The *Botryosphaeria* conundrum – a New Zealand perspective

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Botryosphaeria fungi can be found in all viticultural regions of New Zealand and in many other winegrowing regions of the world, but despite the fact that they are so widespread and so common, we still do not know very much about their capacity to cause disease.

Botryosphaeria is the name of a genus – in other words, a group of closely related species. In New Zealand, the genus *Botryosphaeria* includes at least five different fungal species that are commonly found in grapevines:

- *Botryosphaeria lutea*
- *Botryosphaeria dothidea*
- *Botryosphaeria parva*
- *Botryosphaeria obtusa*
- *Botryosphaeria stevensii.*

Other species of *Botryosphaeria* are found in grapevines in many other countries, and species of the genus are also found in an extensive range of other woody hosts in New Zealand and elsewhere. Such host plants include avocados, oaks, apples, pears, olives, *Prunus* species, poplars, pines, ashes, elms, and various berries.

Botryosphaeria fungi are already known to be involved in a number of different grapevine diseases, including bunch rots, cane die-backs, dead-arm conditions, graft failures, bud deaths, and, especially, trunk diseases. In the latter case, the fungus causes damage or decay in the wood inside of the vine trunk.

As in so much of the research involving grapevine diseases, the advent of advanced DNA technology has had a major impact upon ▸

the taxonomy of *Botryosphaeria* (that is, the exact identification and classification of the fungal species). For that reason, the taxonomy must be considered a "work in progress" – much of what was previously received as "understood" has since been shown to be wrong, or at least in need of a major review. Bearing that caveat in mind, our work at Linnaeus laboratory has been greatly assisted by Alan Philips from the New University of Lisbon (Universidade Nova de Lisboa), Portugal, who has helped us with the identification of the different species of *Botryosphaeria*.

A riddle wrapped in a mystery inside an enigma

While species of *Botryosphaeria* are often found in vines with disease symptoms, they are also frequently found in vines where no particular disease or health problems are evident. *Botryosphaeria* fungi may simply exist inside or on the outside of the vine without apparently causing any disease.

This conundrum raises several questions. Do all species of *Botryosphaeria* cause disease or are some not as pathogenic as others? Do species of *Botryosphaeria* cause disease on their own, or do they require other fungi to be present? Or might they only cause disease when a vine is already compromised – say, by another infection or by stress from some other source? Does the mere presence of a *Botryosphaeria* fungus in a vine mean that it is just a matter of time before a disease condition becomes evident?

Fungi of grapevines are described as "weak" or "strong" pathogens (fungi that cause disease) or as "saprophytes" (fungi that live only on dead tissue and do not cause disease). A strong pathogen will cause disease on its own, whereas a weak pathogen generally will not. If, however, a vine is compromised by stress, injury, or some other disease, then a weak pathogen may become opportunistic and start to behave as a stronger pathogen.

Recent research into the area of grapevine fungal diseases indicates we are dealing with "complex diseases." Such diseases may also be described as stress-related diseases, and they frequently occur where one or more "weak" pathogens are apparently causing fairly serious problems. Researchers who work with fungi (mycologists) would probably describe most of the *Botryosphaeria* species as "weak" pathogens, or, for some species, even as "saprophytes."

What makes for a complex disease?

While the presence of one or more fungal culprits is an essential component of the complex disease, other factors also come into the equation. Certainly, the physical condition of the vine needs to be considered as well. If the vine is very young and trying to establish itself, it may perhaps be more vulnerable to fungal attack. If the graft union is not of the best quality, or if the vine has a pre-existing and debilitating viral disease, it may also be more vulnerable to fungal attack.

Complex diseases are also critically influenced by environmental factors, including:

- water stress (either deficit or excess)
- nutritional deficiencies or toxicities
- adverse soil conditions, such as heavy clays, or extreme pH
- insect predation, such as white-fringed weevils, black beetles, and grass-grubs
- excessive heat or frost damage
- any other adverse site-related issues.

A complex or stress-related disease can be described then as a disease condition resulting from a three-way interaction between the vine, the fungi and the environment. Only further research on this interaction will help us to deal with *Botryosphaeria* fungi in the vineyard.

***Botryosphaeria* in diseased vines**

Table 1 illustrates the distribution of *Botryosphaeria* species found on or in diseased vine samples coming through Linnaeus laboratory. The species and location of the fungi provide an interesting comparison when linked to the following grapevine disease symptoms:

Table 1: Distribution of *Botryosphaeria* species on samples of diseased vines

	Trunk	Graft	Cordon	Buds	Leaves	Excoriose
B. lutea	10	2	7	2	4	4
B. parva	4	1	1	1		
B. dothidea	3		2			
B. obtusa			2			
B. stevensii		1				
Unidentified	21	6	5			7

Trunk disease

The wood inside the vine trunk has a dark wedge that radiates out from the centre and is clearly visible on cross section. The vine has stopped growing or has collapsed. This wedge symptom looks a bit like Eutypa but the vine does not have the same leaf symptoms. In more severe cases, the wedge may have expanded to occupy around half or even all of the interior of the trunk (see Figures 1a and 1b). The staining may be seen also on transverse sections and can often be traced to a pruning wound or other injury. Samples like these often produce *Botryosphaeria parva* or *B. lutea* in culture. We suspect *B. parva* is a stronger pathogen than other *Botryosphaeria* species.

There are dark spots inside the wood of the trunk that are clearly visible on cross section and the vine may be growing very

Fig. 1a. The wood inside the trunk has a dark wedge that radiates out from the centre.

Fig. 1b. In severe cases the wedge may expand to occupy around half or even all of the interior of the trunk.

slowly, or may have stopped growing (see Figure 2).

There are light brown discolourations inside the wood of the trunk or the cordons which are often traceable through the vine to pruning wounds, and the vine may be growing slowly, or have stopped growing. Sometimes only one side of the vine is affected. Shoots may be shortened and leaves may be smaller than normal (see Figures 3 and 4).

Cane die-back

The green cane wood may be stained either a dark or light brown in colour on ▸

Fig. 2. Dark spots on the inside of the trunk.

Fig. 3. Light brown discolourations, often traceable to pruning wounds.

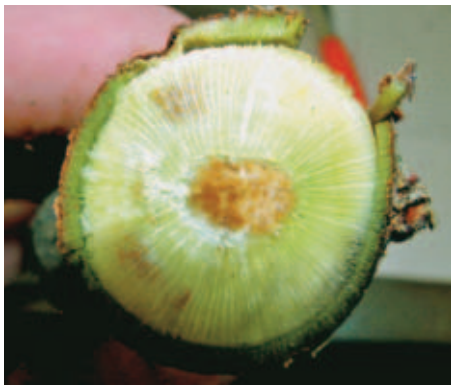

Fig. 4. Sometimes only one side of the vine is affected.

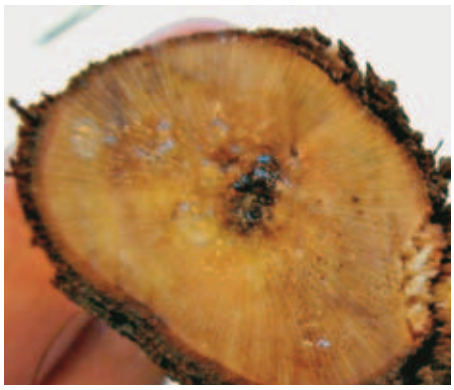

Fig. 5. The central pith is often darkened.

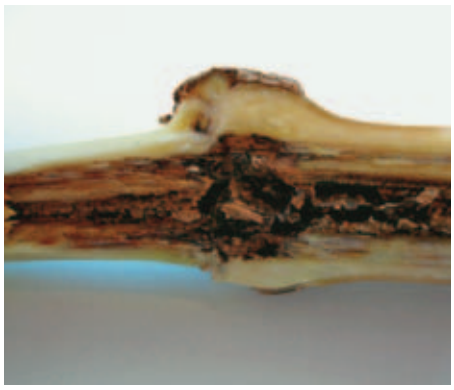

Fig. 6. The inside of the graft is blackened and necrotic.

Fig. 7. Bud necrosis.

the inside and this is visible on cross section. The central pith is often darkened, and the leaves may be reduced in size and may be wrinkled (see Figure 5). The following season, these canes will be dead. These symptoms often produce *B. obtusa, B. lutea* or *B. dothidea*.

Graft failures

The inside of the graft is stained dark brown or is blackened and necrotic. The dark staining seen inside the graft region may extend a short distance up or down the interior of the vine (see Figure 6). These symptoms may be associated with any of the *Botryosphaeria* species.

Bud necrosis

The buds may fail to burst and on sectioning may be seen to be brown and necrotic. This usually affects the middle areas of a laid-down cane or cordon (see Figure 7) and has been associated with *B. lutea*.

Excoriose

This is where the external bark and wood of the vine is bleached a light whitish-grey in colour and there are many fine dark spots visible. These are fungal spore bodies that can release fungi in rain or wet weather (see Figure 8). European researchers consider excoriose to be an unimportant "condition" rather than a serious problem, but we have our doubts. We often find excoriose in association with *B. lutea*.

Fig. 8. With excoriose fine dark spots are visible on the external bark and wood.

Leaf spots

Small necrotic spots form on the leaves and the surface of the leaf becomes wrinkled.

Here, there and everywhere

Most mycologists who work with *Botryosphaeria* understand the fungi to be spread by means of air-borne spores. Cankers and other structures that contain these spores form on the surfaces of wood and in cracks; the spores are typically released in response to rain and then dispersed by wind or rain splash.

The very wide host range of *Botryosphaeria* fungi means that there will always be abundant sources of inoculum. Every shelter belt, orchard tree and nearly all other trees must be considered as potential sources of infection: they are probably an integral part of the natural lifecycle of the fungi. Pruning cuts, training wounds and re-trellising cuts are probably prime sites for infection to enter the vines.

The near-ubiquitous distribution of *Botryosphaeria* fungi in nature will ensure that the chances of a vine escaping infection at some point during its life are extremely low.

So what initiates a disease condition?

If you cut a section in the trunk of an old vine – as we have done on many occasions – you will nearly always find abundant signs of fungal trunk disease. Most of the vines will probably have blackened or dark brown areas inside them, some will have soft-rots in the heart wood, and many interesting and

potentially pathogenic fungi, as well as a range of supposedly saprophytic fungi, will be revealed in culture.

But what do the symptoms really mean? Such vines are often cut down simply because a change in cultivar has been called for by the winery, not because of any apparent health problems. The vines themselves are often producing well and are apparently in good health.

Does disease only develop when the vine is under stress and the fungi turn opportunistic, causing stress-related diseases? Or do *Botryosphaeria* species require the presence of other fungi and cause a complex disease that way? We often find species of *Botryosphaeria* in conjunction with other fungi, such as *Cylindrocarpon destructans* and *Phaeomoniella chlamydosporum*, the fungi associated with the diseases known as "Black foot" and "Black goo" (Petri Vine Decline) respectively.

We are not suggesting that these different fungi must be present in order to cause disease. Rather, it appears likely that if increased numbers of different fungi are present, then there is a greater likelihood of disease developing.

The situation in New Zealand

Botryosphaeria fungi are commonly found in vines in all viticultural regions of New Zealand. They are often found on or in vines where no particular disease condition is evident. But *Botryosphaeria* does seem to play a role in a number of problems that have recently emerged in newer viticultural areas, especially around the Marlborough region. Some of these newer developments are also on suboptimal sites that have heavy soils (often poorly drained) – and that may place the young vines which are trying to establish themselves under undue stress. Development of suboptimal sites is a general trend in many countries: new plantings are made in attempts to extend the boundaries of already established and successful regions. Often, a range of diseases and other viticultural problems plague such developments.

Control

Without a detailed understanding of how *Botryosphaeria* diseases are spread – which we don't yet have – methods of control must necessarily focus on controlling the means of inoculation. Since air-borne fungal spores can potentially infect pruning wounds and other injuries on vines, it would make good sense to use pruning wound treatments on any open cuts.

Nursery contamination is obviously another potential source of infection, as has already been shown for *Phaomoniella chlamydosporum*. Good hygiene at the nursery level and correct sanitation of grafting wood and hydration water will minimise this risk. Nurseries should also consider treating propagation material if infection is seen on the wood surface (excoriose). Professor Doug Gubler, at the University of California, Davis, is trying an interesting approach and treating wood with lime-sulphur before propagation.

During the first couple of years of a vine's establishment, there is some evidence to show that foliar applications of phosphorous acid can stimulate an anti-fungal response. These preparations have been shown to sometimes be of benefit with specific fungal diseases such as Petri Vine Decline (caused by *Phaeomoniella chlamydosporum*). Trials on the use of phosphorous acid preparations against *Botryosphaeria* fungi have not yet been done, so such applications come with no guarantee of success. Furthermore, concerns have been raised in Australia about the possible presence of residual chemicals called "phosphonites" in wines made from vines treated with multiple applications of high-dose phosphorous acid preparations.

Mycorrhizae fungi are naturally occurring symbiotic fungi that inhabit the roots of many different plants and are known to assist in the uptake of nutrition. *Mycorrhizae* are considered to be a potentially useful treatment against fungal diseases, especially root-borne ones, probably by simply improving the overall health of the plants. While *Botryosphaeria* are not root-rotting or root-inhabiting fungi, using *Mycorrhizae* may help to combat disease by generally lowering stress. To the best of our knowledge there has been no work done on this possibility, but we would be keen to try it.

Where to from here?

New Zealand Winegrowers (the national industry body) has recently funded a research project to be run by researchers at Lincoln University, in conjunction with the New University of Lisbon in Portugal. The project is designed to research the epidemiology, control and identification of *Botryosphaeria* fungi in New Zealand vineyards, and we look forward to the results of their work over the next few years. ■