Improving Fungicide Control of Botrytis Bunch Rot



FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: SAR 01/02

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Research Organisation: South Australian Research and Development Institute

Date: 30th June 2002

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GWRDC Final Report

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(SARDI)

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1. Abstract

Laboratory and field studies were undertaken to determine if potassium metabisulphite (KMS) controls *Botrytis* bunch infection when applied near harvest.

The laboratory studies showed that 3 g/L KMS inhibited the germination of *Botrytis* spores and that the effect was greater when the spores were incubated in grape juice. Volatiles emanating from suspensions of KMS inhibited germ tube elongation but not spore germination. Experiments using single inoculated berries showed that KMS inhibits the development of *Botrytis* when applied up to 48 hours after inoculation. However both laboratory and field studies showed that KMS had little effect on sporulation from berries infected more than 48 hours previously. Applications of 4 g/L KMS on one or two occasions at various times between veraison and harvest did not inhibit sporulation from infected berries. Further work needs to done to evaluate KMS and other fungicides for use just before harvest.

2. Executive Summary

Numerous laboratory and field studies were undertaken to evaluate the efficacy of potassium metabisulphite (KMS) for the control of Botrytis bunch rot. This material is frequently applied to grapes following rain near harvest that splits berries and encourages the development of Botrytis bunch rot. Despite the widespread use of this product in the industry there has been no critical studies showing that the treatment is effective.

Initial laboratory studies were carried out to measure spore germination of *Botrytis* spores when various levels of spores were mixed in different concentrations of KMS solutions. These studies showed that 80% or more of the spores failed to germinate in the presence of KMS, and there was little difference in efficacy with different spore loads or KMS concentration. Tests also showed that the efficacy of KMS was improved when grape juice was added to the suspension.

In another experiment *Botrytis* spores failed to germinate after being washed from berries using a spray of 3g/L KMS. Other laboratory studies showed that volatiles emanating from KMS solutions inhibited germ tube elongation but not spore germination.

Individual grape berries (table grapes) were inoculated by spraying with *Botrytis* spores and then either dipped or sprayed with KMS solutions 24 or 48 hours after inoculation. These results showed that KMS inhibited the production of spores at both times of inoculation but that the degree of inhibition was not complete. Similar experiments assessed up to 4 days after treating with KMS, Peratec (a formulation of hydrogen peroxide) or Rovral showed that only Rovral significantly inhibited sporulation 4 days after treatment.

Nine field trials were undertaken to evaluate the effect of KMS applied on either one or two occasions at various times after individual berries on bunches were inoculated. In some experiments, KMS was compared with Peratec, and fungicides such as Sumisclex and Switch. In another experiment KMS was compared with and without the addition of Citowett wetting agent.

None of the treatments significantly inhibited sporulation from previously infected berries.

Overall this work has shown that KMS sprays prevent the germination of *Botrytis* spores but not growth and sporulation, from berries infected with *Botrytis* more than 48 hours before spraying.

In practical terms this means that mycellial growth and *Botrytis* sporulating on berries would be killed by applying KMS, but the sprays would be ineffective in preventing further sporulation on the infected berries. In these trials there was no indication of KMS "drying out" previously infected berries.

3. Background

Losses due to Botrytis bunch rot have been significant in some vineyards in recent years, with reported losses of 10 to 30 million dollars (Emmett pers. com.). Botrytis bunch rot is the most difficult fungal disease to control in vineyard, and many of the registered fungicides cannot be applied after flowering if the grapes are destined for export wine production. The main group of fungicides allowed by wineries close to harvest are dicarboximides, and in many areas there is a level of resistance to this group of fungicides. Alternative chemicals with efficacy against bunch rot that can be applied close to harvest will be invaluable to the industry.

The disease can be controlled by fungicides, but often their efficacy is not fully utilised because of the difficulty in obtaining good spray coverage near harvest. Botrytis bunch rot is particularly severe in vineyards where rain causes berries to split on bunches just before harvest. Growers frequently apply potassium metabisulphite (KMS) in these situations to "dry out" the berries and prevent further spread of bunch rot. Unfortunately there is little efficacy data and only anecdotal evidence to support the use of KMS.

The aim of this project was to critically evaluate KMS for the control of Botrytis bunch rot.

4. Project Aims and Performance Targets

The aim of this project was to obtain data on the efficacy of KMS for the control of Botrytis bunch rot and to extend the results to industry. This would be achieved by conducting experiments in the laboratory and the field and presenting the results through industry meetings and industry publications.

5. Methods

LABORATORY EXPERIMENTS

A number of experiments were conducted to evaluate the effect of KMS on the germination and growth of *Botrytis* spores as well as the effect on sporulation on infected berries.

Experiment 1. Effect of Botrytis inoculum level on the activity of KMS

The experiment compared the activity of KMS at 3 g/L on the germination of *Botrytis* spores in suspensions of 10^6, 10^4 and 10^2 spores/ml. Spores were obtained from 14 to 21 day old cultures of *Botrytis* grown on V8 agar. Different concentrations of spores were suspended in KMS solution and placed in individual wells. After incubation at 25^{o}C spore germination was counted on 40 spores per treatment, each replicated 5 times at 24, 48 and 72 hours.

Experiment 2. Comparison of KMS and Rovral on spore germination

The above experiment was repeated with an additional treatment of 1 ml/L Rovral (Iprodione) included for comparison with KMS.

Experiment 3. Effect of grape juice on the activity of KMS

Botrytis spores were germinated in diluted grape juice to determine if media nutrient level influenced the activity of KMS. Suspensions of *Botrytis* spores were prepared and adjusted to 10^6 spores/ml in suspensions of either water, KMS (3 g/L), Rovral (1 ml/L), and grape juice diluted 1:1, or 1:10, with water, mixed to provide total concentrations of either KMS (3 g/L) or Rovral (1 ml/L). This experiment was carried out in wells similar to those used in experiment 1 and 2. Spore germination was measured on 40 spores per well at 24, 48 and 72 hours after inoculation.

Experiment 4. Effect of grape juice on the activity of KMS

This experiment was similar to experiment 3 except that the spore suspensions from each treatment were plated onto tap water agar and incubated for 24 hours. For each treatment 4 plates with 4 drops of 1ml spore suspension per plate were used. Germination of 25 spores per drop was measured.

Experiment 5. Effect of KMS volatiles on the germination and germ tube growth of Botrytis

To determine if volatiles from KMS inhibited the germination and growth of *Botrytis*, droplets of 10^4 spores/ml were placed on potato dextrose agar (PDA) plates, which were then inverted over solutions of either over 3, 4 or 5 g/L KMS. Each treatment was replicated 5 times and germination was measured on 100 spores per plate at 6 and 24 hours after incubation at 24^{o}C. Germ tube growth was also measured on 10 spores per plate at these times.

Experiment 6. (a & b) Effect of KMS on Botrytis *sporulation*

(a) The experiment was undertaken to determine the effect of KMS on sporulation from previously infected grapes. Table grapes were artificially infected by spraying the bunch with a spore suspension of 10^6 spores/ml. Bunches were incubated at room temperature in a humid chamber, and at 24 and 48 hours after inoculation, berries were sprayed with 4 g/L KMS solution and re-incubated to induce sporulation. At least 8 berries per treatment were incubated for 4 days after inoculation before the presence or absence of sporulation was recorded. Also included for comparison were sprays of Rovral and Peratec.

(b) The above experiment was repeated but in this case the treatments were applied to sporulating berries which were assessed immediately prior to spraying them with fungicides as well as 30 hours and 4 days later.

Experiment 7. (a & b) Effect of KMS on spore viability

(a) Berries on Chardonnay vines that had previously been injected with *Botrytis* spores on April 4th were sporulating in the field on April 23rd. At least 20 of these bunches were harvested and returned to the laboratory where individual bunches were sprayed to run off with a solution of 4 g/L KMS or water using a small hand held sprayer. Bunches were rotated to ensure that all sporulating berries were completely covered with spray. Run off spray from the berries was collected and droplets of the solution plated immediately on PDA. Plates were exposed and placed in a laminar flow for 60 minutes to allow the droplets to evaporate. The lids were replaced on the plates and incubated for 12 hours at 25^{o}C before they were examined and spore germination counted.

(b) This experiment was repeated on a further batch of Chardonnay bunches where individual berries had been artificially inoculated and induced to sporulate. At least 4 bunches each with 4 or more sporulating berries were sprayed with either water, 4 g/L KMS or 10 ml/L Peratec and the excess spray collected and plated out as described above. Plates were incubated for 8 hours at 25^{o}C before spore germination was counted, on at least 500 spores per treatment.

FIELD EXPERIMENTS

A number of experiments were undertaken to evaluate the effect of KMS when applied to infected berries in the field. The experiments were carried out at various times on Chardonnay and Sauvignon Blanc vines at the Lenswood Research Centre in the Adelaide Hills and on Sauvignon Blanc vines at the Nuriootpa Research Centre.

In these experiments individual bunches were labelled and 3 to 5 berries per bunch artificially inoculated by injecting each berry with a suspension of approximately 10^6 spores/ml using a fine hypodermic syringe. Spore suspensions were freshly prepared from at least 2 or more different isolates of *Botrytis* grown on V8 agar for at least 7 days. Berries in different experiments were injected between veraison and harvest. KMS and other materials were applied to individual berries using a hand held misting sprayer. Individual bunches were carefully rotated during spraying to ensure that the best possible spray coverage was obtained. In each experiment, between 10 to 25 bunches were used for each treatment. At various times after treatment, bunches were harvested, and placed individually into paper bags to reduce cross contamination during transport to the laboratory. In the laboratory, bunches were removed from the bags and placed into trays each containing a moistened "chux" and then enclosed in a plastic bag. Bunches were incubated at 22^{o}C for 7 - 10 days and assessed for *Botrytis* sporulation using a 0 to 4 scale rating scale where 0 = no *Botrytis*, 1 = 1 to 10%, 2 = 11 to 25%, 3 = 26 to 50% and 4 = greater than 50% of the bunch infected with *Botrytis*.

A number of other treatments were also tested in these experiments. These were KMS at 4 g/L with and without the addition of Citowett wetting agent, Peratec, Sumisclex 1 ml/L, and Switch 0.8 g/L.

At the Nuriootpa site, a misting system was installed along 2 rows of Sauvignon Blanc vines to induce sporulation in infected bunches. This system consisted of a irrigation line installed at the top of the canopy and a series of suspended pulsing misters which were set up to mist inside the canopy and around the fruit zone. In general this system was not as successful as anticipated as it failed to induce sporulation and spread of the disease in the bunches. In addition the exceptional dry period during the harvest period of March/April did not result in natural sporulation and spread of *Botrytis*.

6. Results /Discussion

LABORATORY EXPERIMENTS

Experiment 1.

A lower spore germination rate was found in the higher inoculum level (10^6 spores/ml), where only 38.5% of *Botrytis* spores germinated in water after 24 hours incubation. However by 72 hours incubation both inoculum levels had over 50% germination. At 10^2 spores/ml inoculation level, conidia were difficult to detect and count with the microscope, so further experiments were conducted using spore concentrations of 10^4 spores/ml or greater.

No *Botrytis* spores germinated in the presence of 3 g/L KMS (Table 1).

Table 1. Germination of *Botrytis* spores with various inoculum levels in water or KMS.

	10^4 *Botrytis* spores/ml		**10^6 *Botrytis* spores/ml**	
	KMS (3g/L)	Water	KMS (3g/L)	Water
24 hours	0	56	0	38.5
48 hours	0	53	0	47.5
72 hours	0	55.5	0	50.5

Experiment 2.

This experiment included 1ml/L Rovral (iprodione) for comparison. Spore germination in water was 56% after 24 hours incubation and did not increase with further incubation (Table 2). KMS did not inhibit germination completely in this experiment as 2.5% of spores germinated after 24 hours and 14% germinated after 72 hours incubation. On the other hand no spores germinated in the presence of Rovral after 24 hours and 3% germinated after 72 hours incubation.

Table 2. Comparison of KMS and Rovral on *Botrytis* spore germination.

	Water	**KMS**	**Rovral**
24 hours	56.3	2.5	0
48 hours	55.6	8.1	3.1
72 hours	56.9	13.8	2.5

Experiment 3.

Experiment 3 was undertaken to determine the effect of diluted grape juice on the activity of KMS. Germination in water was 13%, 43% and 80% when spores were incubated for 24, 48 and 72 hours respectively, but in the presence of grape juice diluted 1:1 or 1:10 with water, all *Botrytis* spores germinated within 24 hours (Table 3). No *Botrytis* spores germinated in the presence of KMS or Rovral after 72 hours incubation in water or either dilution of grape juice.

Table 3. Effect of grape juice on the activity of KMS.

Treatment	**Germination (%)**		
	24hrs	**48hrs**	**72hrs**
Spore + Water	13	43	80
Spores + KMS	5	0	0
Spores + Rovral	0	0	0
Spores + Grape juice 10[1]	100	100	100
Spores + Grape juice 50[2]	100	98	100
Spores + KMS + Grape juice 10[1]	0	0	0
Spores + Rovral + Grape juice 10[1]	0	0	0
Spores + KMS + Grape juice 50[2]	0	0	0
Spores + Rovral + Grape juice 50[2]	0	0	0

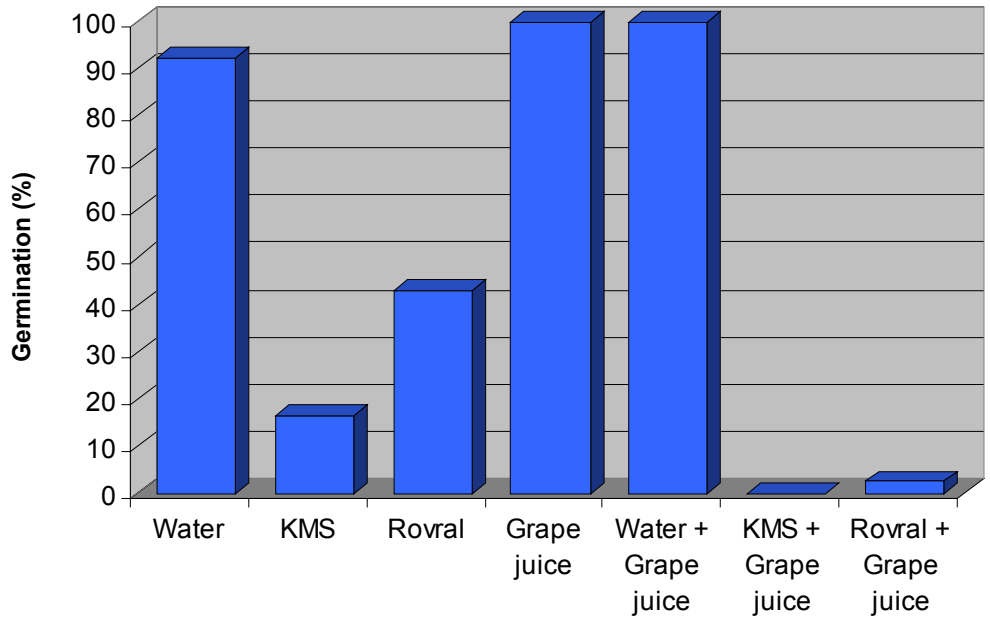
[1] Grape juice diluted 1:10 with water

[2] Grape juice diluted 1:1 with water

Experiment 4.

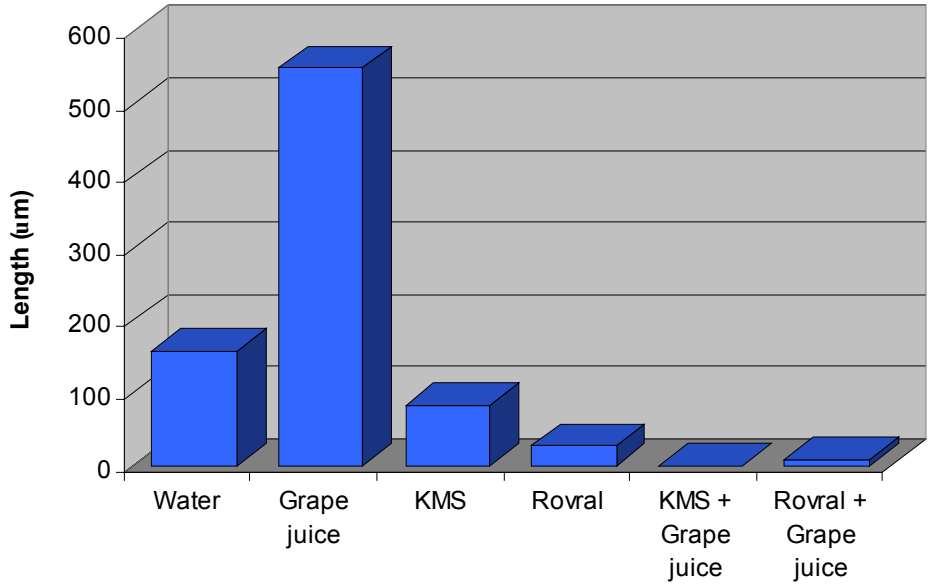
Some of the treatments in experiment 3 were repeated, but the spore suspensions were plated onto tap water agar to enable measurements to be made on germ tube elongation. The results show a similar response to the previous experiment except that 16% and 43% of spores germinated in the KMS and Rovral treatments respectively (Fig 1). The reasons for this are unclear unless resistant strains were selected out. On the other hand, KMS was effective when grape juice was added and only 2.5% of spores germinated in the presence of Rovral and grape juice. These experiments indicate that the presence of even small amounts of grape juice not only increase sporulation, but enhance the effect of the chemicals in inhibiting that sporulation.

Figure 1. Effect of KMS and Rovral on the germination of *Botrytis cinerea* spores after 24 hours incubation on water or grape juice (diluted 1:10 with water).



Germ tubes grew to around 160µm after 24 hours incubation in water whereas growth was 550µm in the presence of 1:10 grape juice. The addition of KMS and Rovral did not inhibit elongation completely as growth was 84µm and 27µm respectively (Fig 2). When added to diluted grape juice however, elongation was inhibited completely with KMS and almost completely (4µm) with Rovral. Overall these results show that both KMS and Rovral are inhibitory to germ tube growth with the effect greater in the presence of grape juice.

Figure 2. Effect of KMS and Rovral on germ tube growth of *Botrytis cinerea* after 24 hours incubation in water or grape juice (diluted 1:10 with water).



Experiment 5.

Where the effect of volatiles from KMS solutions was evaluated, none of the treatments inhibited spore germination (Fig 3). While volatiles from all concentrations (3 to 5 g/L) significantly inhibited germ tube elongation, none completely inhibited the elongation (Fig 4). The effect was most obvious after 24 hours incubation.

Figure 3. Effect of KMS volatiles on the germination of *Botrytis* spores.

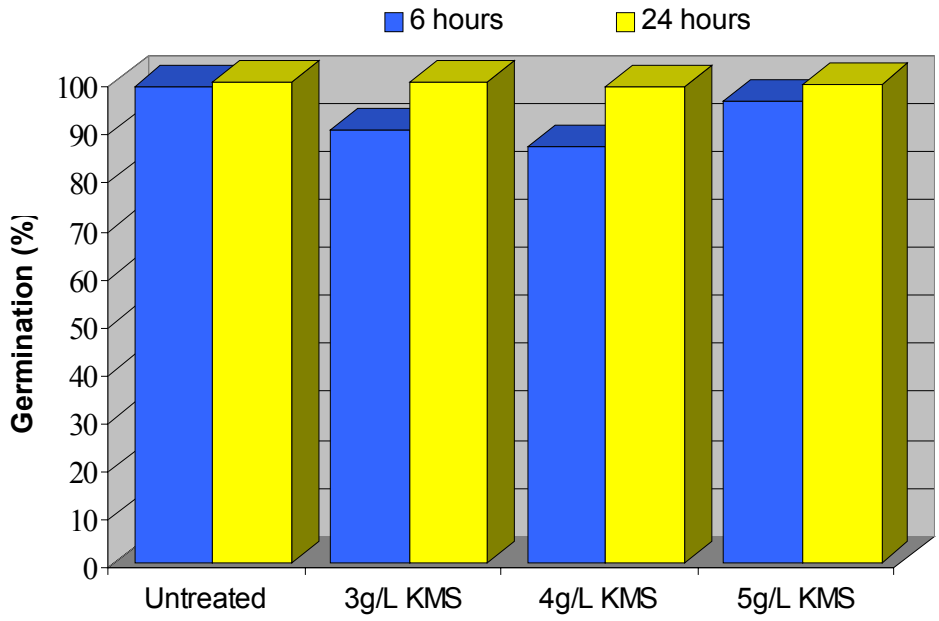
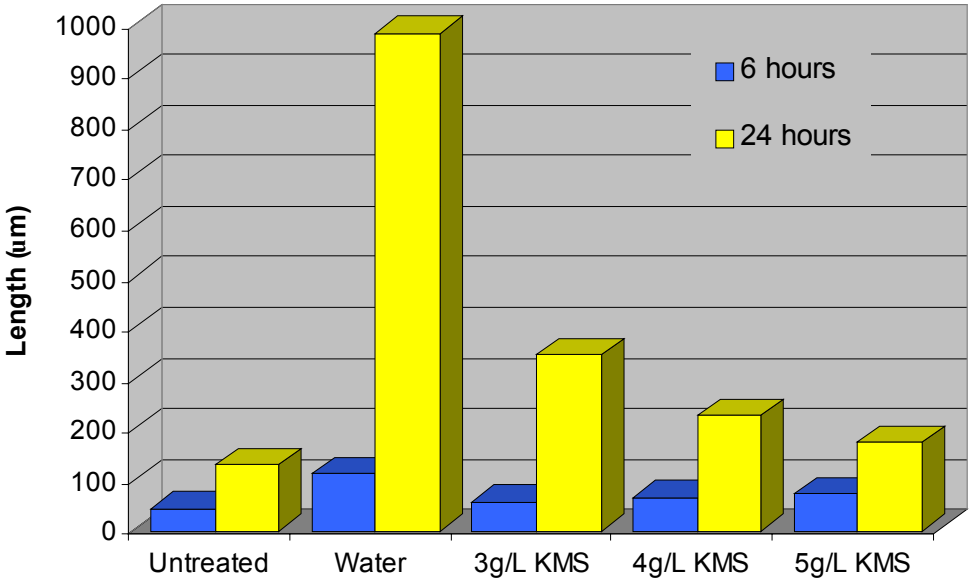


Figure 4. Effect of KMS volatiles on the length of *Botrytis* germ tubes.



Experiment 6. (a & b)

Applying KMS either 24 or 48 hours after inoculation significantly inhibited sporulation on infected berries. The inhibition with KMS was greater than that achieved with Peratec but not significantly different from that achieved with Rovral (Fig 5). Where this trial was repeated and the development of sporulation monitored for up to 4 days after spraying, sporulation on the KMS treatment was inhibited for at least 30 hours after treatment. After 4 days however, sporulation had increased to a level similar to that in the water only treatment (Fig 6). Rovral was the only treatment where sporulation was significantly inhibited up to 4 days after treatment.

Figure 5. Effect of treatment and spray timing on the area of berry sporulating 4 days after inoculation.

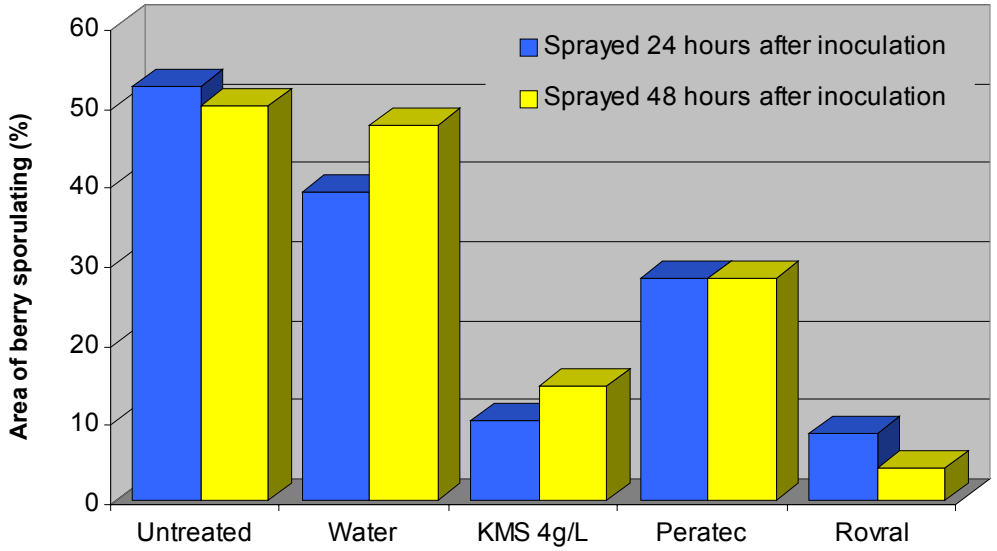
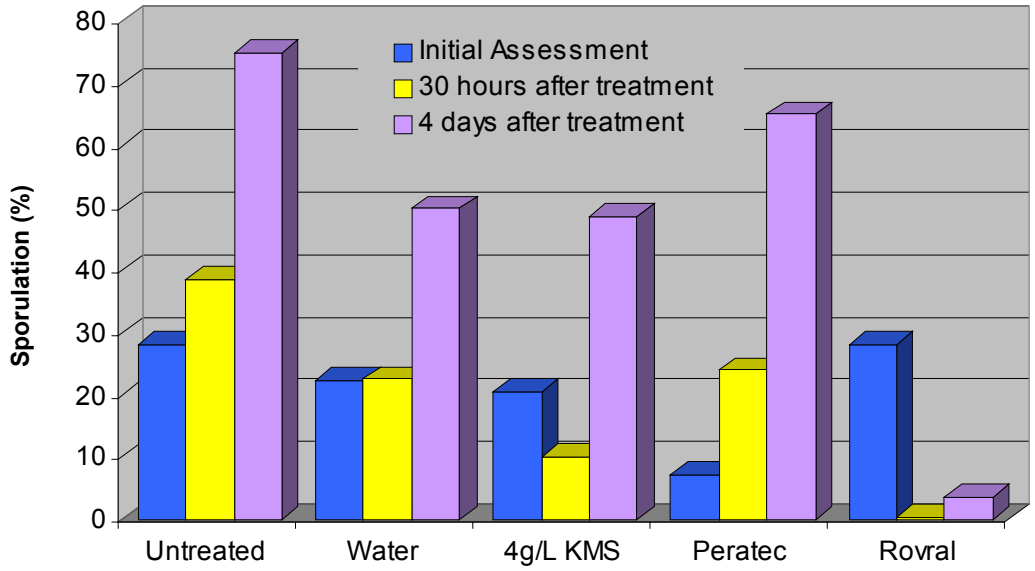


Figure 6. Effect of KMS on sporulating *Botrytis* infections.



Experiment 7 (a&b).

In both experiments where *Botrytis* spores were collected in the "run off" spray from bunches sprayed with either water, KMS or Peratec only spores in the water spray were viable with more than 74% germination.

FIELD EXPERIMENTS

In the nine field experiments that were undertaken, few of the KMS treatments markedly inhibited sporulation from infected berries. For example where Sauvignon blanc bunches were inoculated on March 1 and then treated with 4 g/L KMS on March 25 and April 3, sporulation from sprayed and unsprayed bunches was rated at 3.5 and 3.3 respectively.

Similarly when Sauvignon blanc bunches were inoculated in February and then sprayed with either KMS, Sumisclex or Switch on April 16 and 23 none of the treatments inhibited sporulation on the inoculated berries as mean ratings were 2.9, 2.9 and 3.8 respectively compared to a rating of 2.8 in the untreated.

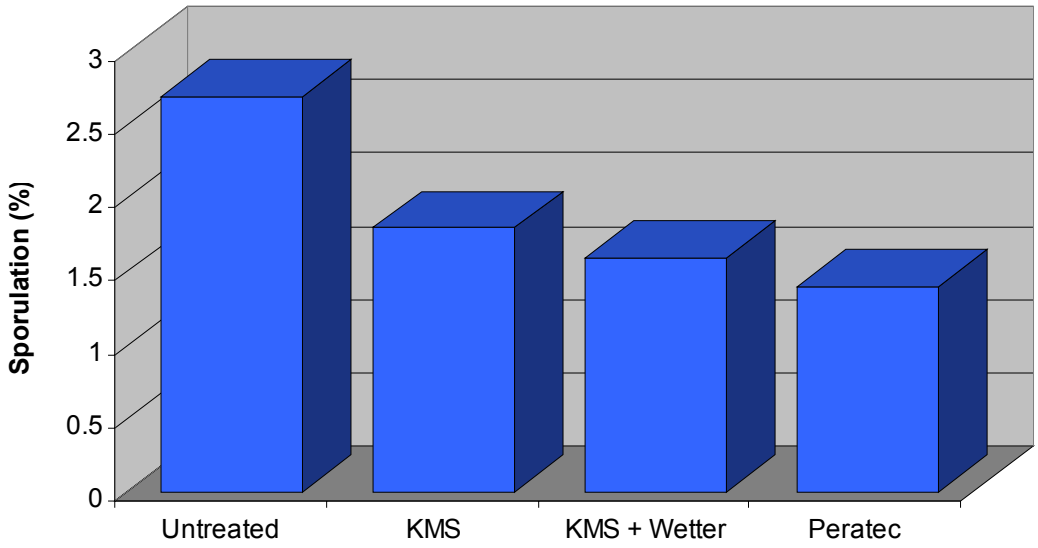
In the one experiment where KMS with and without a wetting agent was compared, the addition of the wetting agent did not improve the activity of KMS. However in this experiment both KMS and Peratec had an inhibitory effect on sporulation (Fig 7). Peratec and KMS were also compared in another experiment on Pinot Noir, where bunches were inoculated on March 24, and treated 7 days later. Sporulation ratings were 2.0, 2.4 and 1.7 for the untreated, KMS and Peratec treatments respectively.

In general there was a slight reduction in the amount of sporulation in some experiments but in commercial vineyards this effect is unlikely to be noticed. This is due to the fact that in the present experiments, individual bunches were sprayed by hand to ensure even coverage of spray over the bunch. In commercial vineyards similar bunch coverage is unlikely to occur with conventional spray machinery and the level of control or suppression of sporulation is unlikely to be as high as that achieved with hand spraying.

In the planting of Sauvignon Blanc, where a pulsating wetting system was installed, different frequencies of misting were imposed on several occasions during the night to induce sporulation on artificially inoculated bunches. Although periods of wetness were not

measured, the misting system was set up to provide a humid atmosphere in the canopy overnight. Despite several attempts with the system, sporulation could not be induced by misting. Further fine tuning of the system needs to be done as it has great potential for inducing disease and evaluating strategies to manage Botrytis bunch rot.

Figure 7. Effect of KMS on *Botrytis* sporulation on artificially inoculated Sauvignon Blanc bunches, Nuriootpa.



7. Outcomes/Conclusion

These studies have shown that KMS kills *Botrytis* spores and inhibits germ tube growth. If it is applied within 48 hours from infection sporulation from infected berries is also inhibited.

However, if applied after this time KMS has little effect on sporulation on infected berries.

This work implies that existing *Botrytis* spores and mycelium on split and infected berries will be killed by KMS sprays, but the sprays would not inhibit further sporulation from these berries.

8. Recommendations

Further work needs to be done to :-

(a) Fine tune and develop the misting system to induce *Botrytis* so controlled field experiments can be carried out with this disease.

(b) Further evaluate experimental products for use near harvest.

9. Appendix 1: Communication

The results of this project have been presented to growers at meetings in Coonawarra, McLaren Vale, and Mt Barker W.A. Further meetings are anticipated particularly Research to Practice workshops. The results from this work have also been presented in the Annual Pest and Disease Technical Issue of the Australian, New Zealand Grape and Winemaker – 2002.

Appendix 2: References

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Appendix 3: Staff

- Trevor Wicks (SARDI – Horticulture Pathology)
- Barbara Hall (SARDI – Horticulture Pathology)
- Robyn McMahon (SARDI – Horticulture Pathology)
- Kent Davies (SARDI – Horticulture Pathology)
- Catherine Hitch (SARDI – Horticulture Pathology)
- SARDI colleagues
- SARDI staff at the Nuriootpa and Lenswood Research Centres

Appendix 4: Budget reconciliation