***Botrytis cinerea* control on Sauvignon blanc using a recycling sprayer**

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Abstract The New Zealand wine industry is strongly committed to sustainable production. The SprayPro R-series[®] sprayer is designed to recycle unused chemical sprays not deposited on the target area. Sauvignon blanc vines in Marlborough, New Zealand, were studied during 2011/2012. The vineyard area was 5 ha at 80% flowering, where the full canopy was sprayed, and 10 ha at pre bunch closure (PBC) targeting the fruiting zone only. Disease control efficacy of spray recycling was investigated using *Botrytis cinerea* as the model pathogen. Pathogen colonisation of necrotic leaf discs placed in the canopy at 80% flowering and PBC showed even control of *B. cinerea*. At PBC, 80% of the water-sensitive papers had adequate to excellent coverage. Botrytis bunch rot incidence and severity were evenly expressed throughout the block but increased during pre-harvest; severity was well below the 3% economic threshold. Recycling of botryticides did not affect disease control.

Keywords SprayPro R-series[®] sprayer, *Botrytis cinerea*, 80% flowering, pre bunch closure.

INTRODUCTION

The application of chemicals to leaves and bunches of grapes is vital to achieve disease control. Modern grape canopies in New Zealand are grown in hedged rows with a range of canopy densities between sites, which change during a season at any one site (Green et al. 2008); spraying technology, therefore, needs to be able to provide coverage under all these conditions. Any technology that delivers good coverage of the target surface must also deposit sufficient active ingredient to the target tissue to control the disease effectively across the whole sprayed area.

The SprayPro R-series[®] sprayer from FMR Group (henceforward 'R-series' sprayer) is a shrouded recovery and recycling sprayer that has been designed and built in New Zealand for use in viticulture throughout the entire growing season. The 'R-series' vineyard sprayer has been designed to meet the increasing needs of the viticulture industry throughout New Zealand, Australia and overseas to address issues of excessive spray drift and the adverse economic and environmental effects of the off-target chemical losses experienced with traditional vine

spraying equipment. The 'R-series' vine sprayer uses a unique (patent pending) application system using a 'revolving vortex' of droplet-laden air produced by off-set tangential fans operating in a vertical mounting within a pair of protective shrouds that guide and contain the vortex whilst facilitating the recovery and collection of 'off-target' drift. A custom filtration system ensures that the recovered liquid is free of material contamination before it is returned to the main tank for re-application.

As the 'R-series' is a new system, questions have been raised as to how effective it would be in controlling disease, in particular *Botrytis cinerea*, when the spray mixture had been sprayed and recollected, potentially several times over. This study evaluated whether there was a change in disease control during the course of the spray run, i.e. whether disease control was more effective at the beginning (before any spray recycling) than towards the end of the tank mixture, when the spray has been recycled more often.

MATERIALS AND METHODS

A vineyard block of 12.2 ha, planted in 2006, in the Lower Wairau area of the Wairau Plains, Marlborough, New Zealand, was selected to conduct the field trial. The vines were Mass

Selected Sauvignon blanc on 3309 rootstock and were planted 1.8 m between vines and 3 m between rows. The sprayer was set up to operate at a pressure of 7 bar, with three nozzles directed at the bunch zone. The tractor travelled at a speed of 7.5 km/h.

Table 1 lists the products applied during the season by the 'R-series' sprayer. For the purpose of the experiment, the spray run always started at the same row (row 1) and followed the same direction and row sequence. The first spray application was on 22 December 2011 at 80% flowering targeting the whole canopy. One tank covered 20 rows or 5 ha. On 16 January 2012, a pre bunch closure (PBC) application was made, targeting the fruiting zone only and covered 40 rows or 10 ha.

Leaf disc bioassay

In order to test the effectiveness of the botryticide applied at 80% flowering and pre bunch closure, irradiated 20 mm diameter grape leaf discs were stapled to flagging tape (5 discs/tape) and were either inoculated pre-spray (1 day before application), inoculated post spray (1 day after application), or not inoculated at each application, with mixed wild type *B. cinerea* inoculum at 10^5 spore/ml, using an atomiser

Table 1: Fungicides applied at 80% flowering and pre bunch closure.

Timing	Product	Target organism	Active ingredient	Rate (g ai/ha)
80% Flowering ¹	Switch®, Syngenta	Botrytis	375 g/kg cyprodinil	300
			250 g/kg fludioxonil	200
80% Flowering	Prodigy™, Dow AgroSciences (NZ)	Insect growth regulator	240 g/litre methoxyfenozide	60
80% Flowering	Systhane™ 200EW, Dow AgroSciences (NZ)	Powdery mildew	200 g/litre myclobutanil	25
Pre bunch closure ²	Switch®, Syngenta	Botrytis	375 g/kg cyprodinil	300
			250 g/kg fludioxonil	200
Pre bunch closure	Quintec™, Dow AgroSciences (NZ)	Powdery mildew	250 g/litre quinoxyfen	50
Pre bunch closure	Du-Wett™, Elliott Technologies	Spreading of fungicides	500 g/litre trisiloxaneethoxylate	200

¹Water rate at flowering was 400 litres/ha.

²Water rate at pre bunch closure was 200 litres/ha.

(Treatments 1, 3 and 6; Table 2). *Botrytis cinerea* was isolated from grape tissues, grown on potato dextrose agar as pure cultures and identified by morphology. Spores were harvested from pure culture plates using 5 ml sterile distilled water with Tween (4 drops/litre) and a glass hockey stick. The suspension was filtered through lens paper before counting and adjusting to the correct spore suspension. The spore suspensions were allowed to dry before further treatments were applied. To determine the effectiveness of the botryticide for the chemical mix over each spray cycle, the leaf discs were applied along the bay in six rows across the sprayed area. Three leaf-disc ribbons per treatment (pre, post and nil *B. cinerea* inoculation) were placed in the fruiting zone of the selected bay. The nine ribbons were randomly placed within a bay.

A comparison of *B. cinerea*-inoculated leaf treatments was applied in an adjacent block where no spray had been applied, in order to determine the amount of naturally occurring *B. cinerea* in the vineyard.

The ribbons were placed in the vineyard the day before the fungicide application and retrieved within a few hours after the spray, when the canopy and leaf discs were dry. The collected ribbons were then incubated in trays lined with damp paper towels for 7 days at 20°C. Each leaf disc on each ribbon was then assessed visually for the percentage area covered by sporulating *B. cinerea*.

Spray coverage

Water-sensitive papers (WSP) were folded in two and one was placed in each bay. They were placed in the canopy at the time of spraying to determine spray coverage in the fruit zone. They were inserted at the front and the back of the fruit area. The WSPs were removed as soon as they were dry and

placed in a zip-lock bag. Each half was scored by determining the overall coverage and effectiveness of the spray on the paper by judging them as Excellent, Adequate or Inadequate coverage, as described by Manktelow (2010).

Field disease observations

Four assessments of botrytis bunch rot incidence and severity in the fruit were carried out from mid-March 2012 until mid-April 2012, just before harvest. Using the area covered by the pre bunch closure spray (40 rows), eight rows with either three or five bays in each row were selected to range from the beginning of the spray run to the end of the run. Fifteen randomly chosen bunches on each side of the vine in a bay were visually scored, consistent with the methods described by Beresford et al. (2006).

Statistical analysis

The results from individual grape leaf discs were averaged for each flagging tape (of five discs) and these averages were analysed using ANOVA to compare the effects of inoculation treatment, spraying, and field row number of the sprayed rows on the mean percentage of the leaf discs covered by *B. cinerea*. The results for incidence and severity of botrytis bunch rot obtained from field assessments of individual bunches were averaged for each bay (of 30 bunches) on each sample date. Then, the effect of field row number on the botrytis bunch rot incidence and severity was analysed using one-way ANOVA on these bay averages for each sample date separately. Following ANOVA, comparisons among means were made using 5% Fisher's Least Significant Difference (LSD). Prior to analysis, all percentages were logit-transformed except for those percentages equal to zero, which were transformed using an empirical logit transformation ($y = \log((p+e)(100-p+e))$), where

Table 2 Treatments applied to grapevine leaf discs.

	<i>Botrytis cinerea</i> Pre-spray inoculated leaf disc	<i>B. cinerea</i> Post-spray inoculated leaf disc	No <i>B. cinerea</i>
Botryticide spray	Treatment 1	Treatment 3	Treatment 6
No spray	Treatment 2	Treatment 4	Treatment 5

p =percentage, and e =half of the smallest non-zero percentage). All results were back-transformed for presentation.

RESULTS

Water-sensitive papers

When the WSPs were visually assessed for coverage, 23.3% were Excellent, 56.7% were Adequate and 20% were Inadequate.

Botrytis cinerea on leaf discs

At the 80% flowering stage, the percentage area of leaf discs covered with *B. cinerea* varied significantly with the *B. cinerea* inoculation timing ($P<0.001$) and spraying treatments (sprayed versus unsprayed) ($P<0.001$) (Table 3). The pre-inoculated discs had significantly greater *B. cinerea* sporulation coverage than the post-inoculated discs, which in turn had greater mean coverage than the uninoculated discs.

The discs that received the botryticide spray had significantly lower coverage than the unsprayed discs. Although there was no overall field row effect ($P=0.26$), there was a significant field row $\times$ inoculation interaction ($P=0.018$) (Figure 1). While there were no significant differences in *B. cinerea* sporulation coverage among the uninoculated rows, a couple of the post-spray inoculation rows differed from each other, as did several of the pre-spray inoculation field rows.

For botryticides applied at the pre bunch closure stage, the percentage area of leaf discs covered with *B. cinerea* sporulation varied significantly with both *B. cinerea* inoculation timing ($P<0.001$) and botryticide ($P<0.001$) treatment (Table 4). The discs that received the botryticide spray had

Figure 1 Mean percentage area showing sporulation with *Botrytis cinerea* on grape leaf discs after incubation for 7 days at 20°C. Leaf discs were placed among vines at 80% flowering and were inoculated with *B. cinerea* either before or after fungicide spraying with the 'R-series®' or not inoculated. Error bars are 95% confidence intervals for the mean. Means within a graph with the same letter are not significantly different (LSD $P<0.05$). One spray tank covered 20 rows or 5 ha.

Table 3 Back-transformed mean and 95% confidence interval for the percentage of grape leaf disc area covered with botrytis at the 80% flowering stage.

Inoculation	No botryticide spray		Botryticide spray		All	
	Mean	95% C.I.	Mean	95% C.I.	Mean	95% C.I.
Pre-botryticide spray	35.0	(21.7, 51.1)	7.2	(3.8, 13.1)	17.0	(11.3, 24.6)
Post-botryticide spray	25.1	(14.7, 39.4)	2.1	(1.1, 4.0)	7.8	(5.0, 11.9)
None	0.8	(0.4, 1.5)	0.2	(0.1, 0.4)	0.4	(0.3, 0.6)
All	10.1	(7.1, 14.2)	1.5	(1.0, 2.1)		

Table 4 Back-transformed mean and 95% confidence interval for the percentage of grape leaf disc area covered with *Botrytis cinerea* at the pre bunch closure stage.

Inoculation	No botryticide spray		Botryticide spray		All	
	Mean	95% C.I.	Mean	95% C.I.	Mean	95% C.I.
Pre-botryticide spray	21.0	(10.5, 37.5)	0.5	(0.2, 1.1)	3.5	(2.0, 6.0)
Post-botryticide spray	10.5	(4.9, 21.0)	0.6	(0.3, 1.3)	2.6	(1.5, 4.5)
None	0.4	(0.2, 0.8)	0.2	(0.1, 0.4)	0.3	(0.1, 0.5)
All	4.6	(2.9, 7.1)	0.38	(0.2, 0.6)		

significantly lower *B. cinerea* coverage than the unsprayed discs. The uninoculated discs had low *B. cinerea* coverage in both the sprayed and unsprayed areas of the vineyard, and were not significantly different in the *B. cinerea* sporulation observed. In contrast to the results at 80% flowering, at pre bunch closure there was no significant effect of field row ($P=0.97$) and there was no inoculation $\times$ row interaction ($P=0.40$) (Figure 2).

Field disease observations

The incidence of botrytis bunch rot increased during the field assessment period from a mean

of 2.1% on the first date to 28% on the final date (Figure 3). The mean percentage incidence did not differ significantly between the vine rows on the first two sample dates (14 March, $P=0.99$; 21 March, $P=0.50$). There was a significant difference on the third assessment date (5 April, $P=0.037$) but not on the final date (14 April, $P=0.81$).

The severity of botrytis bunch rot was generally very low throughout the assessment period, with a mean of 0.8% on the final assessment date. The mean percentage severity did not differ significantly between the vine rows on the sampling dates (14 March, $P=0.93$; 21 March, $P=0.46$; 5 April, $P=0.054$; 14 April,

Figure 2 Mean percentage area showing sporulation with *Botrytis cinerea* on grape leaf discs after incubation for 7 days at 20°C. Leaf discs were placed among vines at pre bunch closure and were inoculated with *B. cinerea* either before or after fungicide spraying with the 'R-series' or not inoculated. Error bars are 95% confidence intervals for the mean. One spray tank covered 40 rows or 10 ha.

Figure 3 Botrytis bunch rot field incidence (%) in different rows of vines on four sample dates. Data are back-transformed mean percent incidence of botrytis from 30 bunches assessed in each of three bays in each row. Error bars are 95% confidence intervals for the mean.

$P=0.32$) with mean severities of 0.02, 0.04, 0.254 and 0.81%, respectively.

DISCUSSION

The water-sensitive paper results concur with the findings of Manktelow (2010); 80% of the water-sensitive papers were found to have adequate to excellent coverage. This showed that operating pressure, tractor speed and nozzle orientation gave good spray deposition at the bunch line.

When botryticides were applied as a protectant (determined when *B. cinerea* inoculum was applied to the leaf discs after the botryticide spray), *B. cinerea* colonisation and sporulation were low, indicating that the botryticide had good efficacy and there had been no change in the efficacy of the recycled spray mix across the block. The amount of sporulation on leaf discs that had been inoculated the day before the botryticide spray was also reduced but the results were more variable than with the post-

spray inoculated discs. The amount of *B. cinerea* sporulation on non-inoculated and non-sprayed leaf discs was low at 80% flowering and pre bunch closure, indicating that natural field inoculum was low at the time of field exposure.

At 80% flowering, the leaf discs inoculated with *B. cinerea*, either pre- or post-botryticide spray, showed some differences in sporulation among the field rows, albeit in different patterns (Figure 1). These differences were not observed at pre bunch closure application. This would indicate that there was not a systematic treatment effect across the block and therefore no pattern of change in efficacy of the recycled spray mix was produced. It is considered that the differences were the result of the methodology and that there was possibly a canopy effect in which denser parts of the canopy prevented the spray from reaching the target discs.

At pre bunch closure there was no interaction between the row and disc inoculation, which

demonstrates that the spray mix being applied by the 'R-series' sprayer was equally effective across the treated area (Figure 2). Overall, the results of *B. cinerea* leaf disc sporulation for the 80% flowering and the pre bunch closure applications were similar and it is concluded that the botryticide efficiency of the spray mix was therefore not reduced because of the recycling.

Field disease

Figure 3 shows that, with time, the incidence of botrytis bunch rot increased. There was rain on 21 and 22 March 2012 of 0.8 mm and 24.4 mm, respectively. This probably contributed to the rise in incidence recorded on 5 April 2012. There was also rain of 21.6 mm on 11 and 12 April 2012 and this will have contributed to the increased incidence on 14 April 2012. Although disease incidence increased during the season, botrytis bunch severity was generally below 1%. The severity scores remained well below the 3% commercial threshold at harvest.

The data collected on 5 April 2012 suggest there were some hot spots of disease that were identified by the intensive sampling that took place. The variation between sampling dates is a result of randomly choosing bunches; therefore at each assessment different bunches were scored. In order to limit variation, the same people scored the bunches each time and on the same side of the vine.

This study has shown that there is no deterioration of the efficacy of the spray mix as a result of recycling. A further advantage of the sprayer is that at 80% flowering it was able to cover up to 6 ha with a single tank (2000 litres), compared with 5 ha for a conventional air-shear sprayer unit. At pre bunch closure, 11.5 to 12 ha were covered by the 'R-series' treated compared with 10 ha by conventional sprayers. These are time and cost benefits associated with the 'R-series', which will be examined in another study.

The botryticides applied at 80% flowering and at pre bunch closure are the most critical spray times for controlling botrytis bunch rot (Wicks & Hall 2005). From the data collected, botryticides applied by the SprayPro R-series

from FMR Group sprayer at these two times effectively controlled *B. cinerea* growth across the block. Recycling of the spray solution did not reduce botryticide efficacy on necrotic leaf discs, in field assessments. Whilst the incidence of botrytis bunch rot increased with time, the severity remained below the maximum threshold (3%) stipulated by many wine companies in New Zealand. The recycling of the spray solution also increased the area covered by a conventional air-shear sprayer.

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