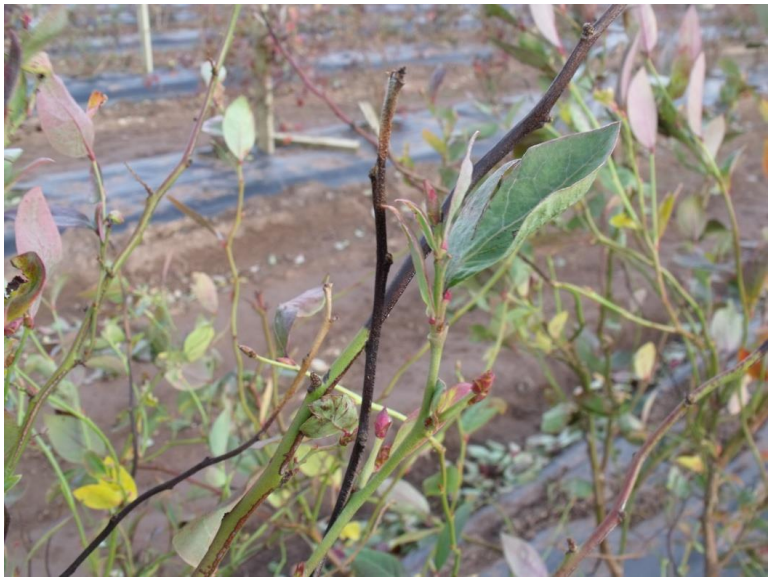

Figure 5. Symptoms similar to those illustrated by Figure 1 but in a field that declined rapidly the following summer

Plants may be damaged or otherwise made vulnerable to infection during propagation or early establishment. Such infections may, at first, be very slow to progress.

Cultivars notably affected by one or more of the symptom types were ‘Aurora’, ‘Bluecrop’, ‘Chandler’, ‘Darrow’, ‘Draper’, ‘Duke and ‘Legacy’.

As part of SF 132, a number of samples from affected plantations were submitted to Fera for laboratory testing. A range of different fungi was isolated from the affected plants, as summarised in Table 1.

There were a few differences noted between symptom types, their position in the plant and the genus of fungus recovered. *Phytophthora* sp. was found infrequently and inconsistently in association with crown decay symptoms, whilst dieback originating from the flowers was most likely to harbour *Phomopsis*. However, many of the fungi were found in association with a range of symptom types.

Of the fungi listed in Table 1, some are well-known pathogens of blueberry. In particular, *Diaporthe* / *Phomopsis* species, *Botryosphaeria* / *Neofusicoccum* species and *Botrytis cinerea* are frequent causes of twig blight and/or stem blight and cankering in many countries. Further work was undertaken to identify isolates of *Diaporthe* / *Phomopsis* and *Botryosphaeria* / *Neofusicoccum* to species level. This would enable comparison of the species found with those identified as causing disease on blueberries elsewhere in the world. It was also necessary to determine whether the quarantine organism *Diaporthe vaccinii* had been isolated from any of the samples.

Table 1. Incidence of fungi as a percentage of all isolations (many samples contained more than one fungus). Leaf spot, fruit and root samples are excluded.

Fungus	**Incidence in samples (%)**
Diaporthe / Phomopsis	30
Phoma	12
*Phomopsis / Phoma**	11
Botrytis	14
Fusarium	12
Cytospora	9
Botryosphaeria / Neofusicoccum	7
Phytophthora	7
Coniothyrium	5
Cylindrocarpon	2
Ceratocystis	2
Ascochyta	2
None	2

*Precise genus not identified

Three *Diaporthe / Phomopsis* species were identified: *Phomopsis viticola, P. eres / conorum* complex and *P. theicola.* No *D. vaccinii* was found. Two *Botryosphaeria / Neofusicoccum* species were identified: *Neofusicoccum australe* and *Botryosphaeria obtusa.*

Host inoculation tests were undertaken with these isolates to test for pathogenicity on blueberry. The technique used is known as Koch's postulates and is described below. This technique has been used for many of the potential pathogens of blueberries found in other countries, and will be referred to throughout the review.

Koch's postulates are undertaken when a potential new pathogen has been found on a plant; they are a set of rules that must be followed in order to prove that the organism isolated from a disease symptom is the cause of that symptom and not just a secondary coloniser or

saprophyte invading the affected plant tissue. The steps to be followed to fulfil Koch's postulates are as follows:

1. The pathogen must be present in association with the symptoms in all (or, more practically, the great majority) of diseased plants examined.
2. The pathogen must be isolated from the symptoms and grown in pure culture, and its characteristics described.
3. From the pure culture, the pathogen must be inoculated into healthy plants of the same type, and must reproduce the same disease symptoms as those from which it was originally recovered.
4. The pathogen must be re-isolated into pure culture from the disease symptoms that developed on the inoculated plants.

Of the three *Diaporthe / Phomopsis* species, *Phomopsis viticola* was the most damaging when inoculated into detached, current-season's shoots. *P. eres / comorum* caused some damage, whilst *P. theicola* did not cause infection. Interestingly, in some cases a different species of *Phomopsis* was re-isolated from the inoculated shoots to that which was used for the inoculations. This indicates that *Phomopsis* was already present in some of the apparently healthy shoots used for the test, presumably as a latent infection or as an endophyte.

Both of the *Botryosphaeria / Neofusicoccum* species proved to be aggressive pathogens in the inoculation tests, causing more damage than the *Diaporthe* species.

Twenty symptomless 'stock' plants were used to provide the detached shoot material for the pathogenicity tests. The plants were retained and grown by Fera, and three (15%) of them died within the following six months. When two of these plants were tested *Phomopsis* was isolated from one and *Coniothyrium* from another. However, *Coniothyrium* was not one of the fungi selected for Koch's postulates tests, so its pathogenicity is unclear.

A brief literature review was carried out as part of project SF 132. However, it was felt that a more comprehensive review was required to gather all available information on the pathogens found to be responsible for blueberry dieback and decline elsewhere in the world, and to see if this information could be related to the findings of SF 132. As a result the review reported here was commissioned as major part of project SF 150.

A widely-used reference publication on diseases of blueberry is the US book *Compendium of Blueberry and Cranberry Diseases*, published by the American Phytopathological Association. However, since this book was published in 1995 there have been considerable

advances and changes. The two greatest changes are probably the increase in the number of countries involved in blueberry production (for example Chile, Argentina, New Zealand and China, in addition to the UK) and the use of new diagnostic techniques (particularly DNA analysis techniques) for the identification of pathogens. As is the case with most other crops, new diseases also emerge continually to threaten production, and existing diseases fluctuate in importance and severity (Cline 2014).

The scientific papers and other information sources identified by the literature searches carried out for this review reveal a complex situation with regard to blueberry dieback and decline, both in the number of organisms potentially involved in the problem and in their interactions (both with each other and with abiotic 'stress' factors). Certain pathogens are considered to be major players in blueberry dieback and have been studied in detail, whilst for others the available information is limited.

This review focuses in detail on fungal pathogens, although bacterial and viral diseases associated with dieback and decline symptoms are described briefly for completeness. The fungal pathogens associated with dieback are split into three groups:

1. Pathogens attacking the aerial parts of the plant (twigs, stems or crowns) to cause lesions and/or dieback;
2. Pathogens attacking the root system (and sometimes spreading from here to the crown or stem bases);
3. Pathogens causing vascular diseases.

The majority of fungal pathogens are to be found in the first group.

Literature Review - Results

Contents

Blueberry stem blight caused by '*Botryosphaeria dothidea'*

Blueberry diseases caused by other members of the *Botryosphaeriaceae*

Godronia or Fusicoccum canker (*Godronia cassandrae*)

Pestalotiopsis and related species

Anthracnose twig blight and fruit rot (*Colletotrichum* spp.)

Botrytis blight (*Botrytis cinerea*)

Other fungi attacking twigs and shoots of blueberry

1b. Fungal and fungus-like pathogens attacking the roots, crowns and stem bases to cause dieback

Armillaria root rot or honey fungus

Phytophthora root rot

Other fungi and fungus-like organisms causing root decay

1c. Fungal pathogens causing dieback as a result of vascular wilt diseases

2. Dieback as a result of infection by bacterial pathogens

Bacterial blight / bacterial canker (*Pseudomonas syringae)*

Dieback caused by other bacterial pathogens

Ralstonia solanacearum

Xanthomonas sp.

Bacterial leaf scorch (*Xylella fastidiosa)*

3. Dieback as a result of infection by viruses

Blueberry scorch virus (BlScV)

Necrotic ringspot disease

Blueberry leaf mottle virus (BlMoV)

1. Diseases caused by fungi and fungus-like organisms

1a. Fungal pathogens attacking twigs, stems or crowns to cause lesions and/or dieback

Many different fungal genera and species are reported in the literature as attacking the twigs, stems or crowns of blueberry to cause dieback, blight or cankering. Some of these are important pathogens of the crop (although the exact diseases found will inevitably vary according to the country in which the blueberries are grown); these are discussed in detail. Others appear to be of minor significance or, whilst still damaging pathogens to the crop in some countries, cause symptoms that are not consistent with those of the dieback and decline problems in the UK. These fungi are therefore described briefly in a final section headed 'other fungi'.

Phomopsis twig blight and canker (Diaporthe spp., asexual states Phomopsis spp.)

A number of *Diaporthe* species have been found in association with disease symptoms on blueberry. The symptoms range from dieback of young shoots and twigs through to death of entire stems and plants. Leaf spotting and fruit decay can also occur.

Diaporthe vaccinii is a cause of significant damage and yield loss on highbush and rabbiteye blueberries in North America, and is the most studied of the *Diaporthe* species found on blueberries. However, the advent of DNA analysis techniques in particular has seen a number of other *Diaporthe* species identified on blueberries suffering from symptoms similar or identical to those caused by *D. vaccinii*. Whilst in the majority of these cases pathogenicity of the various *Diaporthe* species on blueberry has been confirmed by inoculation tests there is far less information available on their epidemiology or impact on the crop.

Diaporthe vaccinii (asexual state *Phomopsis vaccinii*)

The host range of *D. vaccinii* is restricted to *Vaccinium* species, with known hosts including American and European cranberries (*V. macrocarpon* and *V. oxycoccos*), highbush blueberry (*V. corymbosum*), rabbiteye blueberry (*V. ashei*), cowberry (*V. vitis-idaea)* and bilberry (*V. myrtillus*) (EFSA 2014). It was found as a pathogen of blueberry for the first time in 1934 (Wilcox, 1939), causing a twig blight. The fungus has now been associated with a range of symptoms, including twig blight, cankers, leaf spots and fruit rot. Recorded yield losses in the USA from twig blight on untreated, susceptible blueberry cultivars have been as high as 70% (Cline 2002a).

Diaporthe vaccinii is an EU-listed pathogen. It has been found previously in the UK on plantings of imported material (OEPP/EPPO 1997) but did not establish here. Within the EPPO (European and Mediterranean Plant Protection Organisation) region it currently has a limited distribution in Latvia and is described as 'transient, under eradication' in the Netherlands (EFSA 2014).

Symptoms

Milholland (1995a) and Ramsdell (1995a) distinguish two main disease types caused on blueberries by *D. vaccinii*. The first is Phomopsis twig blight and fruit rot, and the second Phomopsis canker. From their descriptions, and from those of other authors, there seems to be some overlap between the symptoms of the two diseases. Schilder *et al* (2006) state that twig blight tends to be more prevalent in blueberry plantations in southern USA, whereas canker is more severe in northern plantations. Descriptions are given below of each of the disease types.

Phomopsis twig blight and fruit rot is characterised by the dieback of one-year-old woody stems carrying flower buds. Cline (2002a) states that infected flower buds turn brown and die (Figure 7), and from these the fungus moves into the twig causing browning and necrosis of the bark around the bud. The fungus then usually spreads down the twig, causing a dark-to-reddish-brown dieback (Figures 6 and 8). Dieback can spread back 15-25cm but normally ceases after the twig is killed and does not progress further down the stem into older wood.

Milholland (1995a) also describes infection of the current year's succulent shoots, which become crooked and have discolouration of the pith in a symptom that is difficult to distinguish from cold injury. This dieback of succulent shoots is also reported from inoculation tests on young plants by Wilcox (1939); in this case the infection progressed back into the previous year's woody tissues below, killing a considerable portion of the inoculated young plants within five weeks.

Leaf spots are reported by Anco and Ellis (2011), Milholland (1995a), and Wilcox (1939 – again from inoculation of test material). Lesions are small and reddish to begin with but increase in size to 10cm.

Infected blueberry fruit are soft, often splitting and leaking juice, with a red-brown and mushy flesh (Milholland and Daykin, 1983).

Figure 6. Dieback due to *Phomopsis* twig blight on cv. 'Draper' (arrows indicate symptoms)

Figure 7. Flower infection cv. 'Draper'

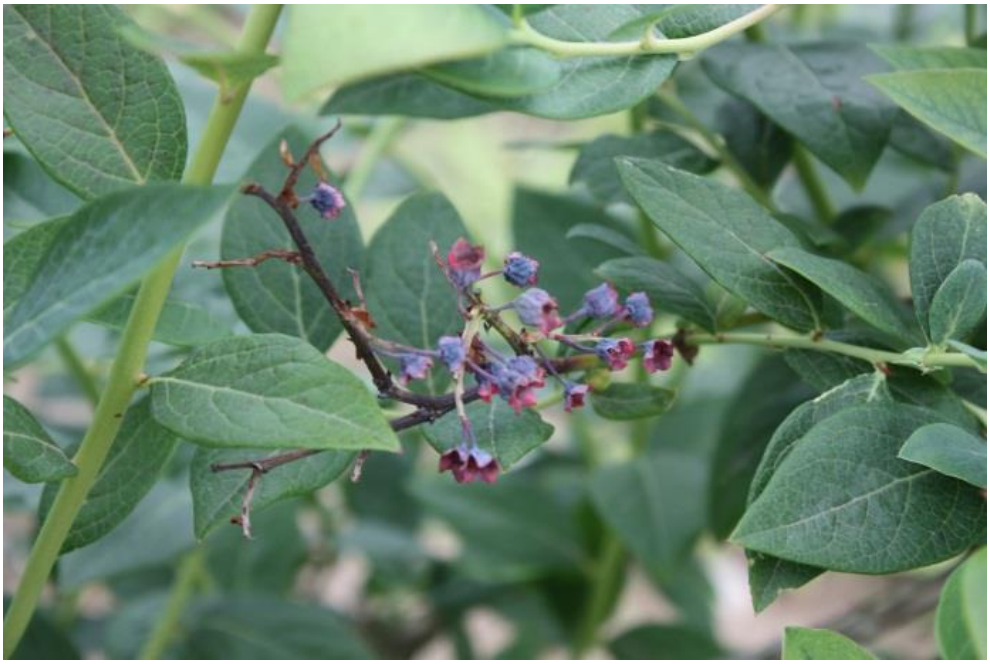

Figure 8. Infection progressing from flowers into twig, cv. 'Aurora'

Symptoms of **Phomopsis canker** can be found from soil level to up to 1.5m above the ground (Ramsdell, 1995a). The disease has been reported from several US states. Cankers may form on one-, two- or three-year-old stems. They are usually brown in colour and range in length from two to over 10cm (Figure 9). They may encircle the entire stem. Old cankers on two- and three-year-old stems become greyish and slightly flattened. Numerous fruiting bodies (pycnidia) of the fungus often form on the surface of these older cankers. Wiengartner and Klos (1975a) state that cankers caused by *D. Vaccinii* tend to be longer and narrower than those caused by *Godronia cassandrae* (Godronia canker).

Below-ground infections of the crown may also occur (Wiengartner and Klos 1975a, Parker and Ramsdell 1977a) and in these cases stem dieback frequently occurs in the absence of obvious canker symptoms on the above-ground parts of the affected stem.

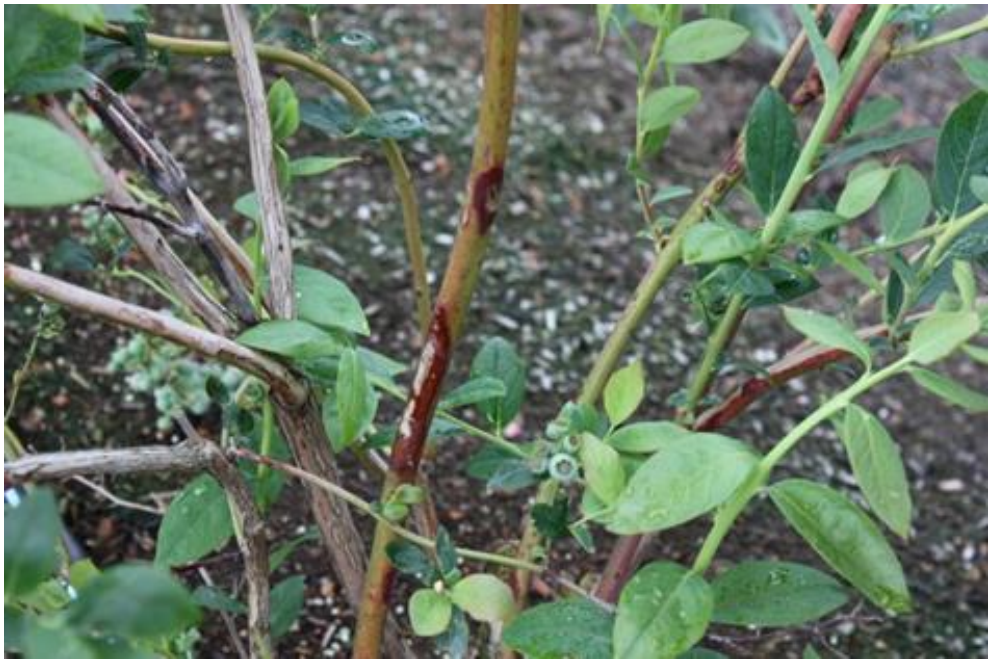

Figure 9. Phomopsis canker, cv 'Liberty'

Epidemiology

The vast majority of publications state that the sexual spores (ascospores) of *D. vaccinii* are not found in the field, although Guerrero and Godoy (1989) report that both the asexual **and** sexual stages of the fungus were found in Chile. It is the asexual spores (conidia) of the *Phomopsis* state of the fungus that initiate infection.

The fungus is capable of overwintering on blueberry twigs infected by twig blight the previous season, and for the stem canker disease as mycelium within the cankers themselves (Milholland 1995a, Ramsdell 1995a). Schilder *et al* (2006) state that another overwintering method for the fungus in some years is within live dormant buds, which die and give rise to twig blight in the spring. Where crowns are infected this would be another method of survival, even when infected twigs, stems and other debris had been removed from the plantation.

Spore trapping work (Milholland 1982, Parker and Ramsdell 1977a) has shown that the conidia are rain dispersed, released during wet periods throughout the growing season of the crop. Ramsdell (1995a) states that as little as 3.8mm of rain triggers the release of conidia. Spore release from the fruiting bodies (pycnidia) present on overwintering twigs and stem lesions is greatest during the flowering period of the plants and declines thereafter, but some are still being released in late summer. From mid-summer, spore numbers are added to by those being produced on new lesions that have formed during the growing season, although these sources do not extend the total period of spore release.

In vitro studies of spore germination by Parker and Ramsdell (1977a) showed that warm temperatures (21 to 27°C) were more conducive to spore germination and subsequent growth of the fungus than cooler temperatures (10 and 15°C).

The method of infection differs between the two disease types caused by the fungus. The main points of entry for Phomopsis twig blight are flower buds and open flowers (Daykin and Milholland 1990, Cline 2002a). Once the flower is infected the fungus spreads down into the twig. Milholland (1982) states that the fungus probably enters the stem from the flower through the vascular tissues, but according to Daykin and Milholland (1990) movement occurs through the cortical tissues, and the vascular tissues and pith are not invaded until the cortex has been completely colonised.

By contrast, stem canker infections generally occur through damaged stem tissues. Parker and Ramsdell (1977a) state that likely causes of such damage are abrasion wounds (including damage from mechanical harvesting equipment), and freezing. They also state that drought predisposes plants to infection.

Detection (see OEPP/EPPO (2009) for full details of diagnostic techniques)

The presence of a *Diaporthe* species can be confirmed on diseased plant parts in a number ways. Fruiting bodies (pycnidia) and conidia may already be present on the plant material when it is first examined, in which case they can be seen under a light microscope. However it is not possible to identify *D. vaccinii* to species level from microscopic examination alone. If no spores are present then incubation of affected plant parts in a humid incubation chamber may encourage their production. Aseptic isolations from symptomatic material onto a suitable agar medium (e.g. potato dextrose agar or sweet clover medium) will be required before a definitive identification can be made.

Whilst it may be possible to obtain a very tentative identification of *D. vaccinii* based on morphological features (such as the size and shape of fruiting bodies, size and shape of spores, and the appearance and growth rate of the fungal colonies on agar), it is not possible to gain a definitive identification in this way. This is because a range of other *Diaporthe* species is known to affect blueberry (see section below headed ‘Other *Diaporthe* species as causes of disease on blueberry’), some of which have very similar morphological and cultural characteristics to those of *D. vaccinii*. It is therefore necessary to confirm the identity of any suspect *D. vaccinii* isolates using DNA analysis techniques.

Control - cultural control measures

Most of the cultural control measures are aimed at either removing sources of inoculum of *D. vaccinii,* or avoiding the conditions that are favourable for infection. These include, where practical, pruning and removal during the dormant season of twigs affected by stem blight (Anco and Ellis 2011, Cline 2002a). Cline (2002a) states that growers who mow bushes after harvest (topping) will also benefit from the removal of blighted twigs. Overhead irrigation should be avoided in order to limit spread of the pathogen.

For Phomopsis canker Ramsdell (1995a) recommends pruning to remove cankered and wilted stems, as deep into the crown as possible. Infected stems should be removed from the field and burned or buried.

Garcia-Salazar (2002) states that as mechanical or low temperature damage are necessary for the development of Phomopsis canker, careless pruning or cultivating and the use of fertilizer late in the summer should be avoided. Keeping plants well-watered during drought and avoiding any other stress factors will also help to prevent the disease.

Control - cultivar selection

Various workers either report on screening test results of blueberry cultivars against *D. vaccinii* or give recommendations as to which cultivars to plant or avoid. Polashock and Kramer (2006, also Polashock, 2006) inoculated a large number of highbush (*V. corymbosum*), lowbush (*V. angustifolium*), half-high (*V. corymbosum* x *V. angustifolium*) and rabbiteye (*V. ashei*) cultivars with *D. vaccinii* and assessed their resistance to Phomopsis twig blight. Most resistant were the half-high cultivars 'Northsky' and 'Chippewa' and the low-bush cultivars 'Blomidon', 'Chignecto' and 'Cumberland'. However, some highbush cultivars such as 'O'Neal' and 'Star' were also relatively resistant, whereas others such as 'Emerald' and 'Legacy' were particularly susceptible.

Cline (2002a) states that the cultivars 'Murphy' and 'Harrison' are highly susceptible to twig blight, 'Croatan' is moderately susceptible, while 'Reveille', 'Cape Fear', 'Bluechip' and 'Wolcott' are relatively resistant. Anco and Ellis (2011) state that 'Bluetta' and 'Elliott' are resistant and 'Rubel' moderately resistant. Cultivars reported as having resistance by Teodorescu *et al* (1985) include 'Atlantic', 'Goldtraube' and 'Heerma'.

Cultivars listed as resistant by Retemales and Hancock (2012) are 'Bluecrop', 'Bluechip', 'Bluetta', 'Cape Fear', 'Elliott', 'Reveille' and 'Rubel'.

According to Ramsdell (1995a) no commercial cultivars show strong resistance to Phomopsis canker. In inoculation tests on two-year-old rooted cuttings carried out by Baker *et al* (1995)

there were significant differences in susceptibility between cultivars, with ‘Elliott’ and ‘Bluetta’ showing most resistance and ‘Spartan’ and ‘Bluejay’ being the most susceptible. However, even the most resistant cultivar ‘Bluetta’ had only a 40% survival rate.

Control - use of fungicides

Chemical control of twig blight can be obtained by applying fungicide sprays on a 7-14 day interval, starting at bud-break and continuing through bloom (Milholland 1995a, Cline 2002a). For protection against fruit rots (including those caused by *D. vaccinii*) sprays need to be continued beyond the flowering period.

There have been many trials evaluating fungicides for the control of Phomopsis twig blight. It is difficult to do a direct comparison between trials because of differences in application timings, methods and rates, and the use of fungicides alone, in mixtures or in alternating programmes. It should also be borne in mind that these trials were carried out in the USA, where active ingredients in commercial products may be present at different concentrations compared to those available in the UK.

A selection of active ingredients that have given control of twig blight in the US trials includes azoxystrobin + propiconazole, benomyl, captan, chlorothalonil, cyprodonil + fludioxonil, fenbuconazole, fenhexamid, fluazinam, propiconazole, pyraclostrobin, pyraclostrobin + boscalid and ziram (Cline *et al* 2008, 2005a, 2004, Cline and Bloodworth 2001, Schilder *et al* 2002, 2000).

A ‘delayed dormant’ application of lime sulphur or calcium polysulphide after leaf buds begin to break is also recommended by some workers (e.g. Anco and Ellis 2011) to reduce early season inoculum. Calcium polysulphide used as a single treatment in this way gave varying results in reported trials (Cline *et al* 2008, 2005b) but performed better when used as a program of sprays from bud break.

The plant defence protein harpin has also been evaluated in trials (Cline *et al* 2003, 2002) and given some control of twig blight, in one case equivalent to that of the grower standard fungicide mixture at the time. The biological control agent *Bacillus subtilis* and the plant defence booster chitosan also gave some reduction in disease levels in a reported trial (Schilder *et al* 2001).

There are fewer reports of control of Phomopsis canker using fungicides, although Garcia-Salazar (2002) states that azoxystrobin + captan, chlorothalonil, fosetyl-aluminium or mefenoxam gave good control. Parker and Ramsdell (1977a) used fungicides in the growing season after heavily diseased bushes had been sawn off just above the soil surface in a bush

rejuvenation test. Fungicide sprays (with benomyl or captafol) throughout the growing season gave disease reductions of between 36% and 58% compared to untreated plants. They speculate that the performance was relatively poor because there may have been below-ground infections in the crowns that infected the new stems as they grew - this type of infection would not be controlled by sprays.

Other *Diaporthe* species as causes of disease on blueberry

The emergence of DNA analysis techniques has enabled more accurate identification of *Diaporthe* species compared with identifications based on the morphological features of isolates. A combination of sequencing of ITS and EF genes and the use of a concatenated tree (see the final report for project SF 132 for further details) has proved particularly useful, although there are still limitations in terms of the lack of available sequence data on Genbank for reference isolates. However, DNA analysis has greatly increased the number of *Diaporthe* species found to be associated with cankering and dieback on blueberry.

Stem canker and dieback is a significant problem on blueberries in Chile, and a range of *Diaporthe* species other than *D. vaccinii* has been isolated from affected plants (Elfar *et al* 2013, 2012a, Latorre, Elfar *et al* 2012, Latorre and Torres 2011, Espinoza, Briceno and Latorre 2008). The symptoms caused are mentioned only briefly but include reddish to brown stem lesions and cankers, vascular discolouration of the internal tissues, and apical necrosis of the shoots.

Identified species of *Diaporthe* isolated from these symptoms were *Diaporthe ambigua, D. australafricana, D. neotheicola, D. passiflorae, D. phaseolorum, D. perjuncta* and *D. viticola.* With the exception of *D. viticola* inoculation tests showed that the various species were pathogenic to blueberry stems, fulfilling Koch's postulates. Elfar *et al* (2013) state that *D. ambigua, D. australafricana* and *D. passiflorae* were highly virulent on blueberry shots, stems and fruits, although in Elfar *et al* (2012a) it is stated that *D. ambigua, D. australafricana* and *D. phaseolorum* were less pathogenic than *Neofusicoccum parvum.*

Elfar *et al* (2013) state that all four species tested (*D. ambigua, D. australafricana, D. passiflorae* and *D. neotheicola*) were also capable of infecting shoots of apple, grapevine and pear, illustrating that unlike *D. vaccinii* many of the other species recovered from blueberries are not host-specific. A number of different host plants are given for many of these species (and others as described below) by Gomes *et al* (2013).

Another feature of the Chilean papers is the statement that the various *Diaporthe* species may be acting alone or together to cause the symptoms, and may also be acting as part of a disease complex with other pathogens such as *Botryosphaeria/Neofusicoccum* species and *Pestalotiopsis* species, as the fungi were often recovered together from the samples with

symptoms. This theory of a disease complex would also fit with the isolation of a range of fungi, including *Diaporthe/Phomopsis* spp. and *Botryosphaeria/Neofusicoccum* spp. from the UK samples tested in project SF132 (*Pestalotiopsis* species were not isolated from the UK plants).

Lombard *et al* (2014) isolated two new species of *Diaporthe* from infected blueberry plants in Italy. The symptoms consisted of cankers at the bases of the plants, and also twig blight. Plant death occurred where cankering was present within the crowns. The species were named as *Diaporthe baccae* and *D. sterilis*, both of which reproduced the disease symptoms on four blueberry cultivars in inoculation tests. Interestingly, none of the isolates of *D. sterilis* could be induced to produce spores on any of the growth media used in the study, meaning that this species could only be separated from other *Diaporthe* species based on its DNA sequence data.

Lombard *et al* also mention working with isolates of *D. eres* and *D. viticola* obtained from blueberry plants from the Netherlands, Chile and the USA, but it is not clear whether these isolates were obtained from obvious disease symptoms on the plants, nor whether Koch's postulates were carried out.

Farr *et al* (2002) examined a number of US isolates of *Diaporthe* obtained from blueberry and cranberry twigs and fruits, and found that whilst most of these were *D. vaccinii* a number (including seven from blueberry) were not. They could not identify these other isolates but speculated that they could have been endophytes rather than active participants in the blueberry diseases. They also noted that other work has shown that 'there are fungal endophytes that under some circumstances behave as pathogens, while under other conditions will remain innocuous'. This aspect of diseases caused by *Diaporthe* (on various hosts) is also discussed by Sinclair and Lyon (2005). They state that some species associate with their hosts as endophytes, apparently establishing latent colonies that are held in check by the plant's defences until that part of the plant dies or is weakened by stress or senescence, at which point the fungus becomes an opportunistic pathogen. They also state that the time of year when infection and damage occurs can be an indication of how aggressive a particular species of *Diaporthe* is, with the more aggressive parasites being able to invade and kill previously healthy tissues during the growing season of the host plant.

Finally, Szmagara (2009) isolated a species from blueberry plantations in Poland that was identified as *Phomopsis archeri*. This was isolated from various symptom types, including widespread necrosis of stems with cracking and peeling of the epidermis (from which it was recovered most frequently), canker spots and necrosis of stem tops. However, a range of

other fungi were also isolated from these symptoms, and Koch's postulates do not seem to have been performed on the *P. archeri* isolates. It also appears from the methodology that species identification was based on morphological characteristics and that DNA sequencing was not used.

Stem canker and stem blight caused by members of the Botryosphaeriaceae

This family of fungi has undergone frequent revisions over the years, based firstly on morphological characteristics and latterly on new information on relationships between the fungi provided by DNA sequence analyses. Many of the fungi in this family are found on plants in both their sexual (producing sexual spores or ascospores) and asexual (producing asexual spores or conidia) states. *Botryosphaeria* is the name for the sexual state of many of the species in this family, but is currently used as the preferred name for only seven species. These include *B. corticis* and *B. dothidea*, the causes in the USA of blueberry canker and blueberry stem blight, respectively. Many of the other species within this family are currently called preferentially by the names of their asexual states, and thus many of the other fungi pathogenic to blueberry are species of *Neofusicoccum.*

As a result of DNA sequence analysis it has been shown that there can be considerable overlap between the species in this family in terms of morphology (e.g. shape, colour and size of spores). This has called into question the accuracy of the species identifications given in earlier work on blueberry diseases. For many years, as stated above, research in the USA distinguished two disease types on blueberry, caused by different *Botryosphaeria* species; stem canker caused by *B. corticis* and stem blight caused by *B. dothidea.* Whilst identifications of *B. corticis* are likely to have been accurate, Phillips et al (2013) state that 'only after gene sequence data were used to clarify species concepts in the genus (*Botryosphaeria*) did it become apparent that some of the earlier reports of *B. dothidea* in association with plant diseases may have been misidentifications. Thus, the earlier reports of *B. dothidea* prior to 2004 should be interpreted with circumspection'.

This means (although it cannot be proven) that some of the cases of *B. dothidea* causing blueberry stem blight, as reported in earlier work from the USA, could actually have been other genera and species within the *Botryosphaeriaceae*, including species of *Neofusicoccum*. There has certainly been a great increase in the number of species (both of *Neofusicoccum* and of other genera in the *Botryosphaeriaceae*) reported as causing stem blight since DNA sequencing began to be used widely in this type of work. However, a further complicating factor is the fact that many of these more recent reports are from countries such as Chile, Argentina and China, as well as the US. It is therefore possible that the increase in

the number of species reported could also be partly due to the greater geographical range over which the blueberry crop is now grown.

In this review the nomenclature of the fungi as given in Phillips *et al* (2013) is used where possible.

Detection

Many members of the *Botryosphaeriaceae* readily produce spores (sexual ascospores, asexual conidia, or both) from fruiting bodies (perithecia or pycnidia) formed on affected plant parts. These may already be present on plant material when it is examined, or can be induced to form by incubating the material in a humid incubation chamber.

The members of this family can usually be readily isolated from affected material by aseptic isolations onto a general purpose agar medium such as potato dextrose agar. Some genera and species can be identified (at least tentatively) based on morphological features, but for many of them DNA extraction and sequencing is required.

Blueberry stem canker caused by Botryosphaeria corticis

Stem canker is a major disease problem on blueberry in some parts of the USA, particularly southern states such as North Carolina. The disease was first seen in the 1930's (Demaree and Wilcox, 1942) and affects both highbush and rabbiteye blueberries (although it does not normally cause significant yield loss on the more vigorous rabbiteye plants (Cline, 2011). The causal fungus, *Botryosphaeria corticis,* affects only *Vaccinium* species (including a number of wild species) and has not been reported outside of the US (Phillips *et al* 2013). The disease is most damaging to younger plants, and is frequently so severe in new plantings that the plantation is destroyed before it comes into full production (Milholland, 1995b).

The identification and designation of *Botryosphaeria corticis* (then called *Physalospora corticis*) from the early cases of stem canker was obviously based on the morphology of the fungus, but fungal isolates from these findings are no longer available for examination. However, Phillips *et al* (2006) tested recent isolates of the stem canker fungus from New Jersey and North Carolina by DNA sequencing and confirmed that it was a separate species from other members of the *Botryosphaeriaceae.* As the morphology of their isolates fitted well with the descriptions of the fungus in the earlier papers they retained the name *B. corticis.*

Symptoms

These begin as small red lesions, appearing on young, succulent stems about a week after infection (Milholland, 1995b). The further development of symptoms is dependent on the susceptibility of the blueberry cultivar affected, although even in susceptible cultivars the symptoms develop slowly, often over a number of years.

In susceptible cultivars the lesions become swollen and conical after six months. After two to three years on such cultivars the lesions develop into large, swollen cankers with deep cracking and fissuring, which may girdle and thus kill the stem. Numerous black fruiting bodies of both the sexual and asexual states of the pathogen develop within the affected area. On moderately susceptible cultivars cankering may be extensive but without conspicuous swelling, whereas on resistant cultivars the cankers are much more restricted in size.

Epidemiology

Succulent current-season stems of blueberry are infected during late spring, and symptoms appear four to six months after infection. Both sexual ascospores and asexual conidia can cause infection; they are released during wet conditions and dispersed by wind (Milholland 1995b, Cline 2011). The stems are infected through the stomata (Milholland 1970) and the infection process is complete within 24 hours. The optimum temperatures for growth, spore production and spore germination are 25-28°C (Milholland 1972a). At a constant temperature of 16°C growth of the fungus was inhibited and the development of symptoms restricted to small red flecks, even on susceptible cultivars. This requirement for relatively high temperatures means that even if the fungus were to find its way to the UK it would be unlikely to prosper in our climate.

The fungus is slow-growing and remains confined to the cortex of the stem for the first year. Eventually it invades other parts of the stem (wood and vascular tissues). Extensive cell division is triggered in susceptible cultivars, resulting in the affected areas becoming swollen, and when the stem is girdled it dies. In resistant cultivars infection still occurs but the fungus remains confined to the epidermis and is visible as small, raised lesions.

Botryosphaeria corticis exists as eight distinct races which differ in the range of blueberry cultivars that they are able to infect (Milholland and Galletta 1969, Milholland 1984, Cline and Milholland 1988). As well as spreading by spores within an affected field, the pathogen can be introduced into completely new areas by the use of infected cutting material (Cline, 2011).

Blueberry bushes that have been weakened by other adverse factors will suffer more severely than otherwise vigorous plants.

Control - cultural control measures

The use of disease-free planting stock is very important, particularly in areas where the disease is not already present. Material for use as cuttings should be selected from disease-free plants, or plants should be obtained from disease-free nurseries. Plant health and vigour should be maintained by appropriate fertiliser, pruning and irrigation regimes, and by the selection of suitable sites for plantations so that plants are not placed under stress. If these principles are followed it may still be possible to grow some of the most susceptible varieties, particularly if the fields in which they are grown are isolated from other blueberry plantations (Cline 2011, Milholland 1995b).

Where the disease develops, pruning to remove canker-affected canes will lower the level of inoculum. Beute and Milholland (1970) examined hot-water treatment in an attempt to eradicate *B. corticis* from propagation material. They found that treatment at 53°C for thirty minutes was enough to kill the fungus in dormant cutting wood, but that the treatment also adversely affected the cuttings themselves.

Control - cultivar selection

The use of resistant cultivars is one of the most important measures in combating stem canker, although as stated previously the fungus exists as a number of different races. Cultivars differ in their susceptibility to these races, and the races present will also differ according to locality. The cultivars grown must therefore be matched to local knowledge of the races present. It is also possible that the resistance in a cultivar may eventually be overcome by the emergence of a new race of the fungus.

Cultivars considered particularly susceptible to the disease include ‘Blueridge’, ‘Legacy’, ‘O’Neal’, ‘Weymouth’ and ‘Wolcott’ (Cline 2011, Retemales and Hancock 2012). Several varieties are listed as highly resistant (depending on race) by Retemales and Hancock (2012), including ‘Croatan’, ‘Emerald’, ‘Jewel’, ’Reveille’, ‘Sapphire’, ‘Santa Fe’ and ‘Windsor’.

Control - use of fungicides

Cline (2011) states that fungicides are partially effective against *B. corticis* but that their use in plantations is not practical. Milholland (1995b) also states that, in general, the use of fungicides for stem canker control has been ineffective.

Blueberry stem blight caused by 'Botryosphaeria dothidea'

This disease was first recorded as a problem on blueberry in 1958 and the causal fungus was identified as *Botryosphaeria dothidea* by Witcher and Clayton in 1963. In more recent American literature on stem blight this fungus is usually still listed as the causal agent, although as mentioned previously Phillips *et al* (2013) believe that identifications of this species prior to 2004 should be treated with caution, and it is possible that in at least some cases other members of the *Botryosphaeriaceae* may have been responsible. For example, isolates of *Neofusicoccum ribis* (identity confirmed by DNA analysis), recovered from stem blight symptoms by Wright and Harmon (2010b), had a morphology that was consistent with Witcher and Clayton's original description of *B. dothidea* in 1963. Nonetheless, *B. dothidea* is still found as a cause of some cases of stem blight in both the USA and other countries, its identity in these cases confirmed by sequencing data (Wright 2011, Choi 2011, Espinoza, Briceno and Latorre 2008).

Symptoms

Symptoms of stem blight differ from those of stem canker (Witcher and Clayton 1963, Milholland 1995b, Cline 2002b). A conspicuous symptom is a rapid wilt of individual branches, with the dead leaves turning brown or reddish and remaining attached for some time. An affected branch is usually very obvious if the rest of the bush appears healthy. These affected branches are often known as 'flags' (although note that similar 'flags' may develop as a consequence of Phomopsis or Godronia canker). This wilting of branches can occur throughout the summer months.

Infected stems will have light brown or tan discolouration of the internal wood – this discolouration is sometimes only a few centimetres in length, but may also frequently extend the entire length of the stem. The discolouration is often confined to one side of the stem, where the disease has originated from a wound or an infected side branch. Infection can also occur through wounds affecting the crown of the plant – in this case the entire plant may die quickly without 'flagging' of individual stems.

The origin of the lesions on stems can usually be traced to a wound of some kind. Infection can also occur near the tips of twigs and in these cases they may be confused with winter injury or other twig blight diseases such as those caused by *Diaporthe* or *Botrytis.* After a few weeks, stems killed by stem blight drop their leaves and turn brown to black in colour. Cline (2002b) states that these dead, infected stems are noticeably darker than stems that have died due to other causes. Black fungal fruiting bodies are produced on the affected parts of the stem just below the surface. Stem blight is particularly damaging on young (one- to two-year-old) plantings of susceptible cultivars.

Epidemiology

Like many members of the *Botryosphaeriaceae, B. dothidea* has a wide host range and has been associated with dieback diseases of many woody plants. Sinclair and Lyon (2005) list over one hundred plant genera in a 'partial' host list. Some of these plants are also important fruit crops, such as apple, peach and currants, and these plants could act as a source of the pathogen for blueberries. Witcher and Clayton (1963) inoculated blueberry shoots with isolates of the fungus from apple, lilac, tupelo gum and the tung tree, and found that all were pathogenic to blueberry.

In a discussion on infection strategies for *Botryosphaeria dothidea* and the closely-related *Neofusicoccum ribis* (syn. *Botryosphaeria ribis*) on various host plants, Sinclair and Lyon (2005) state that the fungi cause diseases on plants stressed by wounds, drought, freezing, defoliation or planting outside their native ranges. They can colonise twigs or branches that are dying or recently dead from other causes. They also occur as endophytes in leaves, fruit and bark; endophytic colonies are 'positioned for rapid exploitation of the substrate when it senesces or comes under environmental stress'.

Sinclair and Lyon also state that plants such as blueberry that are intensively selected for horticultural characteristics are susceptible to attack by species of *Botryosphaeria* under a wide array of circumstances, whereas resistance is the norm for less intensively bred plant species.

In addition to other plants acting as potential sources for the fungus, sources within blueberry plantations are of course very important. The fungus overwinters in dead and infected stems. Both sexual (ascospores) and asexual (conidia) spores are produced from fruiting bodies on the affected stems, and while both can initiate infections the conidia are thought to be more important (Milholland 1995b). Spores can be produced throughout the year; in the south-eastern USA peak production occurs in June and July, with the lowest numbers found between December and February (Creswell and Milholland 1988). Spores are released

during wet conditions; conidia are splash-dispersed while ascospores can also be wind-borne. Most infections arise between March and June, but they can occur for most of the year. The optimum temperature for growth of the fungus is 28°C – no growth occurs below 10°C or above 32-35°C.

Infection usually occurs through a wound, and symptoms are normally seen about 4-6 weeks after infection (Creswell, 1987). The likelihood of infection decreases with increasing age of the wound, but in some cases it has been shown that infection can still occur four weeks after wounding has occurred (Creswell and Milholland 1988). The risk of infection also decreases with increasing age of the stem (Creswell, 1987). Once it is within a stem the fungus spreads in the cortex and the vascular tissues and moves down the stem by as much as 75 millimetres in a month (Milholland 1972b).

Common wound sites leading to infection are those produced by pruning, cultivation and harvesting machinery, low temperature damage, pest damage, and bark damage caused by herbicide applications. Late season cold injury occurring the previous season on succulent shoots is a common entry point (Cline 1994). Temperatures below freezing can cause cracking in the forks of blueberry stems, resulting in wound-related epidemics in March and April (Cline 2002b). The stem blight pathogen can also invade stem cankers caused by other fungi such as *Botryosphaeria corticis* and *Diaporthe* species (Milholland 1995b) – further evidence that disease complexes can occur. Abdelgawad and Hendrix (1986) found that twigs initially killed by *Botrytis cinerea* (grey mould) became colonised by *B. dothidea* after June, which subsequently extended the blighted area.

Cline (1997a) isolated *B. dothidea* (along with other fungi including *Pestalotiopsis* sp.) frequently from necrotic tips developing on hardwood cuttings that had been propagated in outdoor rooting beds and then grown on for six months in a glasshouse. These cuttings presumably appeared healthy when taken, illustrating again that propagation material with latent infection is a potential problem (this was also seen with the blackcurrant dieback pathogen *Diaporthe strumella* in HDC project SF12).

Control - cultural control measures.

As with stem canker, the use of healthy, disease-free planting material is important in the establishment of new plantations. Isolation of new plantations away from those suffering from the disease may also help, but as mentioned previously the pathogen can also be found on a large number of other woody hosts.

Avoiding the creation of wounds is also critical. As stem blight is most damaging to young plantations Cline (2002b) recommends that heavy pruning to promote rapid growth should not be practised in one- to two-year-old plantations, and that pruning in such plantations should be limited to the removal of diseased material.

As late-season cold injury is a common entry point for the pathogen, fertiliser should not be used after mid-summer to prevent the production of late-season succulent growth. Such shoots are often produced around the base of the plant. Cline (1997a) experimented with removing this type of cold-damaged shoot in December, which resulted in lower disease levels the following year.

Site selection should also play a part. Cline (2002b) states that the worst cases of stem blight in commercial plantations occur on soils that are extremely sandy, resulting in drought conditions and poor growth, or on very fertile soils that promote excessive growth. Avoidance of sites where low temperature damage is likely to occur regularly would also be important.

The planting of extra plants in some rows is sometimes practised by growers where disease pressure is high – these spare plants can then be used as replacements for those killed by stem blight as the plantation establishes (Cline 2002b).

Where the disease is present the prompt removal and disposal of infected stems (or whole plants where they have been killed) is important to reduce inoculum levels. Stems should be removed 15-20 centimetres below the point where any internal staining ceases (Milholland 1995b).

Control - cultivar selection

Resistant cultivars are available and should be utilised wherever possible. Cline (2002b) states that this should be a primary consideration in the establishment of new plantings, given that young bushes are the most susceptible. Isolates of *B. dothidea* exhibit a broad range of pathogenicity. Creswell and Milholland (1987) identified two virulence groups (pathogenic races) based on the disease reactions produced by the groups on a small number of blueberry cultivars.

Amongst blueberry cultivars listed by various workers as being particularly susceptible (although there is some variation between their findings and recommendations) are ‘Bluechip’, ‘Bluecrop’, ‘Brigitta Blue’, ‘Bounty’, ‘Duke’, ‘Reveille’, ‘Gulf Coast’, ‘Magnolia’, ‘Jubilee’. Resistant cultivars listed include ‘Cape Fear’, ‘Chippewa’, ‘Elliott’, ‘Murphy’, ‘O’Neal’, ‘Reka’, ‘Springhigh’, ‘Santa Fe’, ‘Star’, ‘Weymouth’ (Polashock and Kramer 2006, Polashock 2006, Smith 2006, Smith 2009, Cline 2002b).

Control - use of fungicides

Both Milholland (1995b) and Cline (2002b) state that fungicides are ineffective or do not provide adequate protection for field crops, and that control is reliant on cultural measures. Nonetheless there has been a small amount of work on the use of fungicides for controlling *B. dothidea* on blueberry. Cline and Milholland (1992) looked at root dip treatments for the control of the fungus in container-grown nursery plants. They found that dipping root systems in benomyl provided good protection against *B. dothidea,* but for a period of three-five months only, not long enough to protect plants subsequently planted out in the field where inoculum would be present year-round.

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In two trials Smith (2009) applied several sprays of various fungicides to a range of pot-grown blueberry cultivars and then inoculated detached stems from the treated plants with *B. dothidea*. Results for the various products often varied between the two trials, but in both of them pyraclostrobin and cyprodonil + fludioxonil showed potential efficacy against the fungus. Smith states that since infections often begin at wounds, fungicide application following a significant wounding event such as mechanical pruning might reduce stem infection.

Blueberry diseases caused by other members of the Botryosphaeriaceae.

In addition to *Botryosphaeria corticis* and *B. dothidea s*everal other members of the *Botryosphaeriaceae* have been reported causing dieback problems on blueberry in recent years, in a number of different countries. This finding of a wider range of pathogens has

coincided with the use of DNA analysis techniques, allowing more accurate identification of genera and species. As stated previously the increased geographical range over which the crop is now grown may also have contributed to this increase in species by exposing the plant to a larger number of potential disease-causing fungi. However, even in the United States, where for many years *B. corticis* and *B. dothidea* were considered the only significant pathogens of the crop from the *Botryosphaeriaceae*, an increased range of species is now being found. Many (but not all) of the species belong to the genus *Neofusicoccum*. This name describes the asexual state of the fungus, which is used for these species in preference to the sexual state. Many of these species were previous known as *Botryosphaeria* species, and may be called this in some papers.

In many of the papers the descriptions of the actual symptoms caused by the pathogens are rather sparse. Stem blight (similar or identical to that described under '*Botryosphaeria dothidea*' above) seems to be the most common symptom type, although cankering at the base of stems is also reported, particularly from Chile.

Dealing with the Chilean papers first, Espinoza *et al* (2009) report the finding of three *Neofusicoccum* species in association with stem canker and dieback, namely *N. parvum* (syn. *Botryosphaeria parva*), *N. arbuti* and *N. australe* (syn. *Botryosphaeria australis*). All three species were pathogenic on a range of blueberry cultivars in inoculation tests (although susceptibility varied with cultivar), with *N. parvum* being the most aggressive. They were also pathogenic on apple and kiwi fruits, indicating that they are not host-specific. Isolates of *N. parvum* tested *in vitro* were highly sensitive to fludioxonil; there was also sensitivity to iprodione although this varied with isolate.

Wounding was required for infection, and the potential routes for infection given by the workers are very similar to those reported previously for stem blight in the US. Finally, the difficulty of field diagnosis is emphasised, given that similar symptoms can be caused on blueberry by species of *Pestalotiopsis* and *Phomopsis*, which sometimes coexist in the same plant.

The optimal growth temperature for all three species was 25°C, and both this feature and the effect of water activity (A_w) were investigated further for the three species by Latorre, Diaz and Reed (2012). They confirmed the optimal temperature, and obtained growth between 10 and 35°C. Growth declined with decreasing water activity. In other work on the effects of temperature Elfar *et al* (2012b) found that the optimal temperature for lesion development by *N. parvum* on detached stems was 30°C. Actively growing (less than one-year-old) stems were more susceptible to infection than dormant, partially lignified one-year-old stems (although the latter could still be infected).

Further work on the use of fungicides, and also biological control agents, against *N. parvum* was carried out by Latorre *et al* (2013). As wounds are the main entry point for the pathogen they looked at the use of pastes or sprays to protect pruning wounds. *In vitro* work showed the sensitivity of the fungus to benomyl, iprodione and tebuconazole. Pyraclostrobin was ineffective, which is interesting as Smith (2009), in work mentioned above, found pyraclostrobin to be effective against *Botryosphaeria dothidea*. The effective fungicides were then tested in field trials on pruning wounds inoculated with *N. parvum* after application of the products. They were also tested, along with the biological control agents, on inoculated detached stems.

The results confirmed that benomyl, iprodione and tebuconazole pastes 'provided considerable protection' of pruning wounds under field conditions, whereas pyraclostrobin was largely ineffective. In the detached stem work it was shown that the biological agents *Bacillus subtilis* and *Trichoderma* spp. were ineffective, as was 75% citrus extract. Pastes containing 5% boric acid were effective but phytotoxic.

In addition to the three species mentioned above, other species of *Neofusicoccum* have also been reported as a cause of stem canker and dieback of blueberry in Chile. In a short report Espinoza *et al* (2008) state that they isolated *Neofusicoccum ribis* (syn *Botryosphaeria ribis), N.mediterraneum* and *N. vitifusiforme* (which they called *N. corticosae*, a synonym) from plants with stem canker and dieback, in addition to some of the other *Neofusicoccum* species already reported above, and also *Botryosphaeria dothidea*; inoculation of detached blueberry stems showed that all of the species were pathogenic. Perez *et al* (2014) found *Neofusicoccum nonquaesitum* associated with stem canker and dieback; pathogenicity was again proven in inoculation tests.

Elsewhere in South America, Wright *et al* (2012) identified *N. parvum* as a cause of twig and stem blight of blueberry in Argentina.

Turning to the United States, Wright and Harmon (2009a, 2009b, 2010a) identified the species in the *Botryosphaeriaceae* causing stem blight of blueberry in Florida. Whilst *Botryosphaeria dothidea* was recovered from the symptoms occasionally (and its identity in this case confirmed by DNA sequencing), the two species found most frequently were *Neofusicoccum ribis* (syn. *Botryosphaeria ribis*) and *Lasiodiplodia theobromae* (syn. *Botryosphaeria rhodina*). Pathogenicity was confirmed by inoculating fresh pruning wounds.

In another paper Wright (2011) confirms *N. ribis* and *L. theobromae* as the predominant causes of stem blight in Florida, but in addition to *B. dothidea* he also lists *B. corticis* and *Diplodia seriata* (syn. *Botryosphaeria obtusa*) as being found infrequently – it is not mentioned whether pathogenicity testing was done with the *D. seriata* isolates. In this work he also tested apparently healthy softwood cutting material of blueberry and found that up to 45% of the cuttings had latent infections, predominantly of *N. ribis* and *L. theobromae*. Wright and Harmon (2010b) had also carried out field trials showing that plants derived from tissue culture survived more frequently and had less stem blight than those derived from softwood cuttings. Koike *et al* (2014) report finding *Neofusicoccum parvum* as a cause of stem blight of blueberry in California, and confirmed pathogenicity.

Elsewhere in the world, the following species have been reported causing stem blight and/or cankering of blueberry (identified by DNA sequencing and pathogenicity proven, unless stated):

Korea: *Botryosphaeria dothidea* (Choi 2011)*, Neofusicoccum parvum* (Choi *et al* 2012)

China: *Botryosphaeria dothidea* (Yu *et al* 2012), *Neofusicoccum vitifusiforme* (Kong *et al* 2010), *N. parvum* (Yu *et al* 2013)

Spain: *Neofusicoccum australe, N. parvum* (Castillo *et al* 2013 – interestingly these workers also obtained isolates of *B. dothidea*, but they were non-pathogenic).

Mexico: *Neofusicoccum parvum, Lasiodiplodia theobromae* (Rebollar-Alviter *et al* 2013)

New Zealand: *Diplodia seriata* (identified from spore morphology) *, Neofusicoccum australe, N. parvum, N. lutea* (syn *Botryosphaeria lutea*) (Sammonds *et al* 2009 – the workers stated that experiments to prove Koch's postulates were ongoing, but no later paper was found giving the results. The fungi were also sometimes found in the roots of plants with dieback symptoms).

Godronia or Fusicoccum canker (*Godronia cassandrae,* asexual state *Topospora myrtillii* syn. *Fusicoccum putrefaciens*)

Stem cankers caused by this damaging pathogen are found in many countries where blueberries are grown. The fungus has a fairly wide host range, and was first reported causing a canker disease of blueberry in Canada in 1931 (McKeen 1958). There are many records from Europe, including some from England and Scotland (Ramsdell, 1995b), although the fungus was not found in blueberry dieback isolations carried out by Fera for project SF 132.

Godronia canker tends to be most severe in young plantings where plants can be completely killed. On older plants the disease may be restricted to a small number of stems, but can still cause significant yield loss.

Symptoms

These are somewhat variable according to the route of infection, but may be first seen in autumn as tiny water-soaked lesions on young stems. The lesions turn red by December (Weingartner and Klos 1975a). In Norway, however, Stromeng and Stensvand (2011) found no sign of the disease on the young stems in autumn, and small red lesions only started to appear in March. The lesions enlarge in the spring (and multiple infections sometimes merge together) to produce an elliptical or circular, reddish-brown, target-like or 'bulls-eye' canker that can range from 1 to 10cm in length. As the canker ages it may become greyish in the centre and often has a reddish-purple margin (Szmagara 2008) (Figure 10). Cankers are usually most abundant at the base of the stem (Figure 11) but may form up to a height of one metre. Numerous black fruiting bodies (pycnidia), 0.5-1 mm in diameter, soon form on the cankered tissues, often in concentric rings. The initial development of a lesion is often around a leaf scar (Figure 12), but they may form at other points on the stem. Leaf and flower buds can also be infected (Sabaratnam 2012); these turn brown and develop pycnidia in spring (Figures 10 and 12), and the fungus spreads from the buds into the stem.

On stems more than two years old, flattening, gnarling and depressions often occur due to infections from previous seasons (Weingartner and Klos 1975a). Stems that are girdled by lesions will wilt during the summer, particularly when fruit are present and the temperature is warm (Ramsdell 1995b), or when the plant is under drought conditions (Parker and Ramsdell, 1977b). The dead brown leaves remain attached and affected stems are prominent amongst the other green, healthy stems as 'flags' (Figure 11), similar those seen with other diseases such as Phomopsis canker and Botryosphaeria stem blight. Localised brown discolouration of the vascular tissues may be visible in the area affected by the canker.

Figure 10. Fully developed Godroniaa canker with grey centre and pycnidia (left); girdling canker on one-year stem (right)

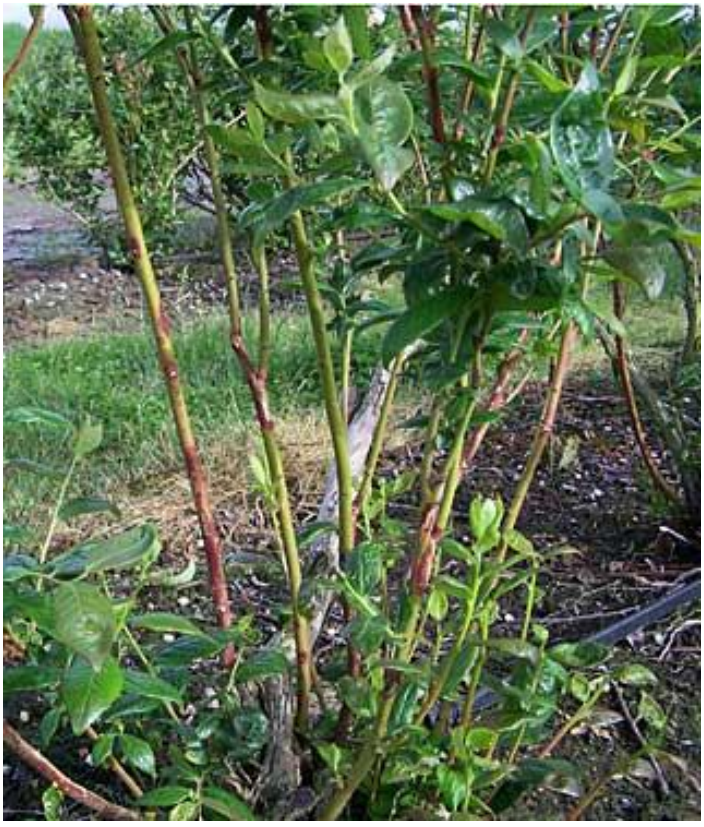

Figure 11. Multiple Godronia canker lesions on young stems, cv. 'Duke'

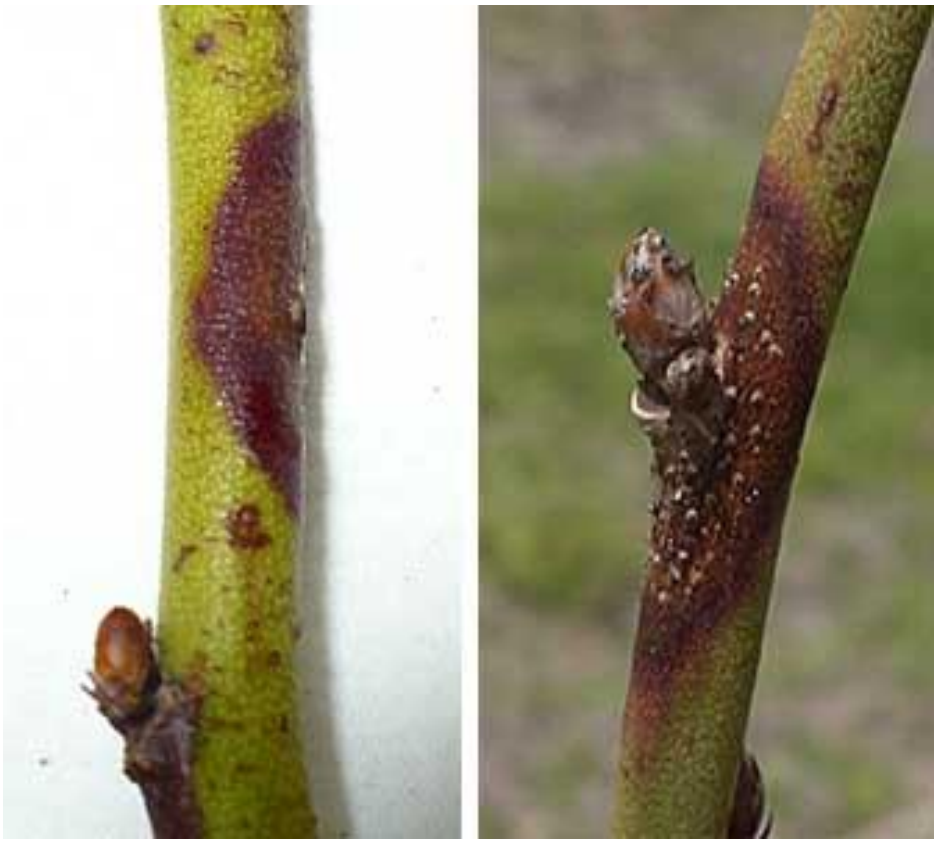

Figure 12. Lesion developing from leaf scar (left); infected flower bud with pycnidia (right)

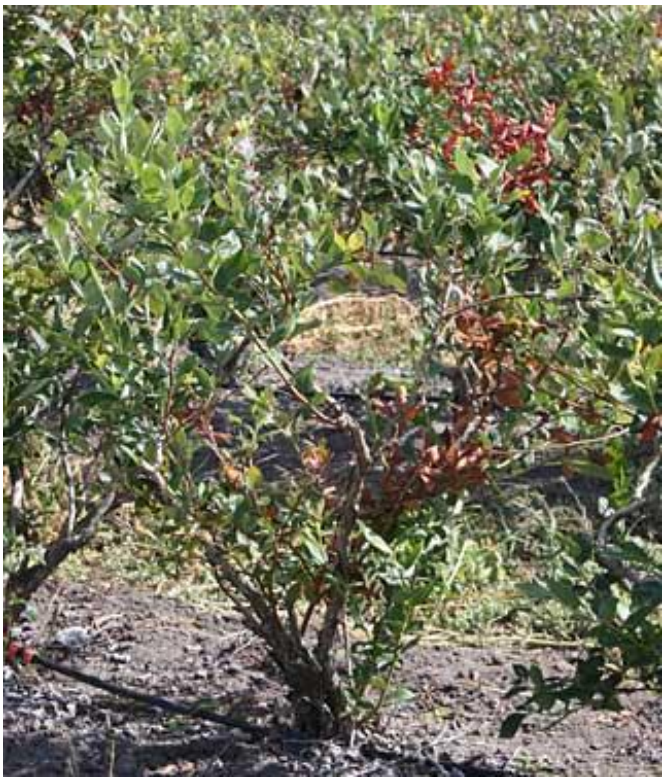

Figure 13. 'Flagging' due to Godronia canker, cv. 'Duke'

Epidemiology

The pathogen survives over winter on infected stems. The disease is spread by asexual spores (conidia) produced within the pycnidia. In North America a second, sexual spore type (ascospore) is also found (Ramsdell 1995b). These are produced by hard, black fruiting bodies 1-2mm in diameter, called apothecia. The apothecia are occasionally found on older dead wood and pruning stubs (more than three years old). However, the ascospores are not thought to play a significant role in infection. Stromeng and Stensvand (2011) state that apothecia and ascospores have not yet been found anywhere in Europe.

The conidia, which are splash-dispersed, can be produced throughout the growing season (and even into December) but are often most numerous in spring and early summer (Parker and Ramsdell 1977b, Stromeng and Stensvand 2011). One- and two-year-old stems can be infected, common sites of infection being leaf scars, petioles, buds, wounds or stomata. The optimum temperature range for growth of the fungus is 14-22°C, but it can grow slowly at 0°C and spores can germinate down to 2°C (Lockhart 1975, Melzer and Hoffman 1980). The pathogen is thus well adapted to cooler climates and conditions and because of this, spring and autumn are the times when infection is most likely to occur. Whilst there are fewer spores released in autumn compared to spring, leaf fall and the subsequent fresh leaf scars produced in the autumn means that there are a very large number of suitable infection sites at this time (Stromeng and Stensvand 2011). Once infection has occurred the fungus invades the cortex of the stem; there is also limited invasion of the vascular tissues.

Control - cultural control measures

Recommendations for cultural management of the disease are given by various workers (Ramsdell 1995b, Stromeng and Stensvand 2011, Sabaratnam 2012), and are similar to those employed for other canker and stem blight diseases. They include using disease-free planting material and practicing best management strategies to ensure that plants are stress-free (such as avoiding drought conditions). Plants should be pruned adequately to ensure good air circulation within the canopy. Overhead irrigation should be avoided on sites where Godronia canker is present, or if it must be used it should be employed in the early morning so that the canopy dries rapidly afterwards. Any stems with symptoms of the disease should be removed and destroyed as soon as they are seen.

Control - cultivar selection

There are noted differences in susceptibility of cultivars to the disease (Ramsdell 1995b, Stromeng and Stensvand 2001, Mukhina *et al* 1993, Lockhart and Craig 1967, Garcia-Salazar 2002, Retemales and Hancock 2012). Amongst susceptible varieties are 'Jersey', 'Bluecrop', 'Johnson', 'Coville', 'Earliblue', 'Collins', 'Duke', 'Ivanhoe', 'Berkeley'. Resistant varieties include 'Goldtraube', 'Hardyblue', 'Bluetta', 'Patriot', 'Rankokas', 'June', 'Weymouth', 'Ama', 'Heerma', 'Spartan'.

Control - use of fungicides

Fungicides can provide preventative control of Godronia canker, but because spores of the pathogen are likely to be present throughout the growing season multiple applications will be required. Ramsdell (1995b) recommends applications from bud burst to early leaf fall. Stromeng and Stensvand (2011) and Sabaratnam (2012) state that the most important times for fungicide use are spring and autumn, during bud burst / early growth and leaf fall. Garcia-Salazar (2002) gives more precise recommendations – four applications from green tip to petal fall, four more from 'first cover' to preharvest and one more postharvest.

Fungicides stated as having activity against the disease include captan (Ramsdell 1995b), azoxystrobin + captan, chlorothalonil (Garcia-Salazar 2002), mancozeb (Szmagara 2008). Szmagara (2007, 2008) also obtained some activity against the pathogen (*in vitro* and/or *in vivo*) from grapefruit extract, chitosan and certain fungal genera (e.g. *Trichoderma* species) that had been isolated along with the pathogen from affected blueberry stems.

Pestalotiopsis and related species

Pestalotiopsis species are isolated very frequently from samples with symptoms such as leaf spots, leaf necrosis, dieback and stem cankers on a wide range of woody plants. Sinclair and Lyon (2005), in a general discussion of diseases caused by these fungi, state that 'they often colonise tissues made susceptible by senescence, damage by other pathogens or insects, freezing, sunscald, or other injuries.' They also state that the fungi can be found as endophytes, saprophytes or pathogens, and that some species can play all three roles.

Most of the reports of *Pestalotiopsis* and the related genus *Truncatella* as pathogens of blueberries have appeared in the last ten years, notably from South America. There is a slightly earlier report from Argentina by Wright *et al* (1998) of stem blight on a range of highbush cultivars imported from the USA (some not yet planted out in the field, others potted plants in a greenhouse). *Pestalotiopsis guepini* was one of two fungi isolated consistently

from the necrosis (the other being *Glomerella cingulata*) and was confirmed by inoculation tests to be a wound pathogen on blueberry twigs and leaves (although less aggressive than the *G. cingulata*).

Espinoza *et al* (2008) found *Pestalotiopsis clavispora, P. neglecta* and *Truncatella angustata* to be associated with canker and twig dieback at 22 locations in Chile (*P. guepini* had been isolated previously from dieback of nursery plants in Chile in 2003). Symptoms consisted of reddish to dark-brown necrotic lesions on twigs, at the basal portion of the main stems, and at the crown. There was extensive necrosis below the bark and dark-brown vascular discolouration. Twig dieback occurred, and in some cases the complete plant collapsed. Black fungal fruiting bodies (acervuli) were present on the affected plant parts.

All three fungi were able to reproduce the symptoms in inoculation tests, fulfilling Koch's postulates. Wounding was required for infection to occur, and the workers speculate that pruning wounds or other stem damage was a likely entry point. The fungi were also able to infect fruits of apple and kiwi, so were not host-specific. *P. clavispora* was isolated most frequently, and *in vitro* tests showed that this species was sensitive to the fungicides fludioxonil and pyraclostrobin.

Finally in this paper, the workers state that whilst the fungi they have found have been shown to be primary pathogens, this does not exclude the possibility that other species or genera such as *Phomopsis* and *Botryosphaeria* may also be involved in the syndrome. In a further short report (Espinoza, Briceno and Latorre 2008) they also found a range of *Neofusicoccum* and *Phomopsis* species to be associated with the symptoms, confirming this hypothesis.

Reports from other countries of the presence of *Pestalotiopsis* in association with dieback of blueberry include (Koch's postulates fulfilled unless stated):

China: (Zhao *et al* 2014) *P. clavispora* causing twig dieback.

Turkey: (Erper and Celik 2011, Dil *et al* 2013) *P. guepini* causing 'blight and drying' of young shoots, and *Pestalotiopsis* sp. causing brown twig lesions with red margins, coupled with leaf necrosis.

Mexico: (Mondragon Flores *et al* 2012, Rebollar-Alviter *et al* 2013) *P. photiniae, P. microspora* and *Pestalotiopsis* sp. The symptoms listed include stem blight, cane blight, cankers and leaf blight.

Uruguay: (Gonzalez *et al* 2012) *P. clavispora* causing twig and branch dieback.

USA: (Cline, 2004) *Pestalotiopsis* sp was one of several fungal genera associated with

dieback of cuttings in propagation beds (Koch's postulates not carried out). The fungus was also found by Weingartner and Klos (1974) (together with many other genera) in association with canker and stem blight affecting blueberries in Michigan.

Anthracnose twig blight and fruit rot (ripe rot) caused by Colletotrichum species (sexual state Glomerella spp.)

Anthracnose, caused by *Colletotrichum* species, is an important disease of blueberries in the USA, Canada and many other blueberry-producing countries. It was first reported by Stretch (1967) causing leaf spots, stem cankers and fruit rot. The fruit rot stage of the disease (often called ripe rot) is the most significant; extensive losses can occur from pre-harvest and, particularly, post-harvest decay. The disease is included in this review as twig blight is one of the symptoms. However, if anthracnose were the primary cause of twig blight and dieback in a plantation, one might also expect to see fruit rotting and leaf spotting as part of the disease syndrome – this has not been reported in the case of the dieback problems in UK crops.

Early reports and papers indicated that the cause of anthracnose was the fungus *Colletotrichum gloeosporioides* (sexual state *Glomerella cingulata*). However, as with many of the fungi described in this review, *Colletotrichum* identification and taxonomy have undergone considerable revision and change over the years. In many cases plant diseases initially identified as being caused by *C. gloeosporioides* are now known to be caused by *Colletotrichum acutatum* or other *Colletotrichum* species. This seems to be the case with blueberries, as when using DNA-based techniques many of the more recent papers identify the fungus causing blueberry anthracnose as *C. acutatum* (e.g. Verma *et al* 2006, Yoshida *et al* 2002, 2007). However, there are still recent cases (such as Xu *et al* 2013b) where *C. gloeosporioides* is still identified as the cause of anthracnose, and confirmed by DNA sequencing. In Slovenia, *Colletotrichum fioriniae* (known previously as *C. acutatum* var. *fioriniae*) has been reported causing anthracnose (Munda 2012).

Both *C. acutatum* and *C. gloeosporioides* are found very frequently in the UK on a wide range of mainly woody plants, and *C. acutatum* can be an important cause of fruit rotting in strawberries (causing the disease known as strawberry black spot).

Symptoms

Many of the papers on anthracnose simply mention 'twig blight' without expanding on the symptoms, but in a couple of cases there are slightly more detailed descriptions. Xu *et al* (2013a, 2013b) describe stem lesions caused by *Colletotrichum acutatum* as dark brown,

originating from infected buds and killing portions of the stem. Lesions have greyish-white centres, with the necrotic areas becoming 6 to 8cm in length. Yoshida and Tsukiboshi (2002) describe a shoot blight with the tips of previous year's shoots turning brown in May, then blighting up to 20cm from the tips.

Leaf spots caused by *Colletotrichum* spp. on blueberry are variable, ranging from small, brown, circular to irregularly-shaped spots to large, black, poorly defined necrotic lesions (Milholland 1995d). Barrau *et al* (2001) report circular lesions which, when well-developed, have salmon-coloured centres and a brilliant red halo.

Infected fruit remain symptomless until they are mature; at this stage the blossom end softens and becomes sunken, and masses of salmon-coloured asexual spores (conidia) are exuded from fruiting bodies called acervuli (Milholland 1995d). Blossom blight can also occur.

Epidemiology

The fungus has a number of overwintering strategies. It survives commonly within blighted twigs, releasing spores from these throughout the following growing season (Milholland 1995d, DeMarsay and Oudemans 2002, Verma *et al* 2006). DeMarsay and Oudemans (2004, 2005) also found that the fungus can overwinter within dormant flower buds formed the previous summer. It can also be recovered from symptomless stems (DeMarsay 2002, Yoshida *et al* 2007).

The fungus infects the fruit either from the colonised flower buds or from conidia splash-dispersed from the blighted twigs. The sexual (*Glomerella*) stage of the fungus is not thought to play a role in infection. The infection remains in a latent state for some time and only becomes apparent as the fruit ripens. Spores produced on the rotting fruit can be a source of secondary inocula. The fungus enters the twigs through blighted flower clusters or rotting fruit pedicels (Hartung *et al* 1981). The optimum temperature for growth of the fungus is 20-27°C, and losses are most severe when there are extended warm, wet periods at flowering and/or just before harvest (Milholland 1995d).

Verma *et al* (2006) infected apple fruit with isolates of *C. acutatum* from blueberry, indicating that the pathogen is not host specific. Both *C. acutatum* and *C. gloeosporioides* are known to have wide host ranges.

Control - cultural control measures

Pruning should be practised to remove affected twigs and flower spurs, and to improve air circulation through the plant canopy. During cropping the fruit should be picked regularly to prevent infection spreading from infected berries to adjacent healthy fruit. Post-harvest cooling of fruit and cleaning and disinfection of handling and storage equipment will also help to reduce losses (Garcia-Salazar 2002). Schilder *et al* (2006) recommend modifying irrigation practices, minimising frequent overhead irrigation and switching to drip or timed irrigation.

Control - cultivar selection

There is considerable variation between cultivars in susceptibility to anthracnose. Those listed as being particularly susceptible include 'Bluetta', 'Blueray', 'Bluecrop', 'Berkeley', 'Coville' and 'Jersey'. These are often cultivars in which the ripe fruit hangs for a long time on the bush prior to picking (Garcia-Salazar 2002).

Resistant cultivars include 'Aurora', 'Bluejay', 'Brigitta', 'Draper', 'Legacy' and 'Toro' (Retemales and Hancock 2012). This resistance relates primarily to fruit infection, but even resistant cultivars can be affected during prolonged weather conditions favourable to infection. 'Reka', 'Burlington', 'Sharpblue', 'Legacy' and 'Elliott' were among cultivars having resistance to foliar infection (Ehlenfeldt and Polashock 2009).

Control - use of fungicides and biological controls

Fungicide programmes are used where anthracnose is a regular problem. Applications start at flowering and can continue at 7-10 day intervals until harvest. Fungicides found to have good activity against anthracnose include cyprodonil + fludioxonil, pyraclostrobin, pyraclostrobin + boscalid, trifloxystrobin, tolylfluanid, azoxystrobin, captan, tebuconazole and fluazinam. (Schilder *et al* 2006, Meszka and Bielenin 2012, Rueegg and Bosshard 2004). Biological control agents were assessed by Verma *et al* (2006) and Meszka and Bielenin (2012). They found *Gliocladium catenulatum, Pythium oligandrum* and *Trichoderma harzianum* reduced disease incidence significantly.

Botrytis blight caused by Botrytis cinerea

The Botrytis blight pathogen *Botrytis cinerea* (also known as grey mould) has a huge host range. It can behave as either a pathogen or saprophyte, and is frequently found colonising plant tissues that have been damaged by other factors (such as weather conditions, cultural

operations, or other diseases and pests). Tender, green tissues are most prone to attack, although the fungus can also sometimes be found on older, woody material. Fruit rots of soft fruit such as strawberries and raspberries are usually initiated from flowering infections that remain latent until fruit maturity, and this is also the case with blueberries.

B. cinerea will usually produce large numbers of grey-brown spores (conidia) when affected material is incubated at high humidity. It is also readily isolated onto general purpose agars such as potato dextrose agar. However, because it is an efficient saprophyte and common coloniser of dead, dying and damaged tissues there can be problems determining whether the fungus is the primary cause of a decay symptom or a secondary coloniser.

Symptoms

Green twigs, blossoms, leaves and fruit of blueberry can be affected (Bristow and Milholland 1995). Vasquez *et al* (2007) also found that the blight affected older leaves and stems. Infected twigs turn a brown to black colour, which later lightens so that they become tan or grey (Figure 14). The symptoms can be mistaken for those of winter injury (Bristow and Milholland 1995) or Phomopsis twig blight (Garcia-Salazar 2002). However, twigs affected by *B. cinerea* often have flattened, black resting structures or sclerotia present, irregularly shaped and up to 5mm in diameter. It is likely that the winter injury itself could act as an entry point for *B. cinerea* (Szmagara 2008, Garcia-Salazar 2002).

Blighted blossoms turn brown and collapse, with masses of powdery, grey-brown conidia present that are easily shed by air currents. Other affected flowers may not show visible signs of infection and the fruit that results from them also remains symptomless until maturity. Decay does not usually develop until after harvest, when the berries shrivel slightly and produce masses of conidia under conditions of high humidity. Leaves can also be infected, particularly via contact with infected flowers. Affected areas first turn yellow and then brown, and conidia may be produced.

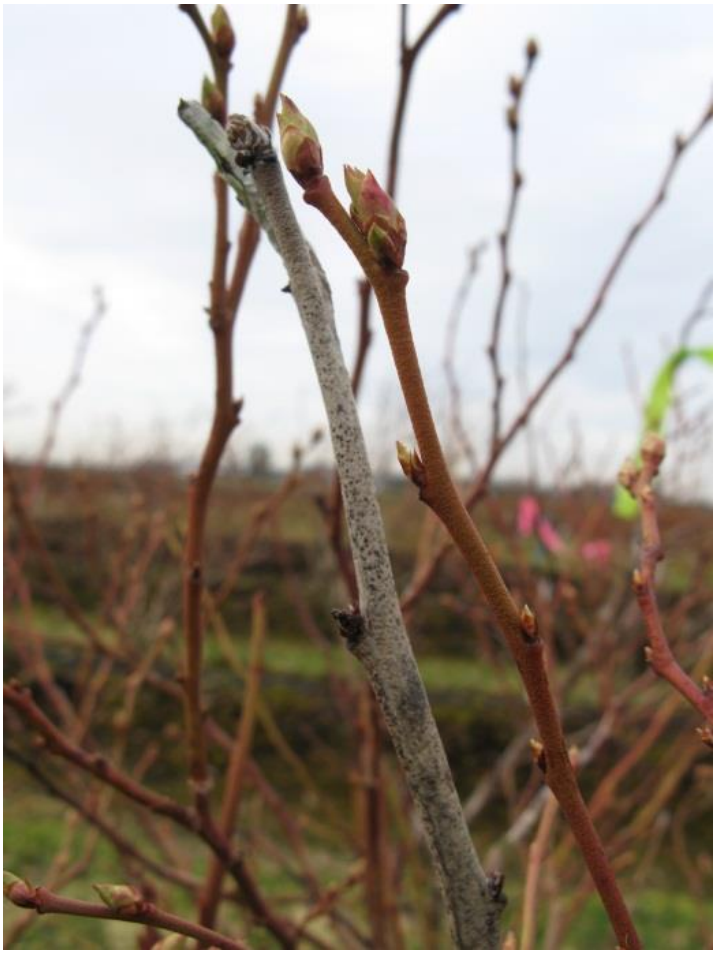

Figure 14. Shoot affected by Botrytis blight

Epidemiology

B. cinerea overwinters in or on affected plant material, or as sclerotia. In both cases conidia are produced in spring. As it is a ubiquitous fungus other crops and plant species may also act as a source of the airborne conidia. Infection of blossoms, twigs or fruit occurs under conditions of high humidity and cool to moderate temperatures of 15-20°C (Bristow and Milholland 1995). The most severe yield losses occur when entire flower clusters are affected by blossom blight so that no fruit are set. It is at this point that twig blight also develops, with the fungus spreading down the peduncle to girdle the stem. The fungus does not usually spread into the twig from infected berries. Bristow and Milholland also state that the fungus does not spread into twigs from affected leaves, as these usually drop before it can spread down the petiole. However Johnston and McKenzie (1982) frequently found *B. cinerea* associated with stem lesions and stem dieback, and found that stem infections often centred on a leaf scar.

Given that *B. cinerea* will readily colonise dead and dying plant tissues it is not surprising that it has been found in joint occupation of lesions with other pathogens. For example Szmagara (2008) isolated the fungus together with *Topospora myrtilli* from cankerous stem lesions. There are also occasions where *B. cinerea* lesions may act as a foothold for other pathogens - Abdelgawad and Hendrix (1986) found that twigs initially killed by *B. cinerea* became colonised by *Botryosphaeria dothidea* after June, which subsequently extended the blighted area.

Control - cultural control measures

Annual pruning to remove affected twigs and to improve air movement within the canopy will reduce the risk of infection (Bristow and Milholland 1995). Excessive use of nitrogen fertiliser in spring can increase the risk by stimulating the rapid production of very tender growth.

Control – cultivar selection

Cultivars with tight flower clusters, such as 'Weymouth', 'Blueray' and 'Rancocas' are particularly susceptible to Botrytis blight (Garcia-Salazar 2002). Finn *et al* (1994) also found the cultivars 'Bluechip', 'Bounty', 'Nelson', 'Berkeley', 'Sierra' and 'Bluegold' to be susceptible.

Control - use of fungicides and biological controls

Fungicide programmes are used in the USA where there is a history of the disease or when suitable conditions of cool, wet weather are forecast. Applications begin at mid-bloom and continue until petal fall.

Other fungi attacking twigs and shoots of blueberry

The fungi discussed so far are those reported most commonly as the cause of blight, dieback and cankering of blueberry. However, the literature searches have revealed that a large number of other fungi have been reported as causes of one or more of these symptoms. Only brief descriptions of these will be given, for one or more of the following reasons:

1. The pathogen can cause a significant disease of blueberries in some countries, but the dieback symptoms caused are not typical of those that are being seen in the dieback cases affecting crops in the UK.
2. In addition to the symptoms affecting shoots, twigs or stems the pathogen causes another symptom as a major part of the disease (such as a leaf spot) which has not been seen in the UK dieback cases.

3. The pathogen is the subject of a ‘new disease report’, after which there are very few or no further papers published on the disease.

Many of the new disease reports come from Argentina, where surveys of diseases in blueberry crops have been undertaken for a number of years.

Unless stated otherwise, all of the fungi described below are either long- recognised pathogens of blueberry or have had Koch’s postulates fulfilled by the workers reporting them as new disease problems.

Checks for UK records of the fungi have been made using the British Mycological Society’s Fungal Records Database of Britain and Ireland (Kirk and Cooper 2009).

Mummy berry (*Monilinia vaccinii-corymbosi*)

The disease has a shoot blight or dieback phase, but this differs from the UK cases of dieback in that woody tissues are not affected. The fungus attacks new vegetative shoots and flowers soon after they emerge in spring. The affected shoots rapidly droop and turn brown, and grey-brown spore masses are produced on the midribs of affected leaves and on dead blossom trusses (making the fungus easy to identify). After the completion of this spring shoot blight phase the vegetative growth of the plant is unaffected for the remainder of the season, but fruit affected by the pathogen become shrivelled and mummified.

Gloeosporium leaf spot and stem canker (*Gloeosporium minus*)

This is a serious disease of blueberries in the southeastern USA (Milholland 1995e, Cline 2002c). The fungus is confined to *Vaccinium* species. However, leaf spotting and defoliation are usually very prominent, which has not been the case in the UK dieback problems. There are no records of this fungus in the UK.

Symptoms sometimes consist of red flecks on the leaves leading to leaf distortion, but also seen are very prominent, large, circular to irregular brown lesions 5-10mm in diameter. Severe defoliation can occur. Stems become infected by the fungus growing down the petiole of infected, attached leaves in mid- to late-summer, or from late summer infection of buds and leaf scars (Milholland 1974a). The stem lesions start as dark red, circular to elliptical lesions. As the lesions enlarge, affected stems turn brown and then grey. Severe stem dieback of up to 50cm can result. Numerous fruiting bodies (acervuli) develop within the affected area – these are found below the surface of the epidermis but exude colourless masses of spores (conidia).

Septoria leaf spot and canker (*Septoria albopunctata*)

This disease is a problem in the southeastern USA and parts of Canada (Milholland 1995f, Hildebrand *et al* 2010). Milholland states that certain stages of the disease on stems may be confused with lesions caused by other pathogens such as *Botryosphaeria corticis* and *Diaporthe vaccinii*. However, leaf spotting and defoliation is again a common and important part of the disease. There are no records of this fungus in the UK.

Leaf spots are small and circular, white to tan in colour with red borders. They may contain a small number of fruiting bodies (pycnidia) of the causal fungus. They are present by early May and increase in number as summer progresses. The stem cankers develop from mid-June to September. They are sunken, with a tan or grey centre and a reddish-brown margin, and may be 5-6mm in diameter. In early spring stem lesions on vigorous shoots may be dark purple in colour.

Alternaria leaf spot, twig blight and fruit rot (Alternaria tenuissima)

Twig blight is a minor aspect of the disease caused by this fungus, which has been recognised as a pathogen of blueberries in the USA since the early 1970's (Milholland 1973). The major problems are premature defoliation caused by leaf spotting, and fruit decay with associated mycotoxin production (Milholland 1995g, Greco *et al* 2012). These symptoms have not been associated with the dieback problems in the UK.

Leaf spots are 1-7 mm in diameter, circular to irregular, light brown to grey in colour with a red border. Fruit develop a greenish-black fungal growth at the calyx end and may leak juice. Twig blight takes the form of reddish, circular spots that may develop into small cankers. Significant twig dieback as a result of these cankers is not reported.

In addition to the USA, *A. tenuissima* was seen for the first time in blueberry in Argentina in 1997, and is now considered to be one of the most important pathogens of blueberry in that country (Wright *et al* 2004, Moschini *et al* 2012). The disease has also been reported from China (Luan *et al* 2007), New Zealand (Johnston and McKenzie 1982) and possibly Mexico (as *Alternaria* sp., Dil *et al* 2013). *A. tenuissima* has a worldwide distribution (including the UK) and is a cosmopolitan fungus, being found on a huge range of plant species (often as a saprophyte).

Twig blight / twig canker and dieback / Coryneum canker (*Discostroma corticale* syn. *Clethridium corticola*; asexual state *Seimatosporium lichenicola,* syn. *Sporocadus lichenicola, Coryneum microstictum)*

It is difficult to resolve the precise nomenclature of this fungus as it has had so many synonyms over many years. It seems to have first been reported on blueberry in the USA as *Coryneum microstictum*, causing Coryneum canker (Zuckerman 1960). Symptoms consisted of girdling stem cankers leading to branch dieback, containing numerous small, black fruiting bodies of the pathogen. There is subsequent report of the fungus, again from the USA, as *Sporocadus lichenicola* (Serdani *et al* 2009) causing twig cankers. In this report the symptoms consisted of multiple, greyish-white cankers with reddish margins. They were associated with the nodes and ranged from 1cm in length to the entire length of the twig. Once again numerous black fruiting bodies were visible.

Work to fulfil Koch's postulates showed that wounding was necessary for infection, but that once this had happened extensive stem cankers, girdling and twig death could occur. Winter injury, sunscald and damage from other sources are postulated by Serdani *et al* as possible infection routes in the field, with plants that are also under additional environmental stresses more likely to succumb.

Seimatosporium lichenicola has a wide host range and is also known to cause canker and dieback diseases on a number of other hosts (e.g. 'Ascospora' dieback of raspberries and blackberries (Sutton and Williamson 1991)). The fungus is recorded in the UK on various hosts, but not on *Vaccinium* spp.

Blueberry blight caused by *Bipolaris cynodontis*

This fungus is reported as a pathogen of blueberry in Argentina (Sisterna *et al* 2009). Symptoms are described briefly as dieback, bud and branch blight. There is also a report of a *Bipolaris* sp. causing bud blight in Mexico (Mondragon Flores *et al* 2012) but apart from this no other literature was found. *B. cynodontis* is found primarily on members of the grass family, causing leaf spots and blights, but has been recorded on a few other broadleaved plants in addition to blueberry. There are no UK records of this particular *Bipolaris* species.

Gibbera twig blight (*Gibbera vaccinicola*)

This fungus is reported in New Hampshire, USA by Smith and Lord (1996, 1997). Twig and crop losses of up to 40% were reported on the cultivar 'Northland'. A detailed description of symptoms is not given, although it is stated that twig and stem infections are characterised

by large, cushion-shaped black stromata (see glossary), bordered by a distinct red margin of host tissue. The stromata, which produce sexual spores (ascospores) may be present singly or in small clusters. Smith and Lord state that the disease was first reported on blueberry in 1936, but that there were no further reports after that, prior to their findings (nor do there appear to have been any subsequently to Smith and Lord's papers).

There are no UK records of *Gibbera vaccinicola.* The species *Gibbera vaccinii* has been reported in the UK on cowberry (*Vaccinium vitis-idaea*).

Leaf spot, shoot and twig blight caused by *Nigrospora sphaerica*

There is a single report of this disease from Argentina (Wright *et al* 2008). Leaf spots are brown and circular, 1-2mm in diameter. Fruiting twig and shoot blight developed from the tips towards the base – no further description is given. Wounds were necessary for infection. *Nigrospora sphaerica* has a wide host range and can act as a weak pathogen or saprophyte. It has been recorded in the UK, but not on *Vaccinium* species.

Stem and branch blight caused by *Fusarium* species

A paper from Argentina (Wright *et al* 2014) reports finding *Fusarium acuminatum* as a cause of a damaging branch blight. Wounding was necessary for infection, and the brown to tan lesions spread from the base of the branches to the tip. In the field, the leaves on dead branches turned brown but remained attached. Young plants could be killed by the disease. Inoculation tests showed that fungal isolates from blueberry were not host-specific, also decaying carrots and onions. In an earlier report Caprara *et al* (2010) found an unidentified *Fusarium* species associated with blueberry dieback and stem blight. They were able to infect both wounded and unwounded test plants with the fungus.

F. acuminatum has been found on numerous plants. It has been associated with root, crown and aerial diseases of a range of hosts, but can also be found as a saprophyte. It is present in the UK, but apparently has not been found associated with *Vaccinium* here.

Leaf blight, twig blight and stem dieback caused by *Aureobasidium* species

Stem dieback of blueberry caused by *Aureobasidium pullulans* is reported from the USA by Caruso and Mika (1991); the pathogen was also able to infect cranberries. Leaf and twig blight caused by *Aureobasidium vaccinii* is reported from Romania by Richiteanu and Teodorescu (1989). Symptoms of infection by *A. vaccinii* include reddish leaf spots leading

to a more extensive leaf necrosis. The fungus progresses down the petioles into the current year's green shoots causing them to die back, often resulting in a characteristic 'shepherd's crook' symptom. The leaves on affected shots shrivel and remain attached. Affected twigs are at first brown to black, becoming grey with weathering. Richiteanu and Teodorescu state that twig blight due to *A. vaccinii* is generally limited to the current season's growth, and any extension of the injury into the older wood is usually associated with other fungi, particularly *Cytospora*. Severely affected bushes look as if they are affected by sun scorch, drought or pesticide toxicity and may be partially or completely defoliated by the end of the growing season.

A. pullulans is also a recognised cause of fruit rotting. Whilst *A. vaccinii* has only been found on blueberry, *A. pullulans* has a worldwide distribution and is also a very common saprophyte and epiphyte (coloniser of leaf surfaces).

Sclerotinia rot caused by *Sclerotinia sclerotiorum*

Dieback of blueberry shoots caused by *Sclerotinia sclerotiorum* has been recorded in New Zealand (Johnston and McKenzie 1982), Japan (Umemoto *et al* 2007) and Argentina (Perez, Farinon and Berretta 2011a). Symptoms are described as shoot blight and blighting of leaves and flower clusters. Johnston and McKenzie state that the symptoms are similar to those of Botrytis blight, but do not say whether woody tissues are affected.

S. sclerotiorum is a common and widespread pathogen in many parts of the world, including the UK (although there are no records of the fungus on *Vaccinium* here). A large number of plant species can be affected. The diseases caused are usually characterised by rapidly spreading rots of soft tissues, although the fungus can be found occasionally on woody tissues. The fungus is readily isolated from diseased material.

Silver leaf caused by *Chondrostereum purpureum*

This disease was reported for the first time on blueberry in 2009, in Chile (France *et al* 2009). Affected plants showed a disease syndrome very similar to that produced by the fungus on many other woody plants such as apple and plum trees. Branch dieback is just one part of the progression of the disease. Other symptoms seen on affected blueberries included silvering of the leaves and necrotic tissue in the centre of old stems. Affected stems showed reduced growth, were easily snapped off and eventually died. Dead shoots or shoots nearly girdled with dead tissues produced typical purplish fruiting bodies of *C. purpureum*, about 5-

10cm in diameter. *C. purpureum* has a widespread distribution and a large host range amongst woody plants. It is common in the UK but has not been recorded on blueberry here.

Stem blotch (unidentified *Cercospora* species)

This fungus appears to be of little importance. It was found on rabbiteye blueberry in Georgia, USA in the 1970's (Milholland, 1977) and has not been found on highbush cultivars. Small, red, slightly raised lesions develop on succulent stems. On woody stems the lesions appear as light to dark red discolouration; they can merge together to encircle the stem within one year and may coalesce to form elongated lesions 1cm in length. Twig or stem dieback as a result of the lesions is not reported.

Other genera of fungi

In addition to the fungi described so far in this review, a number of other genera have been isolated from diseased blueberry stems in countries such as the USA (Weingartner and Klos 1974, Annis and Stubbs 2004), Poland (Szmagara 2009) and Mexico (Mondragon Flores *et al* 2012). Some of these findings are interesting as they represent some of the other fungal genera that were sometimes recovered from the affected blueberry samples in SF132, such as *Coniothyrium, Cytospora* and *Phoma*. However, interpreting the findings in these particular reports (usually the result of disease surveys) is difficult, since pathogenicity testing to fulfil Koch's postulates was not done. It is therefore quite possible that the fungi could have been secondary colonisers, saprophytes or even mycoparasites attacking other fungi within the lesions.

As an example, Szmagara (2009) reports isolating 26 different fungi from various disease symptoms on blueberry stems and twigs, including six species of *Fusarium* and six of *Phoma.* Of the fungi isolated some, such as *Godronia cassandrae* (reported as the asexual state *Topospora myrtilli*), are likely to have been the primary cause of a symptom, whereas others (e.g. *Cladosporium cladosporioides, Penicillium decumbens, Saccharomyces* spp., *Trichoderma* spp.) are highly unlikely to have been pathogenic. This makes it difficult to interpret the findings of some of the other genera (e.g. *Cytospora* and *Phoma*) that could be potential pathogens.

1b. Fungal and fungus-like pathogens attacking the roots, crown and stem bases to cause dieback

Blueberries are susceptible to attack by a number of pathogens that decay the root systems, crowns and stem bases. Affected roots are unable to function properly so that the plant becomes starved of water and nutrients, whilst girdling lesions at the crown or at the bases of individual stems will also prevent water and nutrient flow beyond that point. Stems and branches will therefore die back as a result and, as the decay caused by the pathogen will be at the base of the plant, the symptom could be described as a 'die up' – a symptom commonly reported during on-site examination of affected plants in project SF 132. However, where a root pathogen is involved then root decay will obviously also be an important symptom, which is unlikely to be present in plants affected by, for example, *Phomopsis* or *Botryosphaeria* stem cankers.

The two main diseases causing root and crown rot in blueberry are Armillaria honey fungus and Phytophthora root rot. Brief summaries will be given of the symptoms and epidemiology of these diseases, and mention will also be made of some of the more minor root pathogens found affecting the crop. Further details on features such as cultivar susceptibility and cultural and chemical control measures can be obtained by consulting the original papers in the reference list.

Armillaria root rot or honey fungus

Honey fungus is a general term covering a number of species within the genus *Armillaria*. The name is derived from the mushroom-like fruiting bodies produced by *Armillaria* species, which are often a honey-brown colour. Honey fungus is predominantly a pathogen of woody plants, and has a huge host range, including many different types of top and soft fruit.

Honey fungus is a disease affecting soil-grown plants, and is highly unlikely to be found in those grown in containers. It is a natural component of woodland ecosystem, and is therefore most likely to be a problem where woodland sites have been cleared for planting and infected root fragments from the woodland trees and shrubs remain in the soil. The fungus could also spread into a blueberry planting from affected trees or shrubs in adjacent woodland.

Armillaria root rot of blueberry was initially reported from the USA (Milholland 1995h), where *Armillaria mellea* and *A. ostoyae* are thought to be the main species responsible. Work on the disease has also been carried out in Italy, where *A. mellea* and *A. gallica* were found in 98% of the samples analysed. *Armillaria ostoyae, A. gemina* and *A. cepistipes* were also isolated

(Prodorutti *et al* 2006, 2009). Whilst the published information on Armillaria root rot of blueberry comes predominantly from these two countries it is highly likely that the disease is present in most countries producing soil-grown crops, including the UK. *Armillaria mellea, A. ostoyae* and *A. gallica* are routinely found affecting a wide range of woody plants in the UK, although is this country *A. gallica* is considered to be only weakly pathogenic).

Symptoms and epidemiology

These are described in the publications listed above. Dieback is one of the later stages of the disease syndrome. Affected plants become stunted and lacking in vigour, with small leaves that may show symptoms of nutrient deficiency or redden prematurely in the autumn. Branches may wilt suddenly and the plant is eventually killed (Figure 15). The time from the first appearance of symptoms to the death of the plant may be relatively short (a few months), or it may be a slow decline over several years. Affected plants will often be found in patches, with the problem spreading slowly to adjacent plants.

Examination of the roots and crowns / stem bases of affected plants will reveal that a brown decay is present below the bark. White fungal mycelium, smelling of mushrooms, will also be found below the bark – this is diagnostic for Armillaria root rot and distinguishes the disease from Phytophthora root rot. It may also be possible to find rhizomorphs (commonly called bootlaces) attached to affected roots and stem bases (Figure 16). These are cord-like structures, red-brown when young but soon turning black with a whitish centre; they can be quite difficult to distinguish from plant roots. They are one of the main ways in which the disease spreads, as once come into contact with the root of an adjacent susceptible plant they will attach to it and infect the plant. Root-to-root contact between an infected plant and its neighbour(s) is the other principal method of spread.

Armillaria species are also efficient saprophytes and will readily colonise dead woody material. The fungus was found by Prodorutti *et al* (2009) to be colonising the bark mulch applied to blueberry fields, in addition to spreading into the fields from adjacent trees. The disease can be found on all soil types, although Milholland (1995h) states that blueberries grown on sandy, well-drained soil are more likely to be affected.

Figure 15. Plant killed by Armillaria root rot

Figure 16. Armillaria: white mycelium below bark, and rhizomorph growing from infected root

Phytophthora root rot

This oomycete (fungus-like organism) was first reported affecting blueberries in the USA in 1963 (Royle and Hickman 1963). Reports from other countries include New Zealand (Johnston and McKenzie 1982), where it was the most important and widespread blueberry disease, Canada (MacDonald 1990), Italy (Tamietti 2003), Chile (Larach *et al* 2009) and Estonia (Starast *et al* 2009). However, the pathogen has a worldwide distribution and is a potential problem wherever blueberry is grown. It was detected on UK plants during project SF132. Unlike honey fungus, Phytophthora root rot can affect both soil- and container-grown plants. The species responsible for the problem in blueberries is usually *Phytophthora cinnamomi*, although *P. citrophora* has also been found in Chile (Larach *et al* 2009).

Symptoms and epidemiology

Above-ground symptoms of the disease (Figure 17) are similar to those of Armillaria root rot. Like Armillaria root rot, Phytophthora root rot is characterised by a decay affecting the roots, crowns and stem bases of affected plants (Milholland 1995i, Cline 1997b). Because *Phytophthora* is a microscopic organism, however, there will be no visible evidence of the pathogen itself, and this helps distinguish the two diseases.

It can be more difficult to distinguish Phytophthora root rot from root decay occurring as a result of prolonged waterlogging. In fact the two problems often occur together, as *Phytophthora* spreads by microscopic swimming spores (zoospores) and is thus favoured by poor drainage and waterlogging. In the field, the disease is often found in waterlogged patches and low-lying areas.

Whilst the zoospores are short-lived, *Phytophthora* species can also form long-lived resting spores (oospores and/or chlamydospores). Soil and the standing areas for container-grown plants can therefore be contaminated for extended periods.

Figure 17. Dieback caused by Phytophthora root rot

Other fungi and fungus-like organisms causing root decay

Whilst *Armillaria* and *Phytophthora* are the predominant causes of root and crown decay of blueberry, other organisms are sometimes associated with the problem. These organisms tend to be weaker pathogens, but acting alone or in combination with others could cause dieback and decline of plants. They are more likely to be damaging on young plants or during propagation. For all of the organisms mentioned in this section Koch's postulates have been fulfilled and pathogenicity to blueberry proven.

Pythium species are fungus-like organisms closely related to *Phytophthora*, and are frequently isolated from decaying blueberry roots (often together with *Phytophthora*). They are regarded by most workers as being less aggressive than *Phytophthora* species, and some species of *Pythium* are purely saprophytic. A range of species has been recovered from blueberries – in most cases pathogenicity testing has not been carried out. However, *Pythium sterilum* was recovered from decaying roots of plants affected by a dieback and decline

problem in a poorly-drained area of a plantation in Michigan, and shown to be pathogenic to inoculated plants (Miles *et al* 2011).

In Argentina, *Fusarium solani* was recovered from the roots of plants in a plantation affected by root rot and sudden death symptoms, and caused root and stem decay in wound-inoculated test plants (Perez *et al* 2007). Perez *et al* (2011) also isolated *F. proliferatum* from the roots of plants with 'dry or dead' branches at another site; one-year-old plants whose roots were wound-inoculated with the fungus developed root decay and branch necrosis and died within 90 days.

Fungi documented as causing root and/or stem base decay resulting in the dieback or death of young plants during propagation include the *Calonectria* (asexual state *Cylindrocladium*) species *C. ilicicola* (Milholland 1974b, Haralson *et al* 2013), *C. colhounii* (Sadowsky *et al* 2011) and *C. kyotensis* (Boesewinkel 1979), and the ubiquitous pathogen *Rhizoctonia solani* (Haralson *et al* 2013).

1c. Fungal pathogens causing dieback as a result of vascular wilt diseases

Vascular wilt diseases caused by soil-borne fungi such as *Verticillium* species and *Fusarium oxysporum* are characterised by the pathogen colonising the plant through the root system and subsequently invading the water-carrying xylem vessels. Symptoms develop as a result of factors such as the production of toxins by the fungus, or blockage of the xylem vessels by either the fungus or the plant itself (in a reaction to the infection). The symptoms include wilting and branch dieback and therefore the diseases merit inclusion in this review. However, wilt diseases are also often characterised by widespread discolouration (staining) of the vascular tissues within affected stems, branches and/or crowns. This staining is usually seen as streaks or lines of discolouration below the bark if affected stems are cut lengthways, or as discoloured rings or part-rings when the stems are cut across and viewed end-on. In general the vascular discolouration is often greenish-brown, brown or black in host plants affected by Verticillium wilt, but more likely to be reddish in plants affected by Fusarium wilt.

Both Verticillium and Fusarium wilts have wide host ranges, but whereas Fusarium wilt diseases of different plants and crops are usually caused by host-specific strains of the fungus, Verticillium wilt is not host-specific.

Reports of these diseases in blueberries are sparse, indicating that at present they are not significant problems. However, Fusarium wilt has recently been reported affecting plants in China (Liu *et al* 2014). Affected plants in a field of the cultivar 'Duke' developed symptoms consisting of wilting of the foliage and stunting, and died after 50 to 60 days. The vascular and cortical tissues of the crowns showed a brown to orange discolouration. *Fusarium*

oxysporum was recovered consistently from the plants and confirmed as the cause of the symptoms in host inoculation tests. The fungus was not tested on other plant species to check for host specificity.

Montalba *et al* (2010) report work carried out in Chile on the effects of nitrogen fertilisers on Fusarium wilt in blueberry. They name the pathogen as *Fusarium solani*. However, the precise symptoms of the disease are not described. *F. solani* is not usually regarded as a true vascular wilt pathogen, as it does not colonise the vascular system of affected plants extensively. It is therefore possible that the 'wilt' in this case developed as a result of the fungus decaying the roots or stem base of the plants, as reported in Argentina by Perez *et al* (2007).

Verticillium wilt of lowbush blueberry (*Vaccinium angustifolium*) has been reported in the USA and Canada by Brisson *et al* (1976).

Whilst the majority of diseases resulting in dieback of blueberry plants are caused by fungi, a few are the result of infection by bacterial pathogens or viruses. Whilst no evidence was found in samples tested in project SF132 of bacterial or viral pathogens, these diseases are still potential causes of dieback in the crop and are therefore described briefly and have been identified on UK grown blueberries. Dieback as a result of viral infection will be discussed in the following section. The most common bacterial pathogen causing dieback is *Pseudomonas syringae.* Other bacterial pathogens associated with dieback or death of plants are *Ralstonia solanacearum, Xanthomonas* sp. and *Xylella fastidiosa*.

Bacterial blight / Bacterial canker caused by *Pseudomonas syringae*

This is a damaging disease in many parts of the world where blueberries are grown (Bristow and Moore 1995, Guerrero and Lobos 1989).

Symptoms

Twig dieback and stem lesions are only some of the symptoms caused by the disease; leaf spots are also common. Water-soaked lesions appear on one-year-old canes in late winter / early spring. They develop into reddish-brown to black, irregular cankers with well-defined margins. The cankers can vary in length from a few millimetres to the entire length of the twig, and may girdle it to cause dieback. This twig dieback can be very similar to that caused by Botrytis blight or blueberry scorch virus. Blighting of the flower trusses is also common, as are circular to irregular, brown leaf spots. Where young leaves are affected by patches of tissue necrosis they may become distorted.

Epidemiology

The bacterium exists as a number of strains, some of which are more damaging than others. The strains affecting blueberry are not host-specific and can also be found on other plants. *P. syringae* is an 'ice-nucleating' bacterium; where it is present tissues will freeze at higher temperatures than those normally expected to cause frost damage, and are therefore more susceptible to such damage. Not all strains are ice-nucleating, but when there is a high proportion of such strains on the plant formation of ice crystals within the cells is more likely, and the bacteria can then gain entry to the plant through the resulting damage. The disease is most damaging to young plants as these have softer growth and younger canes that are more likely to be infected and girdled by lesions. The bacteria live commonly on the plant surface or in the buds as epiphytes and can only infect via damage or through natural openings such as leaf scars. The bacteria can be spread by wind, rain, insects or contaminated tools, or within infected young plants or propagation material.

Control

Affected twigs and branches should be pruned out. The cultivars 'Elliot', 'Rancocas' and 'Weymouth' show some resistance to the disease. Late summer application of nitrogen should be avoided. Fungicide sprays are used widely in Canada and the USA, with applications in autumn and spring. Fungicides containing copper are the only ones likely to be effective, although strains of the bacterium resistant to copper are now becoming prevalent in some areas of Canada and the USA (MacDonald *et al* 2002). The biological control agents *Bacillus subtilis* and *Pseudomonas flourescens* have been shown to have activity against the disease.

Dieback caused by other bacterial pathogens

Ralstonia solanacearum

In a summary article of a poster given at a meeting of the American Phytopathological Society, Patel *et al* (2013) report finding blueberry plants exhibiting symptoms of wilting and rapid cane death in the USA. They state that the symptoms superficially resembled stem blight (presumably that caused by *Botryosphaeria/Neofusicoccum*) but that the pattern of leaf discolouration was unique (although the exact leaf symptoms are not described in the summary article). Affected plants also exhibited a watery, grey discolouration of the vascular tissue in affected stems, and significant bacterial 'streaming' was observed when symptomatic wood chips were placed in water. *Ralstonia solanacearum* was isolated from the symptoms and confirmed as the cause by host inoculations.

Ralstonia solanacearum has a wide host range (although races exist that differ in their host ranges). It is an EPPO-listed quarantine organism, which in the UK poses a particular threat to solanaceous hosts (causing the diseases brown rot of potato and bacterial wilt of tomato).

Xanthomonas leaf spot and stem canker

Roberts *et al* (2002) found young blueberry plants (18-24 months old) on nurseries in Florida affected by soft, dark brown to black stem cankers, often resulting in death of the entire plant. The plants also exhibited leaf lesions that were roughly circular, 5-20mm in diameter, reddish-brown surrounded by a yellow halo, frequently merging to affect large areas of the leaf. A *Xanthomonas* species was isolated from the plants and host inoculations confirmed pathogenicity.

Bacterial leaf scorch caused by *Xylella fastidiosa*

Thus disease has been recognised in the southeastern USA since 2004 (Chang *et al* 2009, Harmon and Hopkins 2009). Whilst affected plants eventually die, some of the earlier symptoms of the disease mean that it is unlikely to be confused with other causes of blueberry decline and dieback. An excellent summary of the disease and its symptoms by Brannen *et al* (undated) can be found online:
http://plantpath.caes.uga.edu/extension/documents/BlueberryXylella.pdf

In the early stages leaves show symptoms of marginal browning, sometimes with a darker border where affected meets healthy tissue. Affected leaves can be distributed widely or confined to just a few branches. The leaves are eventually shed and the plant becomes leafless and skeletal. At this stage the leafless stems and branches become yellow (a unique feature of the disease making affected plants very prominent), but both aerial parts and roots appear healthy internally. Eventually the plant dies and at this point the symptoms could be confused with dieback caused by root pathogens.

The bacterium is a vascular pathogen, although staining of the vascular tissues is not seen. It is transmitted by sap-sucking insects such as leafhoppers and froghoppers. It has a wide host range amongst cultivated and wild plants and weeds, and causes important diseases such as Pierce's disease of grapevine and variegated chlorosis of citrus. *X. fastidiosa* is not present in the EU and is an EPPO-listed quarantine organism (as are its non-European vectors). It is a warm weather organism and is only likely to be a significant risk in warmer climates of the EU such as those found in the Mediterranean.

3. Dieback as a result of infection by viruses

A review of the threats posed by viruses to blueberry production around the world has been written by Martin *et al* (2012). Only a small number of these viruses cause symptoms of dieback or decline, however, and in many cases other symptoms are also present that readily identify the cause of the problem as a virus rather than a fungal or bacterial infection.

Blueberry scorch virus (BlScV)

This aphid-borne virus exists as a number of different strains, and symptom production will vary according to the strain of virus, the cultivar of blueberry and the environmental conditions. Symptoms range from none (asymptomatic) to severe damage. In severe cases twig dieback can occur, as well as blighting of young leaves (Figure 18) and flowers. Additional symptoms sometimes exhibited include yellow leaf margins or red line patterns on the leaves in late summer and autumn. The blighted flowers often fall off soon after they develop symptoms, but can sometimes remain on the plant throughout the growing season and into the following winter's dormancy. Plants with severe symptoms bear little fruit and can take on a scorched appearance, giving the virus its common name. Some varieties undergo decline over a few years and eventually die.

The twig dieback symptoms (Figure 19) caused by this virus can be confused with those caused by other pathogens, such as Botrytis blight or Phomopsis twig blight, or abiotic damage such as that caused by frost (OEPP/EPPO 2005). The virus is not present in the UK and is an EPPO-listed quarantine organism.

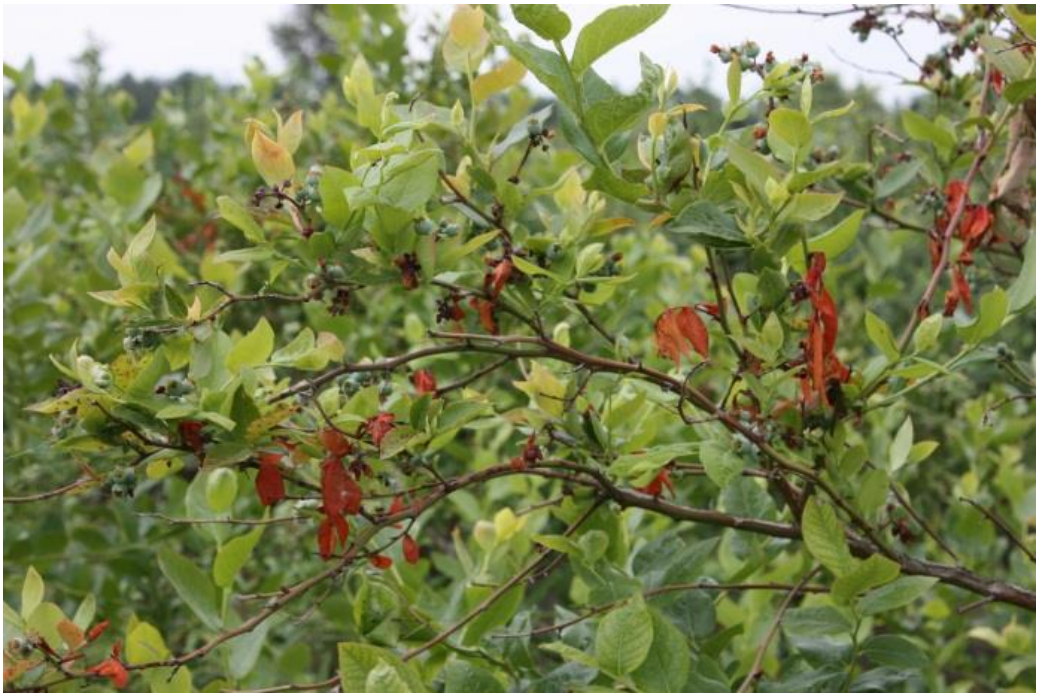

Figure 18. Leaf and flower blight caused by Blueberry scorch virus

Figure 19. Twig dieback caused by Blueberry scorch virus

Necrotic ringspot disease caused by Tobacco ringspot virus (TRSV) and Tomato ringspot virus (ToRSV).

These nematode-transmitted viruses often cause a decline in susceptible cultivars, in some cases leading to plant death. 'Top dieback' has also been reported (Ramsdell 1978). However, foliar symptoms strongly suggestive of virus infection, such as yellow mosaic patterns or necrotic spots and leaf distortion, are also frequently seen.

Blueberry leaf mottle virus (BlMoV)

This pollen-transmitted virus is currently limited to parts of Canada and the USA. The symptoms vary with cultivar. Affected bushes can develop stem dieback, but also show other symptoms such as leaves of reduced size with distortion, yellowing or mottling (Ramsdell and Stace-Smith 1979).

Dieback caused by abiotic factors

A detailed discussion of blueberry dieback caused by factors other than diseases is beyond the scope of this literature review. However, literature searches containing the terms 'blueberry' and 'dieback' inevitably resulted in the retrieval of papers describing dieback caused by abiotic factors. Such factors included frost damage (Entrop and Weber 2013, Weber and Entrop 2013), herbicides (Hodges *et al* 1979), surfactants (Cline and Oudemans 2002) and de-icing salt (Berkheimer and Hanson 2006).

The German papers by Weber and Entrop are of relevance to this review, however. Whilst winter frost damage was determined to be the primary cause of a twig dieback associated with destruction of the vascular tissues, a range of fungi such as *Godronia cassandrae, Phomopsis* spp., *Diplodia seriata, Pestalotiopsis* sp., *Colletotrichum acutatum* and *Fusarium* spp. was also recovered sporadically from the symptoms. These were considered to be opportunistic colonisers of the frost-damaged tissues.

Discussion

Testing by Fera of blueberry samples exhibiting various symptoms of dieback and decline under project SF 132 resulted in the recovery of a range of different fungi. These included species from genera known to cause disease of blueberry in the USA and other countries (such as *Diaporthe/Phomopsis and Botryosphaeria/Neofusicoccum*), as well as species of genera that can sometimes act as pathogens of other plants and crops (such as *Coniothyrium, Phoma* and *Fusarium*). Host inoculation tests using the *Diaporthe/Phomopsis* and *Botryosphaeria/Neofusicoccum* species showed that most were capable of acting as pathogens on blueberry.

These results are somewhat different from the results of research on the dieback problem currently affecting blackcurrants and investigated under Project SF 012, which showed that a single species, *Diaporthe strumella* (asexual state *Phomopsis ribicola*) was predominantly responsible for the problem. The results of the blueberry work are more akin to those found in Project SF 131, investigating dieback problems affecting gooseberries (although in this case the range of fungi recovered was somewhat different and the number most frequently associated with the more severe die-up' symptoms was relatively small.

The information generated by the literature searches undertaken for this review has shown that the results obtained by Fera in their testing of UK blueberry dieback samples are the norm rather than the exception. Many other workers are finding a number of different pathogens to be associated with twig dieback, stem blight and cankering of blueberry. These pathogens might be present in the same plantation, on the same plant and even sometimes within the same lesion. Similar results are being found in most of the major areas in which blueberries are grown, such as the USA, South America, Europe and Australasia.

Many of the papers reporting that several different pathogens might be involved in the problem of blueberry dieback and decline have been published within the last ten years, so is this a relatively new phenomenon? Whilst some of the earlier books and papers on blueberry diseases might give the impression that there are a limited number of dieback, blight and canker pathogens with quite clearly-defined symptoms, a clue can perhaps be found in a paper published in 1974 by Weingartner and Klos, entitled 'Fungi associated with blueberry stems in Michigan'.

During their study of canker and stem blight diseases of blueberries in Michigan Weingartner and Klos identified 24 species of fungi in association with the symptoms (although many were shown to be non-pathogenic in host tests). These included several of the genera that feature prominently in this literature review and/or which were found by Fera in their tests, such as *Godronia cassandrae, Diaporthe vaccinii, Botrytis cinerea, Seimatosporium lichenicola, Coniothyrium* sp., *Phoma* sp., *Fusarium* sp., *Cylindrocarpon* sp., *Alternaria* sp. and *Verticillium* sp.

It would seem, therefore, that the association of a complex of fungi with such symptoms is nothing new. However, there is little doubt that two changes occurring in recent years have increased that complexity still further. The first is an increase in the number of countries growing significant numbers of blueberry plants. The presence of the crop in many new areas means that the plants will inevitably be exposed to a greater range of potential pathogens than might be found in their native North America.

The second change has occurred in the techniques used in the diagnosis of plant diseases. This major change is the development and uptake of DNA analysis techniques, which has changed the whole area of fungal taxonomy dramatically. In terms of blueberry pathogens the most prominent changes are in the taxonomy of *Diaporthe/Phomopsis* species and the members of the *Botryosphaeriaceae* family. Until recently the predominant species of these involved in blueberry diseases (in the USA at least) were *Diaporthe vaccinii* (Phomopsis twig blight and canker), *Botryosphaeria corticis* (stem canker) and *Botryosphaeria dothidea* (stem blight). The work reported in this review (from South America in particular) has shown that a number of other *Diaporthe* species can cause very similar symptoms to those of *Diaporthe vaccinii*. Other fungi from the *Botryosphaeriaceae* family (often, but not exclusively *Neofusicoccum* species) have been shown to cause canker and blight symptoms (including in the USA), whilst identifications of *Botryosphaeria dothidea* itself prior to 2004 may be erroneous.

These groups of fungi are still undergoing revision and it is likely that there will be further reclassification and name changes. As an example, one of the *Diaporthe / Phomopsis* isolates obtained by Fera from the UK blueberry samples (and found to be non-pathogenic in host tests) was named in the reports for SF 132 as *Phomopsis theicola.* The sexual state of this fungus is *Diaporthe theicola.* A synonym of this species is *Diaporthe neotheicola*, and under this synonym it was reported from Chile by Elfar *et al* (2013) as a confirmed pathogen on blueberry. However, a recent paper (Udayanga *et al* 2014) has now included **all** of these names as synonyms of *Diaporthe foeniculina*, based on re-evaluation of DNA sequence data and comparisons of fungal morphology. This serves to illustrate some of the problems encountered in trying to interpret the findings of different papers published over many years.

Of the fungi recovered from the UK samples, the literature clearly shows that both *Diaporthe/Phomopsis* species and *Neofusicoccum/Botryosphaeria* species are considered major pathogens of blueberry in most parts of the world where the crop is grown. As stated above, *Phomopsis theicola* has also been reported in Chile. Of the other species found by Fera in project SF132 (and giving the name used in the project reports for SF132 first):

***Phomopsis eres / conorum* complex**: Found to be pathogenic in Fera host tests. *Diaporthe eres* is mentioned by Lombard *et al* (2014), but it is unclear what symptoms the blueberry isolates were obtained from, nor whether Koch's postulates were carried out.

***Phomopsis viticola*:** Found to be pathogenic in Fera host tests. Isolated from disease symptoms in Chile but found to be non-pathogenic in host tests there (Espinoza, Briceno and Latorre 2008). Also mentioned by Lombard *et al* (2014) but the same uncertainty applies as for *D. eres* above. In discussions with Dr Annemiek Schilder of Michigan State University during a visit funded by an HDC travel bursary (December 2013) Graham Moore was informed that *P. viticola* is isolated frequently from diseased grapevines in Michigan, but is not thought to be an important component of blueberry dieback problems in the state.

***Neofusicoccum australe*:** Found to be pathogenic in Fera host tests. Also found in association with stem blight and/or cankering in Chile (Espinoza *et al* 2009), Spain (Castillo *et al* 2013) and New Zealand (Sammonds *et al* 2009). Pathogenicity confirmed by Chilean and Spanish workers, no reports found of the results of host tests in New Zealand.

***Botryosphaeria obtusa*:** Found to be pathogenic in Fera host tests. Reported (as *Diplodia seriata*) in the USA by Wright (2011) and New Zealand by Sammonds *et al* (2009), but neither mentions any results of pathogenicity tests.

Turning to some of the other genera found by Fera, *Botrytis cinerea* is a recognised and damaging pathogen of blueberry, causing Botrytis blight. *Fusarium* species have been reported as apparently minor dieback pathogens in South America. Species such as *Phoma, Cytospora* and *Coniothyrium* are mentioned occasionally in the literature, but pathogenicity tests don't seem to have been done. *Phytophthora* is a well-known and damaging cause of root and crown decay.

Fungi known to cause dieback, canker or blight problems and found commonly by other workers, but **not** isolated by Fera from the UK samples include *Godronia cassandrae* (cause of Godronia or Fusicoccum canker in many countries), *Pestalotiopsis* species (mainly in South America) and *Colletotrichum gloeosporioides / C. acutatum* (cause of anthracnose in many countries). All of these fungi have been found previously in the UK, although not

necessarily on blueberry – *Pestalotiopsis* species are found very commonly on a wide range of woody plants, as are the two *Colletotrichum* species. *Godronia cassandrae* has been found on blueberry in the UK previously but does not appear common. However, given its presence as a damaging pathogen in countries such as Norway and Poland, and its apparent suitability for growth in the UK climate, it would perhaps not be surprising if it became more widespread.

Of the symptoms found in the surveys of affected UK plantations, there are many pathogens reported in the literature (and discussed in the main body of the literature review) that could be potential causes for the symptoms described as 'limited' and 'progressive' twig dieback. Pathogens commonly infecting through flower buds or open flowers and thus potential causes of the symptom described as 'tip dieback associated with flower infection' include *Diaporthe / Phomopsis* (a well-known route of infection for *D. vaccinii* but also a possibility for other species), *Botrytis cinerea* (Botrytis blight) and *Colletotrichum* species (anthracnose). *Phomopsis* species and *Botrytis cinerea* were both found commonly in association with these symptoms in the UK samples.

Causes for the 'die-up' symptom might include root and wilt disease pathogens (e.g. *Armillaria* spp.*, Phytophthora* spp.*, Fusarium oxysporum, Verticillium* spp.). *Phytophthora* was found in some of the UK samples affected by crown decay, but root rotting (a common symptom of *Phytophthora* infection) was not reported as a widespread symptom in the plantation surveys. The wilt pathogens were not found in any of the UK samples. Other pathogens that are causes of lesions or cankers affecting the stem base and/or crown, and which would not commonly cause associated root decay, are *Diaporthe / Phomopsis* (again a common symptom caused by *D. vaccinii* and also reported for other *Diaporthe* species), *Botryosphaeria / Neofusicoccum* species, *Godronia cassandrae* and *Pestalotiopsis* species. Of these fungi, *Phomopsis / Diaporthe* and *Neofusicoccum / Botryosphaeria* were isolated from crown rot symptoms in the UK samples.

As mentioned previously, the association of a 'disease complex' with blueberry dieback and decline problems is a common feature discussed in the literature. To repeat just a few examples that have been mentioned within the review, Chilean workers have found species of *Diaporthe / Phomopsis, Botryosphaeria / Neofusicoccum, Pestalotiopsis* and *Truncatella* in association with cankering and dieback, with the fungi often co-isolated from the same plants. In Argentina, *Pestalotiopsis guepinii* and *Colletotrichum gloeosporioides* were both found associated with stem blight. In the USA, *Botryosphaeria dothidea* was found colonising and extending twig dieback that had been caused initially by *Botrytis cinerea*, and *B. dothidea* is also known to invade cankers and lesions caused by *Diaporthe / Phomopsis* and other members of the *Botryosphaeriaceae* family. As many of the fungi involved in the dieback, blight and cankering problems are known to be opportunistic pathogens and wound

colonisers, the concept of one fungus taking advantage of damage caused by another is not surprising.

Of course, it is not just tissue damaged as a result of attack by other diseases that allows many of these pathogens to enter the plant. Other forms of damage are also routine entry points, particularly those caused by cultural operations and weather conditions such as frost. The concept of the susceptibility to diseases such as those caused by *Diaporthe / Phomopsis* and *Botryosphaeria / Neofusicoccum* increasing as a result of plant 'stress' (e.g. caused by drought or other adverse growing conditions) is also raised frequently. Such stress could cause weakening or necrosis of the plant tissues that could then be colonised by opportunist pathogens arriving on the plant as spores from external sources, or it could trigger fungi residing within the plant as endophytes into a more damaging parasitic role. This concept is also seen with other disease problems; for example stress is thought to play a significant role in increasing the susceptibility of blackcurrant plants to dieback caused by *Diaporthe strumella*.

The presence of some of the pathogens on or within planting material (in either a latent state or as an endophyte) merits further investigation. Infected blueberry cutting material has been shown to be a source of several species within the *Botryosphaeriaceae* and this could also be the case for some of the other pathogens. An Indication of this source of potential pathogens was found in SF 132, where apparently healthy branch material used in inoculation tests was found to already contain a species of *Phomopsis.* It is also seen with many other crops. Work in project SF 012 found that in some cases *Diaporthe strumella* was present in blackcurrant stock plants used for the production of hardwood cuttings.

Turning to control measures, the avoidance of plant damage or stress would seem to be a key factor in reducing the risk from many of the pathogens. Whilst cultivar resistance has been shown from research to be effective against some of the key blueberry dieback pathogens, this becomes more difficult to implement as a control strategy where a range of different organisms are likely to be acting together to cause the problem.

Control using fungicides and/or biological control agents appears from the literature to be effective against some, but by no means all, of the pathogens involved in blueberry dieback and decline. They work best where there is a clearly defined route and a limited time period for infection (e.g. through flowers or flower buds) that can be protected against spore germination and infection. Thus sprays can work well against twig blight caused by *Diaporthe vaccinii* (and possibly other species of *Diaporthe / Phomopsis*), *Botrytis cinerea* and *Colletotrichum* species. Protection against wound-derived infections is more difficult; it seems to be effective against some pathogens (e.g. *Godronia cassandrae*) but not others (e.g. some

of the *Botryosphaeria / Neofusicoccum* species). Whatever the route of infection, once a pathogen is deep-seated within the stems or the crown of a plant eradication using fungicides is usually ineffective.

Conclusions

- Blueberries are hosts for a wide range of fungi, many of which are capable of causing disease. The number of species reporting has tended to increase in line with the introduction of blueberry growing to new countries and with improved diagnostic methods.
- Symptoms are often associated with the presence of a complex of fungi.
- Studies have shown that several different *Diaporthe* species (asexual states = *Phomopsis*) can cause very similar symptoms. A similar situation exists for some other pathogens, including those from the *Botryosphaeria* family.
- Symptoms may arise as a result of complicated interaction between more than one species of fungus and abiotic factors such as mechanical damage and drought stress. Growers should be aware of the likely importance of irrigation problems (pots and soil) and soil structural factors affecting root growth (organic matter, aeration) as factors in the development of symptoms.
- Species associated with dieback in UK blueberries, for which pathogenicity to blueberries was confirmed by SF132 and which are reported as being responsible for disease in other countries include *Phomopsis eres / conorum* complex*, Phomopsis theicola*, *Neofusicoccum australe, Botryosphaeria obtusa*. A number of fungicide active ingredients and plant defence boosting materials (harpin, chitosan) are reported to contribute to disease reduction. Unfortunately of the fungicides, several have been withdrawn from use in the UK (notably benomyl and ziram) or are not currently registered for use in any similar crop (propiconazole, lime sulphur).
- Epidemiology studies show that the more commonly found species produce spores that survive on twigs and stem lesions and are readily dispersed in wet conditions. Many are more active at temperatures above the normal for UK but that does not preclude infection of material held under warm, moist conditions during propagation and early establishment. It is common practice in propagation to grow plants at very high densities, to trim the plants at least once during the growing season and to employ overhead sprinklers as the main source of irrigation. The use of clean stock and ultra-careful hygiene practices must therefore be given priority both in nurseries

and during crop establishment when plants are grown in pots at high densities and under humid tunnel conditions.

- While fungicides may be useful for disease prevention (blossom, leaf/fruit scar and wound infections) they are not generally effective against established/deep-seated infections. Latent, symptomless/endophytic infections have been demonstrated or strongly suspected as a cause of later plant failure. A controversial subject but one that is no less important for study by blueberry scientists as by those concerned with other crops.

Knowledge and Technology Transfer

SF 150 was funded as an extension to SF 132 and no specific knowledge and technology transfer activities have been undertaken during the period of SF150.

The authors look forward to contributing to industry journals and events following approval of and panel discussion of this report. We would also hope that, if funding is available, an agronomy fact-sheet might be published.

Glossary

Abiotic – non-living.

Acervulus – a type of fruiting body (usually just visible to the naked eye) formed by some fungi, producing asexual spores or conidia.

Apothecium – a fruiting body, very variable in size and sometimes mushroom-like, formed by some fungi; produces ascospores.

Ascospore – a type of sexual spore, produced by many fungi.

Botryosphaeria – the sexual state of several fungi in the family *Botryosphaeriaceae.*

Botryosphaeriaceae – a family of fungi containing genera such as *Botryosphaeria, Diplodia, Lasiodiplodia* and *Neofusicoccum*.

Chlorosis – yellowing of tissue.

Conidium (plural conidia) – a type of asexual spore, produced by many fungi.

Diaporthe – the sexual state of *Phomopsis* species.

Endophyte – an organism that lives in a plant for at least part of its life without causing apparent disease. In some cases such organisms may apparently be capable of causing disease if the plant becomes damaged or stressed.

Epiphyte – an organism living on the surface of a plant.

Genus (plural genera) – a taxonomic rank used in classification. Organisms within a genus are further sub-divided into species.

Inoculum – the source of infection, often spores for a fungal pathogen.

(an) Isolate – a pure culture of a micro-organism.

Isolation – the process by which an organism is recovered into pure culture from its host.

Koch's postulates – a series of steps undertaken to prove that an organism is the cause of a disease symptom (described fully in the Introduction section of this review).

Latent infection – the state whereby a plant is infected by a pathogen, but no symptoms of the disease have yet developed.

Morphology – the structure, appearance and form of an organism.

Necrosis – tissue death

Neofusicoccum – the asexual state of several fungi in the family *Botryosphaeriaceae.*

Non-pathogenic – unable to cause disease.

Pathogenic – able to cause disease.

Pedicel – flower stalk.

Peduncle – stem supporting a group of flowers.

Phomopsis – the asexual state of *Diaporthe* species.

Pycnidium – a type of fruiting body (usually just visible to the naked eye) formed by some fungi, producing asexual spores or conidia.

Saprophyte – an organism deriving nutrients from dead organic matter.

Sclerotium – a resilient resting structure produced by fungi such as *Botrytis* and *Sclerotinia.*

Stroma (plural stromata) – a mass of fungal tissues, or a mixture of fungal and host plant tissues, on which fruiting bodies are formed.

Synonym (often abbreviated to syn.) – in terms of the classification of organisms, an alternative name used for the same organism.

Taxonomy – the classification and naming of organisms.

Zoospores – microscopic swimming spores produced by fungus-like organisms such as *Phytophthora* and *Pythium* species.

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Appendix 1

Organisms documented as directly or indirectly causing shoot or branch dieback in blueberries

All of these organisms are discussed in the main text of the review, with full references quoted. All of the organisms listed here are either causes of well-recognised diseases of blueberry that have been known for many years, or have been isolated more recently from diseased plants. In the latter cases pathogenicity has been proven by host inoculation tests to fulfil Koch's postulates.

1a. Fungi affecting aerial parts - twigs, shoots or stems (occasionally crowns)

Diaporthe / Phomopsis species – Phomopsis twig blight and canker

D. vaccinii - the most well-known species and a quarantine-listed organism in Europe.

Other species:

D. ambigua

D. australafricana

D. baccae

D. neotheicola

D. passiflorae

D. perjuncta

D. phaseolorum (Phomopsis phaseoli)

D. sterilis

Diaporthe sp.

Botryosphaeria and related species, and their asexual states (all members of the *Botryosphaeriaceae* family) – 'Botryosphaeria' stem canker / stem blight

Botryosphaeria corticis - long-recognised cause of stem canker in the USA.

Botryosphaeria dothidea - long-recognised cause of stem blight.

N.B. Phillips *et al* (2013) state that identifications of *B. dothidea* prior to 2004 on host plants should be treated with caution, as they could potentially be other species.

Other species:

Diplodia seriata / Botryosphaeria obtusa

Lasiodiplodia theobromae / Botryosphaeria rhodina

Neofusicoccum arbuti

Neofusicoccum australe / Botryosphaeria australis

Neofusicoccum luteum / Botryosphaeria lutea

Neofusicoccum mediterraneum

Neofusicoccum nonquaesitum

Neofusicoccum parvum/ Botryosphaeria parva

Neofusicoccum ribis / Botryosphaeria ribis

Neofusicoccum vitifusiforme

Other fungi

Godronia cassandrae (asexual state *Fusicoccum putrefaciens* syn. *Topospora myrtillii*) - Godronia or Fusicoccum canker

Pestalotiopsis and related species (causing canker, blight and dieback):

- *Pestalotiopsis guepini*
- *Pestalotiopsis clavispora*
- *Pestalotiopsis neglecta*
- *Truncatella angustata*

Colletotrichum (asexual state) / *Glomerella* (sexual state) species causing anthracnose (damaging fruit rot, but also twig blight, stem lesions and leaf spots):

- *Colletotrichum acutatum / Glomerella acutata*
- *Colletotrichum gloeosporioides / Glomerella cingulata*
- *Colletotrichum fioriniae*

Botrytis cinerea - Botrytis blight of shoots/twigs and blossoms.

Monilinia vaccinii-corymbosi - cause of mummy berry, a damaging fruit rot but also a

Gloeosporium minus - Gloeosporium leaf spot and stem canker.

Septoria albopunctata - Septoria leaf spot and stem canker.

Alternaria tenuissima - mainly leaf spots but also small stem cankers.

Discostroma corticale syn. *Clethridium corticola*; asexual state *Seimatosporium lichenicola,* syn. *Sporocadus lichenicola, Coryneum microstictum* - twig canker and dieback, Coryneum canker.

Bipolaris cynodontis - dieback, bud and branch blight.

Gibbera vaccinicola - Gibbera twig blight.

Nigrospora sphaerica - leaf spot, twig and shoot blight.

Fusarium acuminatum - Fusarium branch blight.

Aureobasidium pullulans - stem dieback.

Sclerotinia sclerotiorum - Sclerotinia rot / shoot blight.

Cercospora sp. - Cercospora stem blotch of rabbiteye blueberry

Chondrostereum purpureum - silver leaf dieback.

In addition to those listed above, a number of other fungi have been isolated by various workers from stem necrosis symptoms, including species of *Coniothyrium, Cytospora* and *Phoma.* They are not listed separately here as there are no reports of Koch's postulates being conducted to prove pathogenicity. They are, however, discussed in the review.

1b. Fungi and fungus-like organisms affecting roots, crowns or stem bases (and leading to dieback)

Armillaria species (honey fungus)

Armillaria gallica

Armillaria cepistipes

Armillaria gemina

Armillaria mellea

Armillaria ostoyae

***Phytophthora / Pythium* species**

Phytophthora cinnamomi

Phytophthora citrophora

Pythium sterilum

***Calonectria (Cylindrocladium)* species**

*C. Ilicicola (*asexual state *Cylindrocladium parasiticum)*

Calonectria colhounii (asexual state Cylindrocladium colhounii)

Calonectria kyotensis (asexual state Cylindrocladium floridana)

Fusarium species

Fusarium proliferatum - root rot.

Fusarium solani - root and stem rot.

Other

Rhizoctonia solani (stem and root rot, web blight (cuttings))

1c. Fungal wilt diseases

Fusarium oxysporum - Fusarium wilt.

Fusarium solani - Fusarium 'wilt'; more likely to be a root/stem rot.

Verticillium dahliae - Verticillium wilt on lowbush blueberry.

2. Bacterial diseases

Pseudomonas syringae - bacterial blight / bacterial canker.

Ralstonia solanacearum - wilting and dieback.

Xanthomonas campestris - leaf spot and stem canker.

Xylella fastidiosa - bacterial leaf scorch.

3. Viruses

Blueberry leaf mottle virus (BLMoV) - stem dieback part of disease syndrome.

Tobacco ringspot virus (TRSV) and *tomato ringspot virus* (ToRSV) - both causes of necrotic ringspot disease, which can lead to decline symptoms and plant death.

Blueberry scorch virus (BlScV) - flower and leaf blight, twig dieback.

Appendix 2

Table summarising substances mentioned as contributing to improving plant health where dieback problems are a threat

Active ingredient	**Example of UK product name**	**Authorisation status, blueberry**
Azoxystrobin + propiconazole	Headway	None
Azoxystrobin + captan		
Bacillus subtilis	Serenade ASO	EAMU 130706
Benomyl	n/a	All products withdrawn from UK sale
Calcium polysulphide / lime sulphur	None	None
Captan	Captan	None
Captafol	None	None
Chlorothalonil	Bravo	None
Cyprodinil + fludioxonil	Switch	On label
Fenhexamid	Teldor	EAMU 061290
Fosetyl aluminium	Aliette	None
Fluazinam	Shirlan	None
Mefenoxam	Ridomil Gold	None
Propiconazole	Banner Maxx	None
Pyraclostrobin	Comet 200	See below
Pyraclostrobin + boscalid	Signum Bellis	Signum EAMU 12722
Ziram	n/a	All products withdrawn from UK sale
Foliar feeds/plant strengtheners/Plan defence elicitors		
Chitosan	n/a	None
Harpin	Pretect	n/a

Appendix 3

Miscellaneous images of symptoms that diagnostic tests showed a direct relationship with specific fungi

The following images may help to illustrate both the range of symptoms found and the types of fungi commonly associated with them. During the period of SF132 and SF150 many photographs were taken and, given time, it may still be possible to match the best of them with laboratory diagnostic results.

It should, however, be noted that the presence of a fungus associated with a symptoms does not prove cause, hence the importance of Koch's postulates studies and a better understanding of the interaction between species.

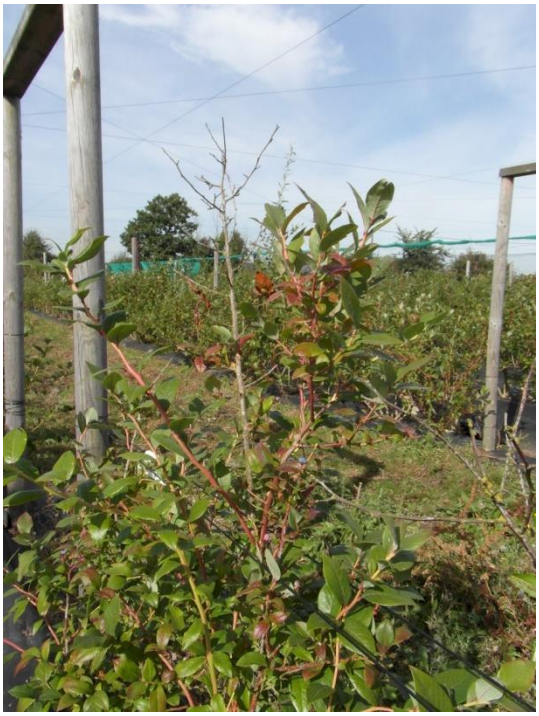

Figure 20. Botryosphaeria

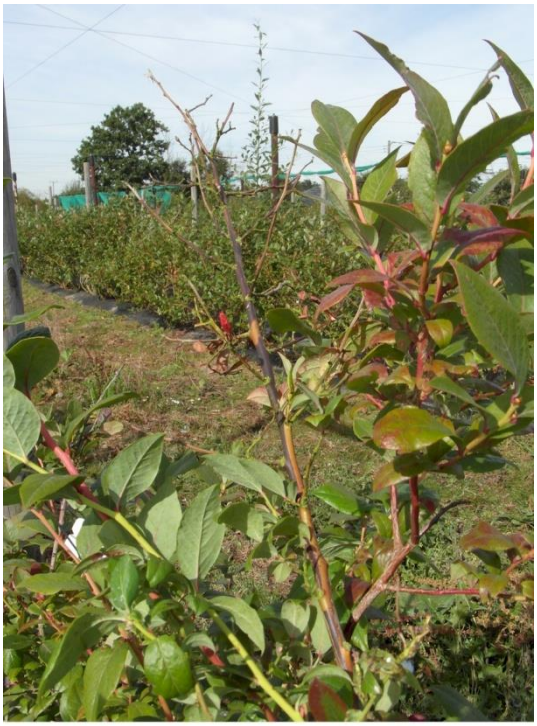

Figure 21. Botryosphaeria

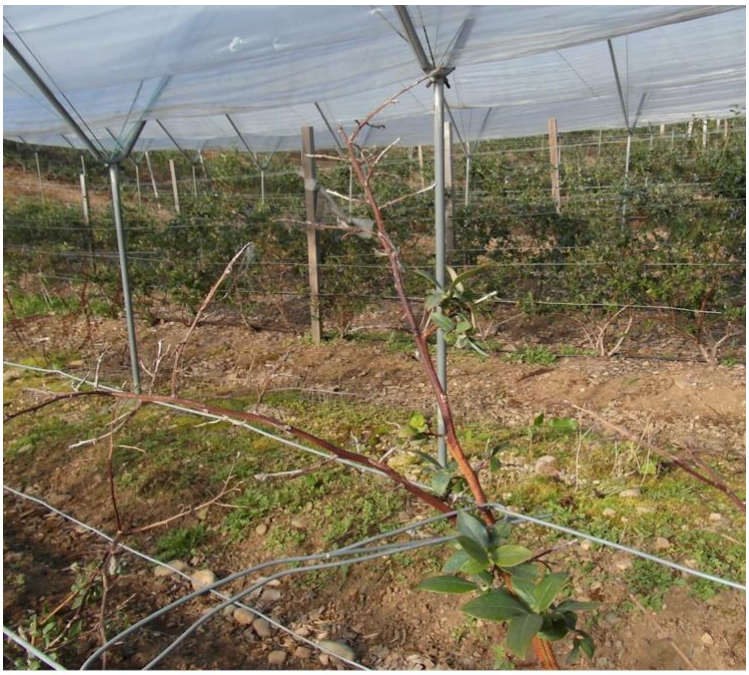

Figure 22. Botryosphaeria

Figure 23. Phomopsis

Figure 24. Phomopsis

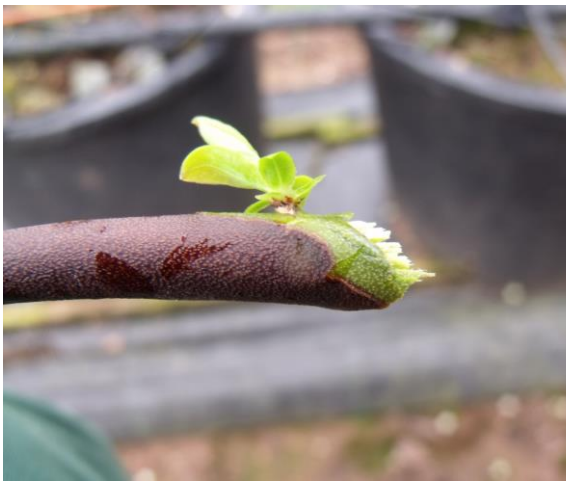

Figure 25. Phoma

Figure 26. Fusarium

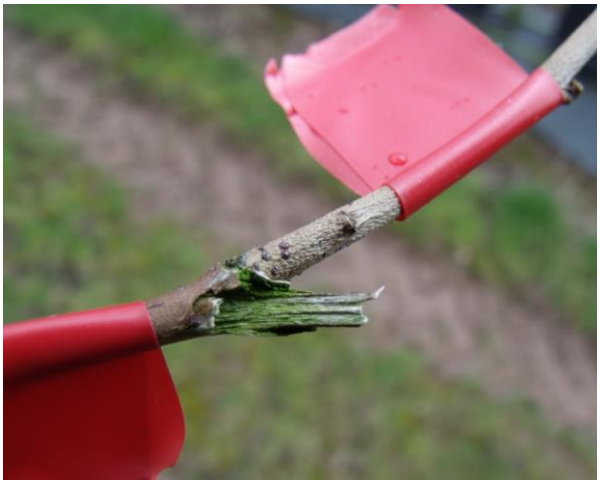

Figure 27. Diaporthe-like (diagnosis not resolved to species)

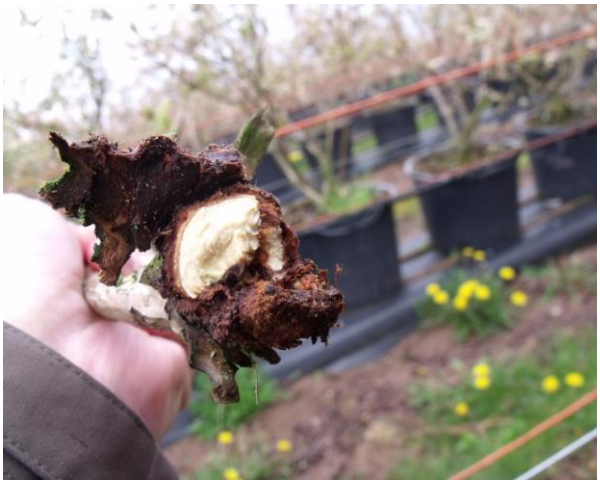

Figure 28. Fusarium

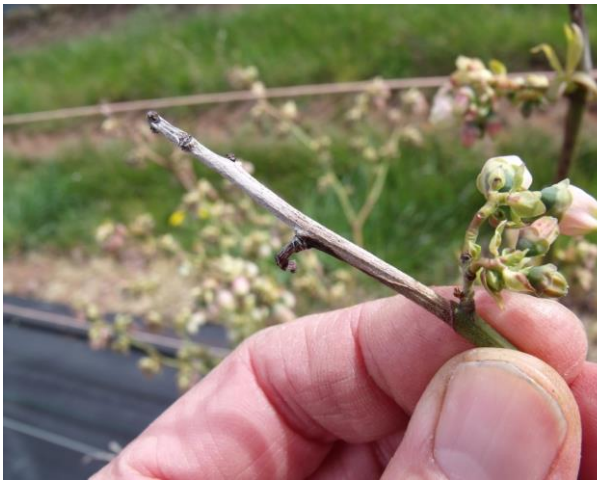

Figure 29. Phomopsis / Phoma (not resolved to species)

Appendix 4

2014 Site Visits Report – EMR team

Blueberry site visits by EMR in 2014

Method

Blueberry plantations identified during visits in 2012 as having die-back and or die-up problems were visited in 2014 to check on the progress of the problem. Two sites were visited.

The names and locations anonymous.

Results

Site 1

The site was visited on 30 October

Established Plantation Mixed cultivars

Main problem in this plantation seen in 2012 and 2013 was crown death. The problem here has continued to progress with many gaps now present in the plantation. Some tip die-back was present but mainly of non-progressive type.

New Plantation cv. Liberty

This was planted in 2012. Very few plant deaths were seen in 2012 but a low incidence of shoot die-back was present. The grower said that in 2013 around 100 dead bushes had been removed from the new planting. In 2014 no further deaths were reported. In general there were no obvious sick bushes or bushes showing premature autumn colouring. In general walk round there was a low incidence of tip die-back, mostly non-progressive. There were samples taken of more progressive die-back and samples were taken of these for laboratory tests. The farm manager reported that the improvement in the plantation was thought to be due to a reduction in irrigation and that the problems seen in 2013 were due to over-watering.

Site 2

The sites were visited on 5 November

Site 2 New planting tunnelled (Mainly cv. Duke)

Block 1

The site was planted out in 2011 from potted blueberries. Field site was on raised beds through mypex and under tunnels. Grower reported the bushes grew well in 2012 but then in

2013 showed bush death or part death, symptoms typical of the problem under investigation. In 2014 there were very few new bush deaths and those present were probably left over from 2013. The main problem observed in 2014 was tip die-back present in about 25-30% of bushes. Some of this was the non-progressive die-back confined mainly to the shoot tip but on about 10% of bushes a more progressive die-back was present. This extended to about a foot but on some bushes the die-back had progressed to the crown and killed the shoot. Samples were taken for laboratory tests.

Block 2

A smaller block of bushes (probably a different cultivar) were also checked. No dead bushes were seen. The incidence of tip die-back was lower - <5% and the progressive shoot die-back rarer <1%. Some samples were also taken.

Isolations from samples

Isolations were made from the die-backs. In all cases *Phomopsis* sp. was consistently isolated and in most cases *Phomopsis* was the only fungus isolated. Isolates were planned to be sent to Fera for species identification but unfortunately the cultures became infested with mites and had to be destroyed.

Discussion

At both sites the incidence of bush death had continued to decline. The improvement on bush health in the sites suggests that growing conditions in 2012/2013 and 2014 may have been very favourable for bush growth so that the bushes out grew the infections. This supports the idea that the blueberry death issue may be a complex interaction between, fungi already in the plant and the growth of the plant, rather than one particular disease problem. Certainly there is evidence for *Botryosphaeria* existing as an endophyte in apple trees and conditions such as stress, cold winters, fungicide use determining whether the fungus causes disease symptoms on the tree. In this respect it may be worth evaluating alternative chemicals, in particular plant strengtheners, elicitors, growth promoter type products for their effect on disease expression.

Of note was the incidence of progressive shoot die-back present at one site. Unfortunately it was not possible to get the species identified. This emphasises the need to include methods to control *Phomopsis* etc in the nursery and to develop a test to check nursery material to ensure freedom from certain fungi.

Appendix 5

2015/15 Monitoring Comments - FAST team

FAST continued to visit key sites ‘on the back of’ routine advisory work.

General impressions were as follows:

- Plant health improved at some sites – even those that were suffering particularly severe symptoms prior to the start of SF 132. Improvements have largely arisen for a combination of actions:
    - Removal of sick plants (including some replanting of planting stations in soil grown fields)
    - Improved irrigation and soil management practices (surface amendment with organic materials)
    - Regular use of available plant protection materials and foliar feeds – mimicking, where possible, the approach of American blueberry growers.

- At some sites bushes have continued to decline – invariably where it has not been possible to rectify root environment problems which would tend to confirm the close relationship between abiotic factors and disease progress

- While tip dieback rarely progresses further than a few tens of centimetres it is often an indicator of other, more serious problems with root and crown health.

