Tendrils as a source of seasonal carryover of *Botrytis cinerea* in vineyards

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Abstract Botrytis bunch rot is a disease that requires management under New Zealand conditions in order to prevent financial losses for wine grape producers. A survey was conducted to investigate the potential spore production of tendrils in 16 vineyards from the Marlborough and Hawke's Bay wine growing regions. Significant differences in spore production potential of tendrils were detected between sampling before flowering and after fruit set. Significant regional differences were also detected, with tendrils from Marlborough producing fewer spores. Under ideal laboratory conditions for incubation of the fungus, spore production by tendrils was low at the start of the season and was reduced further following application of the industry standard fungicide programme. Based on these experiments and a review of other data it is recommended that tendril removal is not required at pruning as part of vineyard disease management.

Keywords tendrils, botrytis bunch rot, vine management, overwintering, *Botrytis cinerea*.

INTRODUCTION

Botrytis bunch rot (BBR) is a major disease of grapes internationally resulting in losses of both table and wine production grapes (Elmer & Michailides 2007). Economic costs of BBR to the New Zealand wine industry include possible direct crop losses (\$5000/ha) and control costs (at least \$1500/ha) (Hoksbergen 2010). One management action used for control of BBR has been to minimise sources of overwintering *Botrytis cinerea* in the vineyard by removing or mulching material that may harbour the fungus. These sources include infected canes,

rachides, leaf petioles and tendrils. However, as each management action has a cost, questions have been asked about the importance of these various inoculum sources on the carryover of *B. cinerea* between seasons.

Pruning as a control measure is used to remove tissue that has become infected with *B. cinerea* during the season and also prevents the fungus from surviving as a saprophyte on the dead tissue. Saprophytic growth by *B. cinerea* allows the fungus to grow when susceptible grape tissue is not available, and produces large numbers

of spores to infect susceptible tissue when it is available. Following successful infection, *B. cinerea* becomes a necrotrophic pathogen, killing the tissue surrounding the infection to produce disease symptoms (van Kan 2006). Reducing the plant material in the vineyard on which the fungus can grow as a saprophyte is therefore a key step in the cultural control of botrytis bunch rot. The relative importance of different tissues as reservoirs for inoculum production in vineyards is not fully understood but has been the subject of investigation (Seyb 2004). The investigation by Seyb (2004) and other related work are reviewed in Mundy et al. (2012).

Mulching of plant material is commonly done in the inter-row, where the material breaks down during the next season. In some cases, under-vine sweepers are used before mulching mowers are run over the inter-row to increase the percentage of pruned material mulched. Some growers have taken the mulching one step further, by composting the pruned tissue to ensure that the material does not provide a reservoir of inoculum (Agnew et al. 2002).

This study gathered evidence for the importance of tendrils as a source of overwintering *B. cinerea* by examining their ability to produce conidia during the ensuing summer period.

METHOD

Grapevine tendrils from commercial vineyards in two grape-growing regions of New Zealand, Marlborough and Hawke's Bay, were collected in early spring and maintained at one vineyard in each respective region until sampled for *B. cinerea* conidial production at the flowering and bunch closure growth stages. The majority of tendrils were collected from Sauvignon blanc vines but in Hawke's Bay, Cabernet Sauvignon, Merlot and Chardonnay tendrils were also collected (see Figure 1).

Tendril preparation and placement

In Marlborough, six replicate tendril samples were sourced from nine vineyards for each of the sample times. Seven replicate tendril samples were sourced from seven vineyards in Hawke's

Bay for each sample time. All samples were collected by the vineyard managers.

Tendrils from each individual vineyard were stapled onto colour coded flagging tape in lots of ten, with the replication necessary for each region. In mid September 2011, these tapes were placed back out into the field on one generic vineyard in each region to mimic normal weathering before sampling. Samples were collected pre-flowering on 28 and 30 November 2011 in Hawke's Bay and Marlborough, respectively. The second samples were collected on 16 and 25 January 2012 in Hawke's Bay and Marlborough respectively, following pre bunch closure botryticidal applications. The tapes were analysed at Plant & Food Research, Marlborough. In Hawke's Bay the tendrils received Pyrus® 400SC, Gem® fungicide and Diva™ sprays for BBR, and in Marlborough Shirlan®, Scala® and Switch®, between sampling times.

Surface area

All tendrils were photographed with a measurement scale included and the images were processed to determine the tendril surface area. Grape tendrils were photographed on a white background using a Nikon D700 digital camera. Tendril images were uploaded into Adobe Photoshop and processed into a two-shade image: black tendrils on a white background. The scale was uploaded into the Adobe Photoshop 'Analysis' option. On command, Adobe Photoshop calculated the area (cm²) of both the white and contrasting black regions. It is important that all images are created using the same scale. Before the start of the experiment, a subsample of tendrils was processed using both this method and the calliper calculation method used by Seyb (2004). Results for the two methods were found to be equivalent and so the photographic method was used for this experiment.

Tendril analysis

Each of the strips was incubated in a sterile plastic container lined with paper towels, with 10 ml of water added. They were left on the bench with the air conditioning set at 20°C to

induce sporulation of existing infections. After 7 days, the tendrils were prepared for spore counts.

All the tendrils in each replicate were removed from the tape and placed into a plastic bag. Twenty ml of sterile distilled water with a small amount of Tween® 80, and 2 ml of 0.05 g/litre Trypan blue in a 1:1:2 solution of lactic acid, distilled water and glycerol dye was then added (Mundy & Agnew 2004). The bags were gently shaken to remove the *B. cinerea* conidia and conidiophores from the tendrils. The bags were left for 10 min to allow the conidia to dehisce from the conidiophores. Conidia were counted using an Improved Neubauer Hemocytometer (any conidiophores that had failed to dehisce were not counted).

The number of conidia in each of the replicates was then calculated, to give a value for potential spores per millilitre of solution from a known surface area, so that the total spores per unit of surface area could be calculated. This calculation has been called the tissue-specific sporulation ability (TSSA) in other publications (Jaspers et al. 2012; Seyb 2004).

Statistical analysis

Analysis of differences between regions, vineyards within regions, and sampling times was done using a linear mixed model using GenStat 14th edition software (VSN International Ltd). Time was treated as a 2-level factor within the linear mixed model. The data values were log-transformed before analysis to equalise the variances and then back-transformed for the presentation of results.

RESULTS

The mean spore density differed significantly between the two regions ($P < 0.001$) and sampling times ($P < 0.001$) (Table 1). Marlborough had lower mean spore densities than Hawke's Bay, and the spore density decreased from pre-flowering to bunch closure (Table 1).

The mean spore density differed significantly between the vineyards within regions ($P < 0.001$) and there was a significant time $\times$ vineyard interaction ($P < 0.001$). The pattern of differences between the vineyards at pre-flowering differed from that at the post-bunch closure sample time (Table 1 and Figure 1).

Figure 1 Mean density of spores (number/cm²) on tendrils following incubation, from 16 vineyards in Hawke's Bay (filled bars) and Marlborough (unfilled bars) at two sampling times (pre-flowering and post-bunch closure). Error bars are 95% confidence intervals for the mean.

Table 1 Mean density of spores (number/cm²) on grape tendrils collected in two regions and at two sampling times in 2011/12.

Region	Time	Mean	95% C.I.
Hawke's Bay	Pre-flowering	3904	(2866, 5318)
	Bunch closure	967	(710, 1317)
Marlborough	Pre-flowering	2181	(1693, 2809)
	Bunch closure	294	(229, 379)

DISCUSSION

The results of this study are consistent with the findings of Seyb (2004) for rachides in the canopy of a range of Marlborough vineyards. In that study, vineyard to vineyard variation was observed, but a mean of 0.2 rachides per vine was measured following pruning, with a tissue-specific *Botrytis* sporulation ability (TSSA) per rachis of 7.5×10^3 conidia (Jaspers et al. 2012). This level was at the upper end of results from the present study at Marlborough sites pre-flowering. Seyb (2004) did not investigate other tissue types in the canopy although the research did look at a range of tissues on the vineyard floor where spore production by *B. cinerea* on rachides was higher than on tendrils.

The tendril sporulation potential in the present study decreased significantly with time in all sampling sites in both regions. This is also consistent with the findings of Seyb (2004), who reported sporulation potential for rachides decreased over time for all three vineyards studied, regardless of the placement of the rachides in the canopy or on ground within the vineyard (Jaspers et al. 2012). Seyb (2004) reported a significant relationship between conidial production and measures of rachis degradation over time, where the sporulation potential decreased by at least 40% between capfall and pre-bunch closure for all sites and treatments (Jaspers et al. 2012).

However, even with the decrease in spore production potential over time and lower total numbers of spores per area of tendrils compared with rachis, many growers may still ask the question, "Should I cut off tendrils when pruning?" A better question to ask would be, "How important are early-season spores for final botrytis bunch rot observed in the vineyard?" Latent flower infections have been shown to be related to

high disease severity at harvest (Keller et al. 2003). Hence many growers apply a botryticide spray at flowering to protect against latent infections (Agnew et al. 2004). However, the importance of early-season latent infections would need to be considered before rejecting tendril removal.

The wine industry has a wide range of nil-residue botryticide control options for the protection of tissues from infection during the flowering and early berry development stages. Since the greatest potential production of spores by tendrils is during this period when susceptible tissues are already protected, tendril removal may not be economically viable. Reduction in spore production over time on the tendrils may have been a result of the fungicides applied between sampling times acting on the *B. cinerea* present inside the tissue. Vineyards in both regions had multiple active chemicals applied over the time between samples.

The present experiments indicate that even under ideal laboratory conditions for incubation of the fungus, spore production on tendrils was low at the start of the season and reduced further following application of standard fungicide programmes. Therefore it is recommended that growers do not need to remove tendrils at pruning for *B. cinerea* control in vineyards.

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