# Effect of Postinfection Application of Phosphorous (Phosphonic) Acid on the Incidence and Sporulation of *Plasmopara viticola* on Grapevine

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**ABSTRACT**

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Phosphorous acid ($H_3PO_3$) applied at 1.2 g/L up to 12 days after infection reduced the incidence and severity of *Plasmopara viticola*. When applied up to 13 days after infection, $H_3PO_3$ reduced sporulation. In some experiments, the postinfection activity of $H_3PO_3$ was better than metalaxyl, while in other experiments it was less effective. Both fungicides applied after infection reduced the incidence and severity of disease on leaves and flower clusters, indicating that postinfection applications should reduce the production of inoculum and the consequent spread of disease within a vineyard. In most cases, the addition of copper oxychloride did not affect the efficacy of $H_3PO_3$.

Downy mildew, caused by *Plasmopara viticola* (Berk. & Curtis) Berlese & de Toni, is a major disease of grapes in many viticultural areas of the world (2).

In most grape-growing districts of Australia, downy mildew causes some crop loss each year, and in wet years, complete crop loss may occur in some vineyards. The disease is controlled with well-timed applications of copper or mancozeb fungicides. Since the mid-1970s, mixtures of metalaxyl formulated with either copper oxychloride or mancozeb have also controlled downy mildew when applied before and after infection.

Fungicides with modes of action differing from that of metalaxyl and related materials are needed in Australia for use in antiresistant strategies. Resistance to metalaxyl has been detected in populations of *P. viticola* in Europe, where in some areas, it is no longer effective against that pathogen (1).

Phosphorous (phosphonic) acid ($H_3PO_3$) was first evaluated as a downy mildew fungicide in Australia during 1986–1987 (3,6), and its potential as a suitable alternative to metalaxyl was demonstrated. This work showed $H_3PO_3$ activity against *P. viticola* and indicated a need for more extensive investigation, particularly into its efficacy when applied either before or after infection. As a result of these and related studies (P. A. Magarey and T. J. Wicks, *unpublished*), a formulation of $H_3PO_3$ was developed and is currently registered in Australia for the control of grapevine downy mildew.

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The objectives of this study were to further evaluate the efficacy of $H_3PO_3$ or an $H_3PO_3$-copper oxychloride mixture when applied at various times after infection.

## MATERIALS AND METHODS

**General methods.** Experiments were conducted in 1988 and 1989 at the research centers of the South Australian Department of Agriculture at Loxton, Nuriootpa, and Northfield, which are approximately 300 km northeast, 100 km north, and 8 km north of Adelaide, respectively. At these locations, vineyards of the *Vitis vinifera* L. cvs. Shiraz, Grenache, White Grenache, and Mataro, which are commonly grown in Australia, were used as test sites. Each site comprised mature vines drip-irrigated and trained on a variety of trellis systems and, apart from the fungicide treatments, standard cultural practices were maintained. Shoots were inoculated by spraying 2–3 hr before dusk a freshly prepared suspension of $1 \times 10^4$ *P. viticola* sporangia per milliliter. The shoots were then sealed in moistened plastic bags to maintain leaf wetness overnight. Fungicides were applied at Loxton with a knapsack sprayer and at Nuriootpa and Northfield with a pressurized hand-wand.

Vines were sprayed to run off, and from 0.75 to 1.5 L of spray liquid was applied per vine depending on the amount of vine foliage.

$H_3PO_3$ as Foli-R-Fos 200, an aqueous solution containing 20% a.i. $H_3PO_3$ neutralized to pH 6.4–6.7 with equal amounts of KOH (U.I.M. Agrochemicals, Queensland, Australia), was used in all experiments. Copper oxychloride (50% a.i. CuOCl) and metalaxyl (25WP) or a formulated mixture of metalaxyl (4%) and mancozeb (64%) as Ridomil MZ-WP, (Ciba Geigy, Aust. Ltd., New South Wales, Australia) were used in various experiments as standard treatments. In some experiments, $H_3PO_3$ was tank-mixed with copper oxychloride immediately before application. The surfactant Citowet (100% alkylaryl polyglycol ether; BASF, Aust. Ltd., North Melbourne, Australia) at the recommended rate of 6 ml/100 L was used in all treatments.

Randomized block designs were used in all experiments. Data were analyzed using the statistical packages GLIM (Numerical Algorithms Group, Oxford, UK) and STATISTIX (NH Analytical Software, Roseville, MN). Weighted regression techniques were used to examine differences in treatment responses over time. Each parameter (for example, the intercept, linear, and quadratic coefficients) of a set of regression curves was tested for equality with an $F$ test. If this was significant, pairwise treatment comparisons of parameters (using Waller-Duncan's $k$-ratio $t$ test) followed.

In some trials it was not relevant to consider relationships over time but rather to compare treatments at particular times. Data of this type were analyzed by first performing an overall test for equality using a chi-square or $F$ test as appropriate. If these were significant, then pairwise treatment comparisons were carried out with $Z$ or $t$ tests.

**Experiment 1—Loxton.** On 11 November 1988, the youngest five fully expanded leaves (>4 cm in diameter) and two bunches on each of four shoots per vine (cv. Shiraz) were inoculated. Single-vine replicates, separated by barrier vines, were each sprayed to run-off once with either 1.2 g/L of $H_3PO_3$, 1.2 g/L of $H_3PO_3$ mixed with 1.25 g/L of copper oxychloride or the formulated mixture of 112 mg of metalaxyl and 1.8 g of mancozeb per liter.

Fungicide treatments were replicated three times and applied at 7, 10, 12, or 14 days after inoculation. Three replicates each of six inoculated vines were left unsprayed as controls. Twenty-four days after inoculation, leaves and bunches were removed from the vine, sealed in moistened plastic bags, and incubated overnight at 23 C ± 1 C to induce sporulation of diseased tissue. The following morning, the incidence of leaves with oilspots was recorded and the extent of sporulation of oilspots as a proportion of oilspot area was rated on

a scale of 0–3 where 1 = sporulation on <1% of the area of oil spot, 2 = 1–25% sporulation, and 3 = 26–100% sporulation.

The production of sporangia in categories 2 and 3 was quantified by selecting 10 sporulating oilspots from each category, measuring the area of each sporulating lesion, and washing the lesions in water. A hemacytometer was used to determine concentrations of sporangia.

**Experiment 2—Nuriootpa.** This experiment was conducted on cv. Grenache vines in April 1988. $H_3PO_3$ at either 1 or 2 g/L was applied to vines at either 1, 3, or 7 days after inoculation. A mean of 16 leaves per shoot on at least five shoots per vine were inoculated on single-vine plots which were replicated four times. Metalaxyl at 250 mg/L was applied to inoculated shoots for comparison. Seven days after the final spray, inoculated shoots were removed and sporulation was induced as previously described. Sporulation was assessed by recording the number of sporulating leaves per shoot.

**Experiment 3—Nuriootpa.** This experiment was also conducted on cv. Grenache to which either 1.2 g/L of $H_3PO_3$, 1.2 g/L of $H_3PO_3$ mixed with 1.25 g/L of copper oxychloride, or 112 mg of metalaxyl plus 1.8 g of mancozeb per liter was applied at either 5, 8, 13, 16, 19, or 22 days after inoculation. A mean of 10 leaves per shoot on five shoots per vine on each of three replicate vines per treatment were inoculated on 9 November 1988. Inoculated shoots were removed 7 days after the last spray and placed in moistened plastic bags to induce sporulation. The extent of necrosis and sporulation was rated on both leaves and flower clusters of inoculated shoots.

**Experiment 4—Nuriootpa.** This experiment was carried out on cv. White Grenache to which the same fungicide treatments as experiment 3 were applied to a similar number of vines. A mean of 13 leaves per shoot on five shoots per vine were inoculated on 22 November 1988 and fungicides were applied at either 6, 9, or 13 days after inoculation. Sporulation was induced as previously described and the extent of sporulation was assessed as a percentage of the total inoculated leaf area.

**Experiment 5—Northfield.** Shoots of cv. Mataro vines were inoculated with *P. viticola* on 10 November 1988 and sprayed 4, 7, and 12 days later with the same fungicides as used in the Loxton experiment. Treatments were applied to single-vine plots replicated six times. A mean of three leaves per shoot on four shoots per vine were inoculated. Treated vines were separated by an unsprayed barrier vine. Shoots were removed from the vines 20 days after inoculation and sporulation was induced and assessed. The incidence of necrotic flower clusters and the production of sporangia on the clusters was also assessed.

## RESULTS

**Experiment 1—Loxton.** Oilspots were first seen 14 days after inoculation, but their incidence was low and variable. For example, on unsprayed vines, oil spots developed on between 7.3% and 35% of the inoculated leaves. Analyses of data indicated that metalaxyl-mancozeb and $H_3PO_3$ with copper treatments applied before oilspot appearance (7 days after inoculation) significantly ($P < 0.05$) reduced the number of leaves with oil spots. $H_3PO_3$ alone was effective only on vines sprayed 10 days after inoculation while a varying response was observed with regard to the level of sporulation (Table 1).

No treatment reduced the number of oil spots per leaf when applied later than 10 days, nor sporulation later than 12 days after inoculation. The level of infection on bunches was low and variable and not suitable for statistical analysis.

Oilspots with sporulation rated category 3 had an average of 630 sporangia/mm$^2$, and category 2 oilspots had 460 sporangia/mm$^2$; these differences were not significant. Sporangia from category rating 1 were too few to measure.

**Experiment 2—Nuriootpa.** Sprays were applied in late April when leaf senescence was beginning and leaves were less susceptible to infection. As a result, sporulation on infected leaves was sparse and only the incidence of sporulating leaves was recorded. Nevertheless, large numbers of leaves on unsprayed vines were infected, resulting in a mean of almost six infected leaves sporulating per shoot. $H_3PO_3$ at 1 or 2 g/L and metalaxyl almost completely inhibited sporulation when applied 3 days after inoculation (Table 2). Seven days after inoculation, metalaxyl was more inhibitory to sporulation than $H_3PO_3$, although each fungicide significantly reduced the incidence of sporulating leaves compared to the control.

**Experiment 3—Nuriootpa.** High levels of infection were achieved on unsprayed vines where sporangia developed on over a third of the leaf area on most leaves on inoculated shoots. In contrast, the metalaxyl-mancozeb formulation applied 5 days after inoculation was the most effective treatment as sporulation on leaves was completely inhibited (Fig. 1). $H_3PO_3$ with or without copper oxychloride was less inhibitory at this time. Sporangia developed on a mean of four leaves per $H_3PO_3$-treated shoot with approximately 12% of the leaf area covered with spores.

The efficacy of all fungicides decreased with time as shown by the regression of incidence of sporulating leaves expressed as a percentage of the control (Fig. 1). Fitted values from the regression equations ($r^2 = 0.96$) are as follows: Metalaxyl-mancozeb, $y = -32.79 + 6.57x$; $H_3PO_3$ + copper, $y = 21.01 + 4.67x$; and $H_3PO_3$, $y = 31.46 + 2.55x$, where $y$ = disease incidence (as percentage of control) and $x$ = number of days after inoculation.

**Table 1.** The effect of fungicides applied after inoculation on the incidence and sporulation of *Plasmopara viticola* on grape cv. Shiraz at Loxton, December 1988

| Treatment rate (g a.i./L) | Spray timing (days after inoculation)[y] | | | | | |
|---|---|---|---|---|---|---|
| | Disease incidence[z] | | | Sporulation[z] | | |
| | 7 | 10 | 12 | 7 | 10 | 12 |
| Metalaxyl-mancozeb (0.112 + 1.8) | 0.0 a | 14.0 ab | 8.9 a | 0.00 a | 0.24 a | 0.11 a |
| $H_3PO_3$ + Cu (1.2 + 1.25) | 3.6 a | 10.9 ab | 18.3 a | 0.07 a | 0.18 a | 0.30 ab |
| $H_3PO_3$ (1.2) | 23.7 b | 5.2 a | 22.2 a | 0.36 b | 0.10 b | 0.54 bc |
| Unsprayed | 19.8 b | 19.8 b | 19.8 a | 0.72 b | 0.25 a | 0.92 c |

[y] Within columns, different letters denote significant differences ($P < 0.05$). $Z$ statistics were used in pair-wide comparisons of incidence and $t$ statistics for weighted mean sporulation scores.

[z] Assessments of percentage inoculated leaves diseased and mean sporulation rating per leaf were mean scores from 60 leaves from each sprayed treatment and 360 leaves from untreated vines.

**Table 2.** The effect of $H_3PO_3$ and metalaxyl applied 1, 3, or 7 days after inoculation on the incidence of sporulation of *Plasmopara viticola* on grape cv. Grenache at Nuriootpa, April 1988

| Treatment rate (g a.i./L) | Spray timing (days after inoculation) | | |
|---|---|---|---|
| | 1 | 3 | 7 |
| Metalaxyl (0.25) | 0.05[z] | 0.05 | 0.05 |
| $H_3PO_3$ (1) | 1.5 | 0.33 | 2.60 |
| $H_3PO_3$ (2) | 0.40 | 0.85 | 1.50 |
| Unsprayed | 5.80 | 5.80 | 5.80 |
| LSD : $P < 0.05$ | 1.7 | 1.60 | 1.50 |

[z] Values are mean number of sporulating leaves per shoot from a maximum of 17 leaves per shoot.

Overall, the efficacy of $H_3PO_3$ with or without copper was less than that of the metalaxyl-mancozeb formulation.

Necrosis and/or sporangia were evident on 56% of inoculated clusters on unsprayed shoots. All fungicides applied 5 days after inoculation prevented necrosis of flower clusters and when applied up to 13 days after inoculation restricted flower infection to 3%. Fungicides applied from 16 to 22 days after inoculation gave some control but were less effective with 20–30% of clusters diseased.

**Experiment 4—Nuriootpa.** Sporulation covering slightly more than a third of the leaf area developed on a mean of 12.5 leaves on unsprayed vines (Table 3). When applied 6 days after inoculation, all treatments significantly reduced both the number of sporulating leaves and the area of sporulation, however, the metalaxyl-mancozeb formulation was the most effective treatment. Sparse sporulation developed on a mean of 2.5 leaves per shoot in the metalaxyl-mancozeb treatment compared to sporulation on more than six leaves per shoot on the $H_3PO_3$ treatments. When applied 9 days after inoculation, metalaxyl-mancozeb and $H_3PO_3$ without copper reduced the numbers of sporulating leaves, and all treatments significantly inhibited the area of sporulation when compared to the unsprayed control.

Fungicides applied 13 days after inoculation did not reduce the number of sporulating leaves but all significantly inhibited the area of leaf sporulating compared to the control.

The addition of copper oxychloride did not affect the activity of $H_3PO_3$ except for the application 9 days after inoculation where an increase in the number of sporulating leaves and the area of leaf sporulating occurred.

**Experiment 5—Northfield.** In this experiment, 68% of inoculated shoots on unsprayed vines developed sporulating oilspots with a mean of 2.3 sporulating leaves per shoot (Table 4). The metalaxyl-mancozeb treatment and $H_3PO_3$ with or without copper oxychloride almost completely inhibited sporulation when applied at either 4 or 7 days after inoculation. Fewer than two leaves with sporulation were detected on all shoots of the 4- or 7-day treatments. At 12 days after inoculation, $H_3PO_3$ with or without copper oxychloride severely inhibited sporulation. By contrast, the formulation of metalaxyl-mancozeb applied at this time was less effective as sporulation developed on a mean of 1.4 leaves per shoot (Table 4).

Neither necrosis nor sporulation developed on most clusters sprayed with the $H_3PO_3$ or $H_3PO_3$ plus copper treatments up to 12 days after inoculation. Similar effects were found on clusters sprayed with the metalaxyl-mancozeb formulation 4 and 7 days after inoculation, but at 12 days, necrosis and/or sporulation developed on 65% of the clusters. On unsprayed vines, sporangia developed on 75% of the flower clusters, most of which developed extensive necrosis.

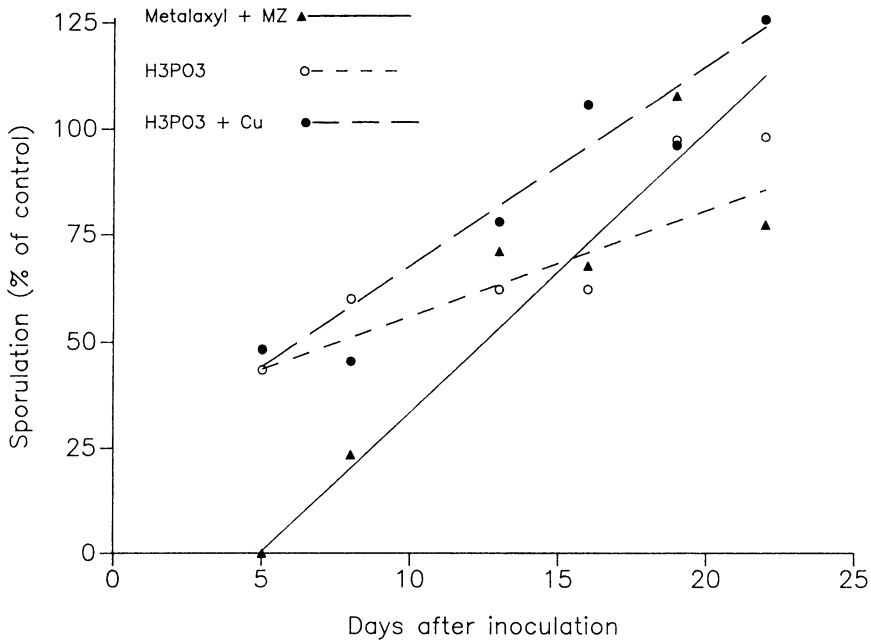


**Fig. 1.** The effects of single sprays of fungicides applied between 5 and 22 days after inoculation on the incidence of sporulation of *Plasmopara viticola* on leaves of cv. Grenache at Nuriootpa, November 1988.

**Table 3.** The effect of fungicides applied 6, 9, and 13 days after inoculation on sporulation of *Plasmopara viticola* on grape cv. White Grenache at Nuriootpa, December 1988

| Treatment rate (g a.i./L) | Spray timing (days after inoculation) | | | | | |
|---|---|---|---|---|---|---|
| | Sporulating leaves per shoot | | | Area of leaf sporulating[a] | | |
| | 6 | 9 | 13 | 6 | 9 | 13 |
| Metalaxyl-mancozeb (0.112 + 1.8) | 2.5 | 6.4 | 15.3 | 0.6 | 10.1 | 5.1 |
| $H_3PO_3$ (1.2) | 7.3 | 6.4 | 14.9 | 16.5 | 13.9 | 17.3 |
| $H_3PO_3$ + Cu (1.2 + 1.25) | 6.7 | 10.7 | 12.8 | 16.3 | 22.7 | 15.3 |
| Unsprayed | 12.5 | 12.5 | 12.5 | 35.0 | 35.0 | 35.0 |
| LSD : $P = 0.05$ | 2.6 | 3.0 | 3.0 | 7.5 | 7.7 | 6.5 |

[a] Percent of leaf area with sporulating oilspots.

**Table 4.** The effect of fungicides applied 4, 7, and 12 days after inoculation on the incidence of sporulation of *Plasmopara viticola* on grape cv. Mataro at Northfield, November 1988

| Treatment rate (g a.i./L) | Spray timing (days after inoculation) | | |
|---|---|---|---|
| | 4 | 7 | 12 |
| Metalaxyl-mancozeb (0.112 + 1.8) | 0.04[a] | 0.01 | 1.4 |
| $H_3PO_3$ (1.2) | 0.08 | 0.3 | 0 |
| $H_3PO_3$ + Cu (1.2 + 1.25) | 0.1 | 0 | 0.004 |
| Unsprayed | 2.3 | 2.3 | 2.3 |
| LSD : $P = 0.05$ | 0.66 | 0.66 | 0.8 |

[a] Values are numbers of sporulating leaves per shoot.

## DISCUSSION

These results show that $H_3PO_3$ applied after infection significantly inhibits the development of *P. viticola* on both leaves and flower clusters. In some experiments, the effect of $H_3PO_3$ applied 7 to 12 days after infection was equivalent to or better than that achieved with metalaxyl or a metalaxyl- mancozeb mixture applied at a similar time. In other experiments, however, the effect of $H_3PO_3$ applied 7 days or less from inoculation was less effective than metalaxyl. The postinfection activity of $H_3PO_3$ was demonstrated on four cultivars of *V. vinifera*; whether similar activity occurs on American

varieties and hybrids of *Vitis* needs to be evaluated.

All experiments showed that either metalaxyl or $H_3PO_3$ applied after infection reduced the incidence of disease and the level of sporulation, indicating that postinfection applications of either of these fungicides should effectively reduce the production of inoculum and the consequent spread of disease.

Currently, $H_3PO_3$ is registered for postinfection use on vines in Australia and is approximately two-thirds the cost of metalaxyl. Therefore, it offers a relatively inexpensive alternative to metalaxyl and related fungicides. The mode of action of $H_3PO_3$ is also likely to differ from that of metalaxyl and other phenylamide fungicides. Alternating these fungicides in spray programs should reduce the likelihood of populations of *P. viticola* developing resistance to either fungicide. $H_3PO_3$-copper oxychloride mixtures were evaluated in these experiments because previous work (4) had shown that the protectant activity of $H_3PO_3$ was limited to less than 7 days after application, probably because of the rapid translocation of $H_3PO_3$ from sprayed leaves. The use of a $H_3PO_3$-copper oxychloride mixture should provide at least 7-day postinfection and 14-day protective activity for each application. The present experiments showed that the postinfection activity of $H_3PO_3$ was in most cases unaffected by the addition of copper oxychloride. This was not unexpected because previous studies (5) had shown copper oxychloride to have no postinfection activity.

These studies have shown that the use of either $H_3PO_3$ or metalaxyl can control *P. viticola* when applied after infection. Efficient use of either of these materials will rely on the development of disease forecasting systems that monitor climatic conditions and accurately predict the occurrence of disease.

The application of $H_3PO_3$ alone could be effective if applications were made within a few days from the start of an infection period. In many viticultural areas, accurate disease forecasting systems are not yet available, and the most appropriate use of $H_3PO_3$ would be in a mixture with copper oxychloride or another protectant fungicide such as mancozeb.

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