

# Evaluation of fungicides and fungicide-adjuvant mixtures for post-infection control of

## Phomopsis cane and leaf spot of grape

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### Introduction

The fungus *Phomopsis viticola* (Sacc.) is the causal agent of phomopsis cane and leaf spot of grape. This disease was previously known as "dead-arm" in American literature (Cucuzza and Sall 1982; Erincik et al. 2001). Various parts of the vine, such as shoots, rachis, leaves, and fruits are susceptible to infection, especially when tissues are immature. Leaf symptoms are small irregular, or round shaped, yellow spots with dark centers (**Figure 1**). Infected canes and rachises show dark necrotic irregular shaped lesions (**Figure 1**). Infected fruits appear as brown shriveled berries near harvest. Typically, control of the disease is obtained with calendar-based applications of protective fungicides such as mancozeb or captan.



**Figure 1** Typical leaf and cane symptoms caused by *Phomopsis viticola* on grape (cultivar 'Seyval')

A disease-predictive model for *Phomopsis* cane and leaf spot was developed by Erincik et al. (2003). The model is based on the relationship between wetness duration ( $W$ ), air temperature ( $T$ ), and level of infection. Essentially, prediction of *Phomopsis* cane and leaf spot of grape involves monitoring leaf wetness and air temperature with electronic sensors, and infection level (assuming available inoculum) is predicted using a previously developed nonlinear model. By incorporating the model into a fungicide application program, growers may be able to reduce the number of applications, or improve application timing. Thus, they can improve disease control while saving money and reducing environmental impacts from unnecessary fungicide applications.

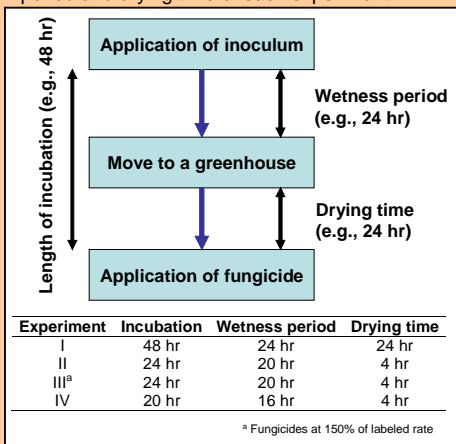
Ideally, the fungicide used with a predictive system should have curative activity, (i.e., control the pathogen after inoculation and pathogen establishment takes place) although predictive systems can also be used to optimize scheduling of protectant fungicides. There are no known curative fungicides effective against *Phomopsis* cane and leaf spot of grape. However, mixtures of some fungicides with adjuvants can enhance curative effects, possibly by increasing uptake of the fungicide by the plant. **Our objective was to evaluate fungicides and fungicide-adjuvant mixtures for their potential curative activity under controlled environment conditions.**

### Materials & Methods

Grape vines (cultivar 'Seyval') grown from cuttings in 25.4-cm pots for 3-6 mo were used. A total of five young leaves, usually less than 3-cm in length and less than 1 wk old, and five internodes between these young leaves were selected per vine for each treatment.

A culture of *P. viticola* grown on PDA for about 3 wk was used for preparation of inoculum. A petri dish was flooded with distilled water, and conidia were collected through four layers of cheesecloth. Spore density was adjusted to  $5 \times 10^6$ /ml using a hemacytometer. Inoculum was sprayed onto leaves and internodes with an atomizer (pressure of 51.7 kPa) until run off. After inoculation, plants were placed in a growth chamber, and temperature was maintained at  $\sim 22^\circ\text{C}$ . A mister within the chamber maintained high RH and free water on leaf surfaces. Three different wetness periods were evaluated in different experiments. Each experiment consisted of a set of wetness duration and drying time to produce an infection period (**Figure 2**). Labeled rates of fungicides were used in experiment I, II and IV (**Table 1**), and in experiment III, 1.5 times the labeled rate (150%) was used (**Figure 2**).

**Figure 2** Time flow diagram of post-inoculation application of fungicides and a list of wetness period and drying time of each experiment



Protectant (pre-inoculation) sprays of mancozeb or liquid lime sulfur were applied with a hand atomizer, at least 3 hr, but no more than 6 hr, prior to inoculation. Plants were dry at the time of inoculation. Post-inoculation treatments (mancozeb, thiophanate-methyl, azoxystrobin, and myclobutanil) in combination with adjuvant (JMS stylet Oil or Regulaid), and mancozeb and lime sulfur alone were sprayed on the plants after the inoculation and drying period.

**Table 1** List of fungicides and dosage used

Fungicide	Dose
Mancozeb(mancozeb)	4.5 kg/ha
Topsin-M (thiophanate-methyl)	1.7 kg/ha
Nova (myclobutanil)	0.3 kg/ha
Abound (azoxystrobin)	1.1 m <sup>3</sup> /ha
Lime sulfur	4.7 m <sup>3</sup> /ha
JMS Stylet-Oil	3.8 m <sup>3</sup> /ha
Regulaid	2.4 m <sup>3</sup> /ha

After 4 wk, disease severity and incidence on internodes and leaves were visually evaluated. The direct estimation of percentage of diseased area was used for internode disease assessments. Disease severity of leaves was assessed by estimating the number of lesions on each leaf using a scale with seven levels (0 = no spots, 6 = more than 100 spots [=lesions]). Data were analyzed using a general linear mixed model (PROC MIXED in SAS) in order to determine effects of spray timing, fungicide, and adjuvant on disease. Contrasts were used for comparing groups of treatments. Disease incidence and severity values were transformed using an angular transformation ( $\arcsin \sqrt{\%}/100$ ) for internode severity, or a square-root transformation for lesion counts on leaves, then back-transformed after the analysis to calculate means of severity.

## Results

Vines not treated with fungicides had 76-93 lesions per leaf and 3-12% severity on internodes (Table 2). Pre-inoculation application of fungicides significantly reduced severity in all experiments compared with the control (Tables 2 and 3). On average, severity was reduced by 96 % with pre-inoculation application of fungicides. Results were very similar for the different experiments even though wetness periods and fungicide dose varied.

However, disease severity with post-inoculation application of fungicides was not different from the control in most experiments. Furthermore, use of adjuvant did not reduce severity compared to no adjuvant in most experiments (Table 3), and the two adjuvants gave very similar results.

## Discussions and Conclusions

Adjuvants are used to increase adhesion of fungicides and other pesticides to plant surfaces, and to potentially increase absorption of the chemical by plant tissues. It has been shown that adjuvants are particularly effective with herbicides, whereas their use with fungicides is less clear. Specificity of adjuvant chemicals, mode of action of fungicidal materials, type of fungus, and environmental conditions, all can affect outcomes. Some adjuvants, such as JMS Stylet Oil, have fungicidal activity; however, this activity appears limited to specific diseases, such as powdery mildew of grape. JMS Stylet Oil did not show any protective activity with *Phomopsis* cane and leaf spot of grape in a controlled environment study (unpublished).

Some fungicides such as Nova (myclobutanol) and Abound (azoxystrobin) have locally-systemic activity, and mixing with an adjuvant could potentially aid in tissue penetration and disease control. Since these fungicide gave no control when applied after inoculation, there was no evidence that enhancement of chemical uptake by the grape plant occurred.

Also, it should be noted that applications of Nova onto young succulent tissues of cultivar 'Seyval' resulted in exhibition of some phytotoxic effects. Phytotoxicity observed as a suppression of leaf expansion, curled tips of leaves, and a mosaic-like discoloration.

**Overall, there was no indication of curative activity from any of the fungicides applied after initiation of infection, whether mixed with adjuvant or not.**

**Table 2**

Effects of various fungicides and fungicide-adjuvant mixtures

Treatment	Experiment							
	I		II		III		IV	
	Leaf # <sup>a</sup>	Node % <sup>b</sup>	Leaf # <sup>a</sup>	Node % <sup>b</sup>	Leaf # <sup>a</sup>	Node % <sup>b</sup>	Leaf # <sup>a</sup>	Node % <sup>b</sup>
Mancozeb+ Regulaid	66.2 a	5.0 ab	78.8 a	12.2 a	56.9 a	1.4 ac	63.9 ab	5.6 ab
Mancozeb+ Stylet Oil	59.8 a	3.0 bc	57.4 ab	2.2 bcd	82.9 a	2.7 ac	66.9 ab	8.4 ab
Topsin-M + Regulaid	68.3 a	3.8 ac	57.2 ab	3.3 ad	42.2 a	0.7 ac	57.4 ab	4.0 ac
Topsin-M + Stylet Oil	70.5 a	5.1 ab	77.4 a	0.7 bcd	62.1 a	1.8 ac	55.6 ab	7.5 a
Nova + Regulaid	83.1 a	6.7 ab	69.6 a	3.5 ad	67.4 a	3.1 ac	45.1 bc	5.2 ac
Abound + Regulaid	56.9 a	3.7 ac	94.6 a	4.9 ab	67.0 a	3.2 ac	48.2 ac	5.3 ac
Mancozeb	63.5 a	8.8 ab	85.1 a	2.8 bcd	94.0 a	1.7 ac	30.6 c	7.0 a
Lime Sulfur	75.9 a	8.3 ab	99.1 a	2.1 bcd	70.4 a	2.3 ab	45.7 bc	10.6 a
Lime Sulfur (pre-inoculation)	4.1 b	0.3 c	25.7 b	0.2 cd	1.4 b	0.2 bc	0.8 d	0.2 bc
Mancozeb (pre-inoculation)	0.5 b	0.3 c	0.4 c	0.1 d	1.1 b	0.1 c	2.9 d	0.2 c
Control	77.0 a	12.2 a	92.7 a	4.7 ac	84.1 a	3.2 a	76.2 a	7.3 a

<sup>a</sup> mean lesion number

<sup>b</sup> mean percentage of internode tissue infected

**Table 3**

Contrasts of various combinations of treatments based on the disease severity with different infectious periods and fungicide dosage

Treatment	Experiment							
	I		II		III		IV	
	Leaf	Node	Leaf	Node	Leaf	Node	Leaf	Node
Control vs. pre-inoculation treatments	45.2*	17.8**	34.2**	6.0**	46.4**	5.3*	93.6**	80.8**
Control vs. post-inoculation treatments	1.0	3.8	1.4	0.2	0.7	0.1	5.7*	0.05
Pre- vs. post-inoculation treatments	82.0**	15.4**	55.8**	9.9**	88.9**	10.5**	193.1**	17.0**
Post-inoculation treatments: with adjuvant vs. without adjuvant	0.02	3.1	3.2	0.7	1.5	0.3	6.8**	1.0
Post-inoculation treatments: Stylet Oil vs. Regulaid	0.01	0.03	0.01	6.0**	1.9	1.5	0.0	1.0
Post-inoculation treatments: local-systemic vs. protectant	0.06	0.5	0.4	0.6	2.0	0.03	0.02	1.1
Post-inoculation treatments with adjuvant: local-systemic vs. protectant	0.15	0.2	0.3	2.3	0.8	0.03	2.8	0.3
Mancozeb: with vs. without adjuvant	0.02	2.0	1.5	1.2	1.2	0.4	11.1**	0.0

<sup>a</sup> \*\* = significant at 0.01 level,  
\* = significant at 0.05 level

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