Grape tendrils as an inoculum source of *Botrytis cinerea* in vineyards – a review

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Abstract *Botrytis cinerea* is a fungus responsible for considerable damage to a wide range of crops worldwide, including grapes. Botrytis bunch rot caused by *B. cinerea* is the major disease problem that must be managed by the New Zealand wine industry each season. However, the fungus is not easily managed as it can be both necrotrophic and saprophytic, with a range of overwintering inoculum sources. New Zealand grape growers have asked whether it is necessary to remove tendrils at the time of pruning in order to minimise botrytis bunch rot infection at harvest. This review provides a summary of the information currently available on the importance of tendrils in the epidemiology of botrytis bunch rot under New Zealand conditions. Gaps in knowledge and areas for further investigation are also identified.

Keywords *Botrytis cinerea*, inoculum, tendrils.

INTRODUCTION

*Botrytis cinerea* is a fungus able to infect a wide range of plants and result in disease (Williamson et al. 2007). Both necrotrophic (killing living cells and colonising the dead tissue) and saprophytic (drawing nutrients from dead plant tissue) growth of *B. cinerea* are important in the life cycle of this fungus (van Kan 2006; Elmer & Michailides 2007). Information on the epidemiology of diseases caused by *B. cinerea* is widely available for a range of horticultural crops, including boysenberry (Walter et al. 1997; Walter et al. 2004), kiwifruit (Elmer et al. 1995), pear (Spotts & Cervantes 2001; Spotts & Serdani 2006), strawberry (Braun & Sutton 1986, 1987; Sutton et al. 1988; Sutton 1990; Boff 2001; Boff et al. 2001; Stromeng & Stensvand 2003; Stromeng et al. 2009), sweet cherry (Borve & Stensvand 2004; Borve et al. 2005) as well as grape (Kochenko 1974; Nair & Nadotchei 1987; Nair et al. 1988; Elmer et al. 1994; Nair et al. 1995; Seyb 2004; Elmer & Michailides 2007; Beresford et al. 2009).

It is estimated that, internationally, the financial impact of grape crop losses from *B. cinerea* is up to US$2B annually (Elmer & Michailides 2007). In New Zealand’s wetter regions, botrytis bunch rot costs the wine industry up to NZ$5000/ha in direct crop losses and an additional NZ$1500/ha in control costs (Hoksbergen 2010). Past research in Marlborough has shown that reductions
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in control costs can be achieved without increasing direct crop losses when knowledge of the epidemiology of the disease under local conditions is understood and applied (Agnew et al. 2004). Seasonal and regional differences in climatic conditions present particular problems in managing the disease under New Zealand conditions. In New Zealand’s cool maritime climate, weather-driven infection events can occur at any stage of the season, requiring management systems to be flexible. Researchers working with the industry have developed tools to provide information on how local weather events may increase local botrytis risk (Agnew 2006; Kim et al. 2010). Different grape canopies can also produce a range of microclimates that may be different from the conditions recorded by a wetness sensor on a central weather station (Henshall et al. 2005). The variable climate can produce wetness periods during different growth stages, and this can result in expression or non-expression of disease from similar starting conditions (Mundy & Beresford 2003; Mundy et al. 2005; Beresford et al. 2008; Beresford et al. 2009).

In order for B. cinerea infections to occur under favourable weather conditions, spores must be present. Dead vineyard tissues can host B. cinerea over the winter and become sources of inoculum during the growing season. Vineyard practice in Marlborough in 2010 was to remove tendrils from the retained canes as part of the pruning process, to reduce possible inoculum production. Removing the tendrils has been estimated to cost 10–15 cents per vine. However, no evidence is available to indicate that this action reduces disease risk. In fact, related work (Mundy et al. 2012) suggests that tendrils may not be a strong source of spores in Marlborough. Potential savings of NZ$2.5M to $5M p.a. for the national wine industry could be made by not removing tendrils during pruning.

Members of the wine industry have questioned the importance of tendrils as part of the epidemiology of botrytis bunch rot of grapes in New Zealand. In response, an experiment has been established to investigate the potential for spore production from tendrils in vineyards in Hawke’s Bay and Marlborough during the 2012 season (Mundy et al. 2012). In addition, this review of literature was undertaken to make relevant information from past research, including an unpublished thesis, more available to the wine industry.

OVERVIEW OF BOTRYTIS CINEREA LIFE CYCLE IN GRAPES

An understanding of the life cycle of B. cinerea in grapes can increase the ability to reduce disease expression in bunches at harvest. Botrytis cinerea is a successful pathogen of grapes because of its dual methods of growth, as both a necrotrophic invader (van Kan 2006) and a saprophytic coloniser of dead tissue (Yunis & Elad 1989). The fungal population of B. cinerea is maintained as a saprophyte throughout the year elsewhere in the vineyard. Saprophytic growth on vineyard residues can be a source of spores for infection during flowering or ripening. Because of the wide host range of the fungus, inoculum may also come from other sources (Williamson et al. 2007), such as dead weeds in the vineyard.

The life cycle of B. cinerea is not simple, as different tissues can become infected at different times during the year and provide pathways for later infection of berries and expression of botrytis bunch rot. The relative importance of infection of flowers and other vine parts, such as dead leaves and bunch trash, has been the subject of considerable study (Wolf et al. 1997; Balasubramaniam et al. 1998; Seyb et al. 2000; Keller et al. 2003; Viret et al. 2004). The susceptibility of berries to infection at ripening has also been studied under New Zealand conditions (Mundy & Beresford 2007; Mundy et al. 2008). Diseased berries at harvest may be the result of latent infections that occur during the early stages of berry growth (Pezet et al. 2003) or direct infections during ripening.

Management options

The polycyclic nature of B. cinerea often requires suppression of the build up of secondary inoculum to reduce the rate of disease progress (Araujo et al. 2005). Inoculum-focused management options
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for the control of botrytis bunch rot in grapes under New Zealand conditions involve minimising overwintering inoculum sources, flower infections, infection of other vine tissues and berry infections. Management options are illustrated in Table 1. An integrated management programme must strike a balance between risk of disease and the cost of management. Each additional management option that is applied increases the cost of production of the crop. In some seasons or regions, not all options will be needed to produce acceptable fruit for wine making.

One important step in the cultural control of botrytis bunch rot is to minimise the carryover of the pathogen from one season to the next (overwintering). This has traditionally been achieved by removing possible sources of inoculum during pruning, including canes, rachii and tendrils. The plant material is commonly mulched in the inter-row, and allowed to break down naturally. In some cases, under-vine sweepers are used before to increase the proportion of pruned material that is mulched. Some growers take the mulching one step further and compost the residue to ensure that the material does not provide a source of inoculum (Agnew et al. 2002).

The saprophytic phase of *B. cinerea* allows it to survive when living grape tissues are not available. It is able to produce large numbers of spores on that tissue, which can infect susceptible tissues when they are once more present. Following infection, *B. cinerea* becomes a necrotrophic pathogen, killing the tissue surrounding the infection to produce disease symptoms (van Kan 2006).

The relative importance of different tissues as reservoirs for inoculum production in vineyards is not fully understood, but has been the subject of some investigation (Seyb 2004). Trash trapped in the bunch after flowering has been shown to be one source of inoculum for infection of berries during ripening. A range of methods has been investigated to reduce the risk of infection from bunch trash (P.N. Wood, unpublished data). Changes in bunch architecture, induced both chemically (Mundy et al. 2011) and mechanically (Neal et al. 2010), have been found to reduce bunch trash, allow better spray penetration, and provide a bunch microclimate less conducive to infection.

Flowers, young berries and bunch trash are often protected from infection by *B. cinerea* by spraying at flowering time with chemical or biological compounds (Elmer et al. 2009).

Cultural methods of disease control such as canopy management have also been reported (English et al. 1989; Agnew et al. 2004) and reviewed (Elmer & Michailides 2007; Mundy 2008). Opening the grape canopy makes the microclimate less amenable to *B. cinerea*

<table>
<thead>
<tr>
<th>Method</th>
<th>Life cycle target</th>
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<tbody>
<tr>
<td>Remove rachii and infected canes at pruning</td>
<td>Overwintering</td>
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<tr>
<td>Remove tendrils at pruning</td>
<td>Overwintering</td>
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<td>Bunch trash removal</td>
<td>Berry infection</td>
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<td>Spray chemicals or biological control agents at flowering</td>
<td>Infection of flowers and other vine parts including latent berry infections</td>
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<tr>
<td>Spray chemicals or biological control agents pre-bunch closure</td>
<td>Latent infection of berries and bunch trash</td>
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<tr>
<td>Leaf pluck and shoot thin canopy to expose bunches</td>
<td>Berry infection</td>
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<tr>
<td>Reduce bunch congestion (includes bunch thinning)</td>
<td>Berry infection</td>
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<td>Berry infection</td>
</tr>
<tr>
<td>Vine nutrition (calcium)</td>
<td>Berry infection</td>
</tr>
<tr>
<td>Management of vineyard floor</td>
<td>Saprophytic infection all year</td>
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Infections, as increased air movement reduces the period of wetness following rain. Additionally, opening the canopy at the bunch zone increases spray deposition and coverage (Elmer et al. 2010). Improved fruit exposure increases berry exposure to sunlight, encouraging thicker berry cuticles and promoting greater resistance to *B. cinerea* and *Erysiphe necator* (powdery mildew; Percival et al. 1993). This reduces ‘diffuse powdery mildew’ infection of berries, which is an entry point for *B. cinerea* (Gubler et al. 1987), and reduces the viability of *B. cinerea* and other fungal pathogen spores without adversely affecting naturally occurring beneficial microorganisms (Percival et al. 1994).

**Integration of management practices**

The options that can be applied in any one season to minimise the risk of botrytis bunch rot in a cost-effective way are complex. In Marlborough, a move to targeted spray application and canopy management in the late 1990s was successful (Balasubramaniam 2000; Balasubramaniam et al. 2000; Agnew et al. 2003; Agnew et al. 2004). Since then, uptake of botrytis bunch rot management options in other New Zealand regions have been the focus of vineyard programmes such as GrapeFutures (Elmer et al. 2010). Information from monitoring and disease prediction models has been integrated into a decision support programme that is now available to the industry (Beresford et al. 2009; Elmer et al. 2010). This has also produced additional tools to increase growers’ technical knowledge, such as standardised sampling methods (Beresford et al. 2006) and a bunch rot assessment training (BRAT) programme (www.bunchrot.co.nz; Hill et al. 2010).

Growers need robust information on the risk profile of any new practice before implementing it, e.g. not removing tendrils at pruning, when removal is the current practice. In order to change current practice, a convincing argument needs to be made that leaving the tendrils on will not increase the risk of loss from botrytis bunch rot. Some information has been collected on the importance of tendrils as an inoculum source, but this information is incomplete and not readily accessible to growers. This review together with a field study of the inoculum production potential of tendrils in Marlborough and Hawke’s Bay (Mundy et al. 2012) will be used to produce a recommendation to growers on the need for removal of tendrils at pruning.

As crop residues are important potential inoculum sources, methods to reduce spore production have been studied. The use of mulches and composts in boysenberries (Walter et al. 2004) and grapes (Mundy & Agnew 2001, 2002; Jacometti 2007; Jacometti et al. 2007) has been investigated. This method also provides other potential benefits to the crop, such as nutrient cycling, and is an acceptable practice for organic growers. Competition with *B. cinerea* for space/nutrients by organisms specialised in decomposing dead tissue may be the mechanism of control here and has been studied as a possible means of biological control (Fowler et al. 1999).

**STUDIES ON TENDRILLS AND OTHER CANOPY SOURCES**

The Marlborough-based study conducted by Seyb (Seyb et al. 2000; Seyb 2004), a summary of which has been published (Jaspers et al. 2012), had four main components: a survey of vineyard ground debris, a survey of vineyards for rachii trash in canopies, sporulation potential of rachides over time and sporulation from rachides in the field. Seyb (2004) did not specifically investigate the potential of spore production from tendrils. Investigations by Balasubramaniam and colleagues in the 1996/1997 and 1997/1998 seasons, also in Marlborough, investigated sources of primary and secondary inoculum of *B. cinerea* that may contribute to disease epidemics (Balasubramaniam et al. 1997). These studies also included airborne spore counts at key growth stages during the season. Unpublished studies in Hawke’s Bay found leaf petioles overwintering on the vineyard floor were the primary sources of *B. cinerea* inoculum (P.N. Wood, unpublished data).

**A survey of vineyard ground debris**

Differences in trash between the under-canopy and inter-row were observed, with significantly
fewer rachides, petioles and less cane length trash detectable in the inter-row than the under canopy in mid-summer (Jaspers et al. 2012). In the under-canopy zone, petiole trash was the most abundant, followed by lengths of pruned cane and tendrils; rachides were the least abundant. However, rachides had the highest tissue-specific \textit{B. cinerea} sporulation ability (TSSA): 3.4 conidia per mm$^2$ and 4.01 conidia per mm$^2$ for under-canopy and inter-row, respectively. Hence rachii were deemed to be the largest potential contributors of spores, as they had both a high surface area and very high spore production per unit of surface area.

These findings are consistent with the observations of Balasubramanian et al. (1997), who rated the incidence of \textit{B. cinerea} sporulation development from overwintering sources from high to low: rachii > tendrils and petioles > canes. In the following season, Balasubramaniam et al. (1998) investigated the importance of sclerotial or mycelial sources of inoculum for each of the tissue types in Marlborough. Sclerotia were most abundant on petioles, but this type of overwintering structure accounted for less than 5% of the observed sporulation. Low numbers of sclerotia in dry climates have also been reported for other crop systems (Elad et al. 1992). Balasubramaniam et al. (1998) observed an average sporulation from mycelia across five vineyards of 73% in rachii, 42% in tendrils, 16% in petioles and 12% from canes.

A survey of vineyards for rachis trash in canopies

Seyb (2004) observed that even after pruning, where instructions had been given to remove rachii during the pruning process, these remnants could still be found attached to the canes that had been laid down. While vineyard to vineyard variation was observed, a mean of 0.2 rachides per vine was observed following pruning, with a TSSA per rachis of 7.5 x 10$^3$ conidia (Jaspers et al. 2012). Therefore, Seyb investigated how these rachii might be contributing to botrytis bunch rot within the vineyard. No other tissue types were measured in the canopy in that study.

Sporulation potential over time

The sporulation potential for rachides decreased over time for all three vineyards studied by Seyb, regardless of the placement of the rachides in the canopy or on ground within the vineyard (Jaspers et al. 2012). As the season progressed, sporulation potential decreased by at least 40% between capfall and pre-bunch closure for all sites and treatments (Jaspers et al. 2012). A significant relationship was observed between conidial production and measures of rachis degradation over time.

Balasubramaniam et al. (1997, 1998) did not measure rachii in the canopy, but they did investigate potential sporulation from bunch trash and airborne spore numbers during the season. Sporulation potential incidence (the number of pieces that sporulated when incubated) for bunch trash ranged from 5 to 40% at pre-bunch closure, but had reduced to less than 5% at all five sites by véraison (Balasubramaniam et al. 1998). However, other potential sources were observed at véraison, with 50–100% of leaf trash harbouring \textit{B. cinerea} that was able to sporulate on incubation (Balasubramaniam et al. 1997). In both the 1997 and 1998 seasons, the highest numbers of spores were trapped at véraison, but the source of those spores was not determined. The total number of spores trapped also varied between seasons. No relationship between spore trapping and final disease was observed.

Sporulation from rachides and other tissues in the field

Spore production in the field decreased over time, with no observed sporulation in March or April (Jaspers et al. 2012). Vineyard spore trapping by Balasubramaniam et al. (1997, 1998) indicated that spore numbers detected in the vineyard were considerably lower than potential numbers measured in the laboratory.

The higher numbers of spores produced early in the season from rachii (and other tissues) may still be important for disease epidemics when a high incidence of latent infection occurs. Balasubramaniam et al. (1998) investigated surface-sterilised and non-sterilised berries at
véraison and detected very little latent infection in that season. Subsequent investigations have found it hard to relate latent infections to observations of disease severity in the field (Obanor et al. 2004). However, other studies of latent flower infections have been shown to be related to high disease severity at harvest (Keller et al. 2003). Hence many growers still apply a botryticide at flowering to protect against latent infections (Agnew et al. 2004). The polycyclic nature of B. cinerea and seasonal differences in the timing of conditions conducive to growth and reproduction of the fungus make it hard to determine when latent infections may have most influence on disease incidence at harvest.

FURTHER QUESTIONS TO ANSWER
While Seyb (2004) studied rachii sporulation potential over time in the canopy, this research did not investigate tendrils under the same conditions. Although in Marlborough tendrils produced fewer conidia per mm$^2$ in both the understory and inter-row than rachii, the relative surface area of this trash type within Seyb’s quadrant studies indicated that tendrils are potentially significant inoculum sources. This culminated in an investigation in 2011 of the importance of tendrils as an inoculum source of B. cinerea under New Zealand conditions (Mundy et al. 2012). The 2011/2012 season study investigated tendril spore production potential on a surface area basis from a number of Marlborough and Hawke’s Bay vineyards at pre-flowering and bunch closure.

The 2011 investigation was also designed to help to answer the question, “Does a high incidence of bunch rot indicate that there may also be a high incidence of tendril colonisation and therefore a greater potential for carry-over of the pathogen? The importance of carry-over inoculum has been studied in New South Wales for grapevines, with an increase in flowering infections where there was a high carry-over, but not always high harvest disease incidence in the second season (Nair et al. 1995).

The relative importance of tendrils as sources of B. cinerea inoculum in New Zealand vineyards has not been fully investigated, and therefore the following hypotheses should be tested:

1. Botrytis bunch rot severity at harvest is correlated to the B. cinerea spore production potential of tendrils in that block the following season
2. Spore production potential of tendrils reduces during the season in the same way that it does in rachii
3. Regional differences exist between the spore production potential of tendrils.

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