Botrytis fungicide evaluation trial

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Because of above-average rainfall in 2011–12, Botrytis infection of mature grapes resulted in them not being harvested in vintages 2011 and 2012. This placed financial strain on producers and caused losses of premium wine.

Rainfall from veraison to harvest is the factor most likely to cause losses from Botrytis infection. Moreover, rain during flowering can cause latent Botrytis to show up as the grapes approach maturity. The incidence of Botrytis is influenced by site selection, canopy management, crop load, vineyard floor management and whether protective spray programs are used.

To reduce the incidence of Botrytis, the NSW Wine Industry Association R&D Committee nominated trials and extension as a number 1 priority. Funding from the GWRDC Innovative Committee has been granted to implement these for the 2013 and 2014 seasons.

Since 2011, a number of Hunter Valley growers have adopted the use of products containing the fungus *Trichoderma* for Botrytis control. This natural predator of Botrytis is now available in sprays for Botrytis suppression and can be used as an alternative to conventional synthetic chemicals registered for Botrytis control. Anecdotally, these growers have had encouraging results using *Trichoderma* sprays in accordance with the manufacturer's recommended protocols rates and timing.

The *Trichoderma* that is present within the grape bunches consumes Botrytis, but it is annihilated by other Botrytis fungicides. No doubt this partly explains why organic vineyards where the *Trichoderma* fungi are not endangered often have lower rates of Botrytis infection, even when the prevailing conditions favour its occurrence.

With the help of Dr Greg Dunn and Dr Melanie Weckert at NWGIC Wagga Wagga, a Botrytis Fungicide Trial was designed to compare an organic botrytis spray program with a conventional Botrytis spray program.

Design

The treatments (30 vines per treatment) were:

T1: Conventional Botrytis spray control

T2: *Trichoderma* products (Colonizer® and Antagonizer®)

T3: Potassium salts of fatty acids (i.e. soap salts; Ecoprotector®)

T4: Control (no Botrytis fungicides).

The experiment was conducted in a 4.35-ha, 12-year-old block of Chardonnay at Glenesk Vineyard at Denman in the Upper Hunter Valley of NSW.

Application

For each treatment we used a separate dedicated hand-spraying knapsack. The conventional spray treatment was applied by knapsack at the same time as it was applied to the rest of the Chardonnay in the block.

Colonizer® (*Trichoderma koningii*) was applied at >20% capfall (on 28 September 2012), with a second spray at >80% capfall on 6 October. Care was taken to ensure that fungicides for powdery mildew and downy mildew were not applied to the whole block within 7 days either side of applying the *Trichoderma* sprays.

As a continuation of the *Trichoderma* treatment, Antagonizer® (*Trichoderma harzianum*) was applied after veraison on 28 December, and again after rain on 24 January in the New Year (2013).

Ecoprotector® was applied at <5% capfall, at pre-bunch closure, and after veraison on 28 December 2012 and on 24 January 2013.

The block was picked on 7 February 2113 at >12.8 Baumé or 23° Brix.

Measurements

Method of monitoring for latent Botrytis infection

The treated plots were monitored for Botrytis infection of the leaves before flowering: the presence of infection in the leaves could increase the spore load, especially if spores became trapped within a bunch.

We assessed the four middle vines of the middle row of each treatment (i.e. row 2, vines 4, 5, 6, 7 of 10 treated vines).

The Chardonnay trial site, Glenesk Vineyard at Denman in the Upper Hunter Valley of NSW *Photo: Richard Hilder*

One week after the 80% capfall spray, four inflorescences per vine from each treatment were randomly selected, secured in four separate ziplock plastic bags, and despatched to Dr Weckert for microscopy.

At inspection of the trial on 25 January 2013 there was no visible evidence of Botrytis infection in any of the treatment plots.

On 4 February, 3 days before harvest and after 117 mm of rain had fallen in the previous 3 days, the vines were inspected again for Botrytis rot.

A day later, three bunches from each treatment plot were randomly selected, secured in separate ziplock plastic bags, and then placed on a bench in the vineyard's recreation room for later observation of Botrytis development.

Bunches were collected only from treatments T2, T3 and T4 at harvest; they were sent to Dr Weckert for analysis.

Laboratory results

Infection on inflorescences

Four inflorescences (immature bunches) were collected from each of six replicates of every treatment 1 week after 80% capfall and sent to Melanie Weckert for fungal analysis.

The inflorescences were frozen for 2 weeks to disrupt the plant cells. The frozen inflorescences were thawed, placed in ziplock plastic bags and incubated for 2 weeks at room temperature.

The inflorescences were then weighed, placed in phosphate-buffered saline (pH 7), and shaken well to dislodge most microbes from their surfaces. The phosphate-buffered saline was serially diluted a number of times, and the diluted washes were spread over DRBC (dichloran rose-bengal chloramphenicol) and Botrytis Selective Medium (BSM) and incubated at 25°C in darkness. After 5 days, Melanie counted all of the colonies at the 10^{-3} and 10^{-5} dilutions. BSM agar turns brown around Botrytis colonies. As none of the fungal colonies was surrounded by a brown zone, this indicated that there was no Botrytis on the inflorescences.

This meant that it was highly unlikely that there was any latent infection at that stage (Table 8).

As expected, inflorescences from treatment T2 (*Trichoderma* Colonizer® and Antagonizer® products) contained significantly higher levels of *Trichoderma* spp., and higher percentages of the total fungi were *Trichoderma* spp. (Table 8).

Infection on berries at harvest

Bunches were collected only from treatments T2, T3 and T4 at harvest. Bunches were frozen at –20°C for 7 days before incubation.

Freezing bunches terminates host resistance and promotes development of the fungal pathogen within the plant tissues, giving a more realistic estimation of bunch rot severity and incidence under the most adverse conditions.

After being frozen, these bunches were incubated at 25ºC for 10 days in ziplock plastic bags containing moist paper towels to increase humidity.

Bunches were scored for coverage of Botrytis bunch rot (i.e. severity). The incidence was 100% (i.e. every bunch had some Botrytis bunch rot). The percentage rot per bunch was significantly decreased by the T2 *Trichoderma* treatments (43% decrease) and by the T3 Ecoprotector® treatment (97% decrease), although the difference between these two treatments was not statistically significant (Table 9).

Conclusions from laboratory investigations

There was no latent *Botrytis cinerea* in the inflorescences. The combined Colonizer®–Antagonizer® *Trichoderma*

Table 9. Incidence and severity of Botrytis on bunches at harvest

Treatment number	Treatment details	Incidence (number of bunches with visible Botrytis)	Severity (% of bunch) covered in *B. cinerea)*
T2	Colonizer® (*Trichoderma koningii*) plus Antagonizer® (*Trichoderma harzianum*)	100%	41.7 [bc]
T3	Ecoprotector® (potassium salts of fatty acids)	100%	29.2 [c]
T4	Control (water spray)	100%	73.3 [a]
P value			0.013

Values down columns with the same letter(s) do not differ significantly ($P < 0.05$).

Table 8. Dilution plating of inflorescences (80% cap fall)

Treatment number	Treatment details	*Botrytis cinerea* on inflorescences?	*Trichoderma* spp. on inflorescences (cfu per g of 5 mm berries) (x 10^6)	*Trichoderma* spp. as % of total fungi
T1	Conventional Botrytis sprays	no	100 [b]	1.9 [b]
T2	Colonizer® (*Trichoderma koningii*) plus Antagonizer® (*Trichoderma harzianum*)	no	3000 [a]	58.7 [a]
T3	Ecoprotector® (Potassium salts of fatty acids)	no	200 [b]	16.4 [b]
T4	Control (water spray)	no	40 [b]	0.3 [b]
P value			<0.001	0.001

Values down columns with the same letter(s) do not differ significantly ($P < 0.05$).

treatment successfully increased *Trichoderma* spp. colonisation of the inflorescences.

Both the *Trichoderma* and the Ecoprotector® treatment decreased colonisation of grape bunches by *Botrytis cinerea* later in the season, although there was no statistically significant difference in the effectiveness of these two treatments.

Results of field observations

Severity and incidence of Botrytis

Table 10 shows the severity and incidence of Botrytis as assessed in the field for each treatment on 4 February (3 days before harvest and after 117 mm of rain had fallen in the previous 3 days).

Table 11 shows the severity of Botrytis infection in the four treatment groups after three bunches from each had been left in ziplock bags on the recreation room benches for 7 days.

Unlike with the method used to obtain the results in Table 9, these bunches were not frozen before incubation; this explained the lesser degree of Botrytis severity in Table 11 than in Table 9.

Season 1 final conclusions

Apart from a few light showers during flowering, rainfall was not a predisposing factor for Botrytis infection until the rain events beginning on 20 January 2013.

The laboratory results indicated that the light rain during flowering at the trial site did not cause latent infection.

Table 10. Results of field observations three days before harvest: Severity and incidence of Botrytis

Treatment	Treatment details	Severity (% of bunch covered in *B. cinerea*)	Incidence (number of bunches with visible Botrytis)
T1	Conventional Botrytis sprays	2%	3%
T2	Colonizer® (*Trichoderma koningii*) plus Antagonizer® (*Trichoderma harzianum*)	2%	2%
T3	Ecoprotector® (Potassium salts of fatty acids)	1.5%	2%
T4	Control	3%	3%

Table 11. Incidence and severity of Botrytis in incubated bunches at harvest (unfrozen)

Treatment	Treatment	Severity (% of bunch covered in *B. cinerea*)
T1	Conventional Botrytis sprays	40%
T2	Colonizer® (*Trichoderma koningii*) plus Antagonizer® (*Trichoderma harzianum*)	30%
T3	Ecoprotector® (potassium salts of fatty acids)	10%
T4	Control	40%

Treated bunch in treatment 1, T1: Conventional Botrytis spray control. *Photo: Richard Hilder*

Treated bunch in treatment 2, T2: Trichoderma *products (Colonizer® and Antagonizer®).*

Photo: Richard Hilder

Image of treated bunch in treatment 3, T3: Potassium salts of fatty acids (i.e. soap salts; Ecoprotector®)

Photo: Richard Hilder

Treated bunch in treatment 4, T4: Control (no Botrytis fungicides). *Photo: Richard Hilder*

At visual inspection of the trial site on 25 January 2013 there was no evidence of Botrytis infection in any of the treatment plots.

However, the 117 mm of rain over a 3-day period before harvest caused Botrytis infection in all treatment plots before the harvest (on 7 February), although the entire trial block was harvested within the winery tolerance of <4% Botrytis incidence.

Recommendations for the future: looking forward

The severity and incidence of Botrytis are influenced by site selection, canopy management, crop load, vineyard floor management, and the use of protective spray programs. Attention to these details helps immensely in reducing losses from Botrytis.

This trial will be continued next season. As a result of this year's findings we plan to introduce several extra treatments, including a combination of Trichoderma and Ecoprotector® together in one program.

These products cannot be tank mixed, and the Trichoderma products need to be sprayed at least 7 days after the Ecoprotector® spray so as not to kill the living spores.

On the basis of our preliminary results, a suggested combination program would be:

1. Ecoprotector® at 5% to 10% capfall
2. Colonizer® at 80% capfall
3. Ecoprotector® at pre-bunch closure
4. Antagonizer® at veraison
5. Ecoprotector® up to 14 days before harvest (before the 14-day withholding period)
6. Antagonizer® if required because of rain (Antagonizer® has a 1-day withholding period).