J. Natn. Sci. Coun. Sri Lanka 1986 14(1) : 145–156

EFFECTS OF FUNGICIDES ON THE DEVELOPMENT OF DOWNY MILDEW DISEASE OF GRAPE VINE CAUSED BY *PLASMOPARA VITICOLA* (BERK. & CURT.) BERL. & DE TONI

N. RAMANATHAN AND A. SIVAPALAN
Department of Botany, University of Jaffna, Jaffna, Sri Lanka.

(Date of receipt : 16.09.85)
(Date of acceptance : 10.09.86)

Abstract : Experiments with sixteen locally available fungicides to control downy mildew disease showed that captan, copper sandoz and thiovit were highly toxic to germination of sporangia. These fungicides acted with an ED_{50} value of < 10 ppm. Germination was completely suppressed with copper sandoz at 100 ppm and with captan, thiovit and pomarsol at 500 ppm. Most of the fungicides tested in this experiment prevented germination at 1000 ppm. Antracol, benlate, brassicol, daconil, bayleton, baycor and sulphur were not very active against germination of sporangia but greatly reduced the development of infection by the fungus, even at low concentrations like 10 ppm. All fungicides prevented development of infection at 1000 ppm. However, copper sandoz, difolatan, trimilton and vondozeb at 1000 ppm were highly effective in curing the already existing young lesions. Sporulation did not occur on leaves treated with any of the fungicides at 500 ppm and above.

1. Introduction

Downy mildew is a destructive fungal disease of grape vine in Sri Lanka. When no protective control measure is employed this malady which has posed a serious threat to the vine growers in Jaffna, can easily ruin the crop in one season. The disease has been found to appear during humid weather in the rainy season from October to March. The infection as observed by Ramanathan and Sivapalan[9] has been found to appear in almost all the vineyards in the Jaffna region; first on the mature leaves, then it spreads to the younger leaves and other parts of the grape vine such as berries, inflorescences, tendrils and young stems. It produces a white downy growth on the lower leaf surface which later becomes necrotic. At later stages of infection, the entire leaf is covered with the fungus, becomes dry and crumpled and finally drops from the plant. Inflorescences affected become withered and stop developing into fruits. The berries affected form brown patches of infection on the surface, become shrivelled and finally shed from the clusters. The affected vine plants showed severe defoliation and fruit fall. There was gradual weakening of vine plants and the yield was reduced. Although the life span of the grape vine is about twenty years, severe yearly occurrence of downy mildew infection greatly reduces its productivity; thereby farmers are forced to destroy the plants in 7–8 years.

Previous studies show that chemical treatment to control downy mildew started with the discovery of Bordeaux[5] and this has been considered as the most effective fungicide in the control of downy mildew.[1,2,3,6,7,11] In addition, many copper salts including the sulphates, oxychlorides and hydroxides were used widely throughout the world.[4] Several organic compounds such as mangozob, folpet (folpel) captafol, captan, dichlorofluanid, mancopper, maneb, metiram – Zinc, propineb and zineb were also found to be effective for the control of downy mildew.[4,6] Difolatan has been found to be significantly superior to the other dithiocarbamates.[10]

Several workers claimed that some fungicides singly or in combination with others have penetrating effects which could last for several days. Metalaxyl or ridomil which is available in a mixture with folpet or copper oxychloride is systemic with an additional property of diffusing as a vapour into moist atmosphere and is capable of eradicating infection throughout.[4]

The recommended dosage of some of the locally available fungicides vary between 2500 ppm – 3500 ppm.

The present investigation is aimed at determining the performances of some of the locally available fungicides on the downy mildew fungus under laboratory conditions.

Table 1 : List of fungicides

Product name	Active ingredient	Manufacturer or distributor
Antracol (Propineb)	Zinc propylene bis dithiocarbamate	Haechem Ltd.
Baycor	Dimethyl thiophanate	Ciba–Geigy
Bayleton (Triadimefon)	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1 H 1, 2, 4-triazole-1-yl)-2 butanone	Haechem Ltd.,
Benlate (Benomyl)	Methyl 1-(butyl carbamoyl)	Lankem (Ceylon) Ltd.
Brassicol (Quintozene)	Pentachloro nitro benzene	Ciba–Geigy
Captan (Orthocide)	N-trichloromethyl marcapto-4-cyclohexene-1,2, dicarboximide	Baur & Co.
Copper sandoz	Cupric oxide and copper oxichloride	Baur & Co.,
Daconil (Chorothalonil)	Tetrachloro-isophthalontrile	Ciba-Geigy
Difolatan (Captafol)	N (1,1,2,2, tetrachloro ethyl thio 4-cyclo hex-4-ene)-1,2 dicarboximide	Ciba-Geigy
Morestan (Quinomethionate)	6 methyl quinoxaline (25%) 2,3 dithiocarbamate (2 – 3%)	Haechem Ltd.,
Morut	Feraminosulf + Pentachloro nitro-benzene	Ciba–Geigy

Contd.

Product name	Active ingredient	Manufacturer or distributor
Pomarsol	1,2 di(3-methoxy carbonyl −2 thiordio benzene	Haechem Ltd.
Sulphur	Inorganic sulphur	Haechem Ltd.
Thiovit (Sulphur)	Inorganic sulphur	Baur & Co.
Trimilton		Baur & Co.
Vondozeb (Dithiocarbamate)	Ethylene 1,2 bis dithio carbamate, Zn, Mn.	Baur & Co.

2. Materials and Methods

Sixteen different locally available fungicides were selected and used throughout the investigations. The fungicides used and their chemical composition are listed in Table1.The effects of these fungicides at concentrations 0 ppm, 100 ppm, 500 ppm and 1000 ppm were studied separately on the germination, infection and sporulation stages of the fungus under laboratory conditions. Also the effects of combinations of compatible fungicides were investigated. Fungicide solutions of concentration 1000 ppm were first prepared in sterile distilled water in sterile glass containers and then were diluted to give other concentrations. Mature healthy leaves detached from grape vine were first inoculated with a sporangial suspension of concentration 2×10^5 sporangia per ml by means of a hand sprayer, allowed to air dry and then sprayed with fungicide solutions of different concentrations. The petioles of the leaves were carefully wrapped with moist cotton wool prior to inoculation and application of fungicides to avoid sporangia and fungicides getting into the cut end of petiole. These leaves were then placed on moist filter papers in plastic trays, sealed with a polythene paper and were incubated at 25°C and 100 per cent relative humidity in an illuminated incubator. At the end of the required incubation period the leaves were assessed for different stages of fungal development. The incubation period was usually twenty four hours for germination studies, six days for development of infection and eight days for sporulation. The treated leaves were removed from the incubator after the required period of incubation. Sellotape impressions of the inoculated surface were prepared and stained with cotton blue in lactophenol on a glass slide. These impressions were observed under the microscope (x 400) and the percentage germination of sporangia was determined from counts of 200 – 300 sporangia in five microscopic fields, randomly selected from each of the leaves. The amount of infection on leaves treated with fungicides was determined by measuring the area of the path under the microscope (x 40). This was expressed as mean percentage area of infection. To assess the sporulation, incubated leaves were shaken with known volume of sterile water and the number of sporangia produced per sq. mm area of the leaf was determined by using a haemo-cytometer.

3. Experiments and Results

3.1 Germination of sporangia

3.1.1 Effects of individual fungicides

The sets of inoculated leaves treated with fungicide solutions and the untreated leaves were incubated under optimal conditions for twenty four hours and then observed for germination of sporangia. The values for mean percentage germination are given in the form of a histogram. The results in Figure 1 show that all sixteen fungicides were effective in reducing the amount of germination at all concentrations tested. With increase in concentration of fungicide the amount of germination decreases and almost reaches the zero level at 500 ppm with captan, copper sandoz, morut, pomarsol and thiovit. Copper sandoz effectively prevented germination of sporangia at even 100 ppm. This experiment shows that among the sixteen fungicides tested copper sandoz, captan, morut, pomarsol and thiovit at concentration of 500 ppm were highly toxic to sporangia of *Plasmopara viticola*. The ED_{50} values calculated for each fungicide shows (Table 2) that the order of effectivity of fungicides on germination of sporangia is copper sandoz = thiovit > captan > pomarsol > sulphur > daconil > morut > difolatan > antracol = brassicol = vondozeb > baycor = bayleton = benlate = trimilton > morestan.

Table 2. Effects of fungicides on germination of sporangia of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h., after 24 hours of incubation.

Fungicide	ED_{50} (ppm)
Antracol	140
Baycor	160
Bayleton	160
Benlate	160
Brassicol	140
Captan	6
Copper sandoz	3
Daconil	40
Difolatan	120
Morestan	530
Morut	110
Pomarsol	10
Sulphur	25
Thiovit	3
Trimilton	160
Vondozeb	140

Table 3: Effects of different combinations of compatible fungicides on germination of sporangia of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h., after 24 hours of incubation.

		Mean % germination (probits)					
	Concentration (ppm)	Antracol	Benlate	Daconil	Difolaton	Morestan	Sulphur
	10	4.6134	4.5310	4.5780	4.8040	4.8170	4.5664
	100	4.3455	4.4422	4.0537	4.3412	4.6308	3.6660
	1000	0	0	0	0	0	0
Antracol	10		4.4799	4.5903	4.3992	4.4670	3.5849
	100		3.9572	3.7139	4.0903	4.2556	3.6035
	1000		0	0	0	0	0
Benlate	10			4.3826	4.4417	4.7063	4.4237
	100			3.9437	4.1494	3.9994	3.4045
	1000			0	0	0	0
Daconil	10				4.2312	4.5770	
	100				3.9682	4.2074	
					0	0	
Difolatan	10					4.3878	
	100					4.4364	
	1000					0	

The probit value for mean % germination on untreated leaves (control) = 4.8498

Fungicide solutions of concentrations 10 ppm, 100 ppm, and 1000 ppm were mixed with equal volumes of another compatible fungicide of the same concentration to give the following combinations: antracol – benlate, antracol – daconil, antracol – difolatan, antracol – morestan, antracol – sulphur, benlate – difolatan, benlate – daconil, benlate – morestan, benlate – sulphur, daconil – difolatan, daconil – morestan and difolatan – morestan. The sets of inoculated leaves treated with different combinations of compatible fungicides and individual fungicides of the same volume and concentration were incubated under optimal conditions. The values for mean percentage germination obtained in this experiment were compared with those obtained with individual fungicide solutions. Table 3 shows that the combinations antracol–sulphur, antracol–daconil and benlate–sulphur effectively reduced the amount of germination at the concentrations tested. All combinations were toxic to sporangia at 1000 ppm and prevented germination completely. Antracol, benlate, difolatan, morestan and sulphur produced better effects when used in combination with any of the above compatible fungicide. The order of effectivity for compatible combination of fungicide was observed to be as follows: benlate–sulphur > antracol–sulphur > antracol–daconil.

Table 4. Effects of fungicides on the development of infection of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h. after 6 days of incubation.

Fungicides	Mean % area of infection Fungicide concentration (ppm)				ED_{50} (ppm)
	10	100	500	1000	
Antracol	6.97	1.25	0.02	0	20
Baycor	1.63	0.04	0.01	0	10
Bayleton	0.45	0.22	0.08	0	8
Benlate	11.05	0	0	0	15
Brassicol	1.00	1.00	0	0	10
Captan	0.003	0	0	0	3
Copper sandoz	0.50	0.25	0	0	8
Daconil	1.11	0.42	0.21	0	15
Difolatan	31.60	17.51	1.60	0	530
Morestan	32.92	13.47	0.26	0	300
Morut	29.34	21.88	0.26	0	190
Pomarsol	35.07	0	0	0	25
Sulphur	3.64	1.82	0.85	0	280
Thiovit	11.22	0	0	0	15
Trimilton	15.25	3.39	2.32	0	530
Vondozeb	47.08	34.40	0	0	200

Mean percentage area of infection on untreated leaves (control) = 65.17

3.2 Development of infection

3.2.1 Effects of fungicides on establishment of infection

Mature healthy leaves first inoculated and air dried were then sprayed with fungicide solutions of different concentrations and incubated together with the untreated leaves which have been sprayed with sterile distilled water, under optimal conditions for six days. The amount of infection was measured at the end of incubation period. The results reveal (Table 4) that all sixteen fungicides effectively prevented the development of infection at 1000 ppm. The fungus failed to infect the host leaves which were treated with benlate, captan, pomarsol or thiovit at the concentrations 100 ppm and above. The area of infection was greatly reduced with bayleton, brassicol, captan and copper sandoz even at low concentration like 10 ppm: The order of effectivity of fungicides on the development of infection was found to be captan > bayleton = copper sandoz > brassicol = baycor > daconil = benlate = thiovit > antracol > pomarsol > morut > vondozeb > sulphur > morestan > difolatan = trimilton.

Table 5. Effects of fungicides on the development of already existing infection of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h., after 4 days of incubation.

Fungicide	Mean % increase in area of infection Fungicide concentration (ppm)		
	10	100	1000
Antracol	106.38	28.24	14.13
Baycor	78.69	24.65	4.69
Bayleton	44.32	35.11	4.79
Benlate	44.06	40.60	10.09
Brassicol	37.17	38.01	17.40
Captan	23.51	32.75	8.95
Copper sandoz	30.08	11.82	0
Daconil	22.2	48.98	12.34
Difolatan	35.15	61.43	0
Morestan	35.53	23.94	13.11
Morut	49.81	25.96	16.55
Pomarsol	34.96	23.61	0.31
Sulphur	21.79	18.47	3.07
Thiovit	54.68	36.92	6.17
Trimilton	46.14	9.03	0
Vondozeb	21.29	19.69	0

The mean percentage increase in area of infection on untreated leaves (control) = 100.38

3.2.2 Effects of fungicides on spreading of established infection

Mature leaves with young lesions were collected from the field and the initial diameters of lesions on each of the leaves were measured. These leaves were then grouped into sets of five, sprayed with fungicide solutions of different concentrations placed on moist filter papers in plastic trays sealed with polythene paper and incubated under optimal conditions for four days. A control was set up by spraying a set of leaves with sterile distilled water. The final diameters of the lesions were measured at the end of incubation period and the percentage increase in area of lesion, if any, was determined. The results indicated in Table 5 show that all fungicides were effective in reducing the rate of increase in area of the already existing lesion at the concentrations tested Copper sandoz, trimilton, difolatan and vondozeb were very effective in preventing further increase in size of lesions at 1000 ppm. The lesions treated with the above fungicides dried up, became brown and necrotic. The sporangia and sporangiophores already present also became dry and distorted and curled up. The fungus was completely killed with these fungicides. From this experiment it could be concluded that copper sandoz, difolatan, trimilton and vondozeb are good eradicant fungicides of high effectivity.

Table 6. Effects of fungicides on sporulation of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h., after 8 days of incubation.

Fungicide	Number of sporangia produced/mm^2 area of host Fungicide concentration (ppm)			
	10	100	500	1000
Antracol	9.42×10^2	1.07×10^2	0	0
Baycor	1.26×10^3	1.26×10^3	0	0
Bayleton	1.26×10^3	3.14×10^2	0	0
Benlate	1.09×10^4	8.79×10^2	0	0
Brassicol	5.59×10^4	8.16×10^2	0	0
Captan	1.19×10^3	8.16×10^2	0	0
Copper sandoz	3.77×10^2	1.13×10^2	0	0
Daconil	1.0×10^3	4.39×10^2	0	0
Difolatan	5.27×10^3	1.44×10^3	0	0
Morestan	1.5×10^3	2.0×10^2	0	0
Morut	9.42×10^2	1.07×10^2	0	0
Pomarsol	1.44×10^3	1.0×10^3	0	0
Sulphur	1.82×10^3	1.13×103	0	0
Thiovit	8.16×10^2	3.77×10^2	0	0
Trimilton	1.0×10^3	5.6×10^2	0	0
Vondozeb	5.65×10^3	8.79×10^2	0	0

The number of sporangia produced per sq mm area of the untreated host leaf (control) = 4.36×104·

3.3 Sporulation

3.3.1 Effects of different fungicides

Mature healthy leaves first inoculated, air dried and then sprayed with fungicide solutions of different concentrations were incubated under optimal conditions for eight days. At the end of the incubation period, the sporulation was assessed on these leaves. Sporulation was expressed as number of sporangia produced per sq. mm area of the host leaf. The results reveal (Table 6) that no sporulation occurred on leaves treated with any of the sixteen fungicides at concentrations 500 ppm and above. Antracol, copper sandoz and morut were very effective in reducing the amount of sporulation even at low concentrations like 10 ppm and 100 ppm. The sporangia and sporangiophores produced were small and the lesions on which they were produced were dry and brown.

Table 7. Effects of combinations of compatible fungicides on sporulation of *Plasmopara viticola* on leaves of grape vine at 25°C and 100% r.h. after 8 days of incubation.

Combination	Sporangial production/mm^2 area of host leaf Fungicide concentration (ppm)		
	10	100	1000
Antracol – Benlate	6.15×10^3	2.5×10^3	0
Antracol – Daconil	1.29×10^4	4.6×10^3	0
Antracol – Difolatan	1.50×10^4	5.58×10^3	0
Antracol – Morestan	9.53×10^3	1.35×10^4	0
Antracol – Sulphur	6.34×10^3	1.57×10^3	0
Benlate – Daconil	5.30×10^3	3.9×10^3	0
Benlate – Difolatan	6.78×10^3	2.26×10^3	0
Benlate – Morestan	5.96×10^2	2.95×10^2	0
Benlate – Sulphur	2.32×10^3	9.41×10^2	0
Daconil – Difolatan	1.17×10^4	5.09×10^3	0
Daconil – Morestan	1.4×10^4	5.3×10^3	0
Difolatan – Morestan	6.03×10^3	3.08×10	0

The number of sporangia produced/mm^2 area of untreated leaves (control) = 4.36×10^4

3.3.2 Effects of combinations of compatible fungicides

Inoculated and air dried leaves were sprayed with fungicide solutions of different combinations of different concentrations. These leaves were then incubated under optimal conditions for eight days and then observed for sporulation. Data obtained on the number of sporangia produced per sq. mm area of the host leaf (Table 7) showed that all combinations of compatible fungicides were effective in reducing the amount of sporulation even at 10 ppm, and preventing sporulation completely at 1000 ppm. However, the values obtained with fungicide mixtures were similar to those obtained with corresponding individual fungicides. The combinations also had some effects on the morphology of the sporangia and sporangiophores produced. The sporangia and sporangiophores were small and short respectively and the lesions on which they were produced were dry and brown.

4. Discussion

Experiments with sixteen commonly available fungicides to control downy mildew disease showed that captan, copper sandoz and thiovit were highly effective at low concentrations in suppressing all three stages of development of the fungus, the germination, infection and the sporulation. The sporangia of *P. viticola* were very sensitive to copper sandoz even at low concentrations. Germination was completely suppressed with copper sandoz at 100 ppm, morut, captan, thiovit and pomarsol at 500 ppm. All fungicides tested in this experiment except bayleton and brassicol prevented germination at 1000 ppm. Pomarsol completely suppressed mycelial development at concentrations 100 ppm and above and prevented germination at concentrations 500 ppm and above but, it did not have remarkable adverse effects on sporulation of the fungus. As far as the development of infection is concerned captan, with very high toxicity towards mycelial development, allowed negligible amounts of infection even at 10 ppm and allowed no growth at and above 500 ppm. Apart from this, copper sandoz at very low concentrations greatly reduced the development of infection. Benlate, pomarsol and thiovit prevented mycelial development at and above 100 ppm while brassicol, copper sandoz, morut and vondozeb showed similar results at concentrations $\geqslant$ 500 ppm. No sporulation occurred on leaves treated with any fungicide at concentrations 500 ppm and above. Antracol, copper sandoz and morut were highly inhibitory to formation of sporangia and sporangiophores even at low concentrations like 10 ppm and 100 ppm. Also these fungicides altered the morphology of sporulating structures. The sporangiophores were shorter and the sporangia were smaller than those produced on control leaves. The lesions became dry and brown.

Captan, copper sandoz, thiovit and pomarsol could be used to control the fungus at germination level or as good protectant fungicides at concentration of 500 ppm.

Antracol-daconil, antracol-sulphur and benlate-sulphur are some of the very effective mixtures or compatible combinations of fungicides which greatly reduced the amount of germination than when they tried individually. But, the different combinations of compatible fungicides did not have any enhanced effect on sporulation of the fungus.

The evaluation of fungicides on young lesions showed that copper sandoz, difolatan, trimilton and vondozeb have eradicating effects on the fungus. These four fungicides at 1000 ppm, completely eradicated the infection already produced on the vine leaves, on the fourth day after application and killed the fungus completely. The lesions turned brown and necrotic.

Captan and copper sandoz which are the most toxic fungicides to the fungus acted with an ED_{50} value < 10 ppm on germination of sporangia and greatly suppressed all three developmental stages. A mixture of antracol-sulphur was the best among the combinations of compatible fungicides and this was found to work on the disease with economy and protect the vine plants from the downy mildew disease.

Acknowledgement

The authors wish to thank the Natural Resources, Energy and Science Authority of Sri Lanka for funding the above project.

References

1. CHAMPAGNOL, F. (1973). Le cuivre, le mildiou et le couiure. (copper, mildew and abortion). *Progress Agricole et viticole* **90** (3) 51 – 59. Abstracts. *Review of plant pathology 53:41.* Cited *in "Evaluation of selected* fungicides for control of grape downy mildew". Pordesimo, A.N. (1976).

2. CHOHAN, J.S. (1965). *Punjab Horticultural Journal* **5**, 181 – 4.

3. EL. RIFAI, I.F. (1970). *Agric. Res. Rev.* Cairo **48** (3):1 – 9. *Review of Plant Pathology* **51:95.**

4. LAFON, R. & BULIT, J.C. (1981). *"Downy mildew of the vine".* The downy mildews ed. D.M. Spencer (1981) 28: 601 – 613.

5. MILLARDET', A. (1885). *J. Agric. Pratique.* 21st Nov. 1885. Cited in "Downy mildew of the vine". Lafon, R. & Bulit, J. (1981).

6. PORDESIMO, A.N. (1976). *Philippine phytopathology* **12**, 60 – 65. January and June, 1976.

7. RAMAKRISHNAN, T.S. & SUNDARAM, N.V. (1955). *Madras Agric. J.* 42, 108 – 15.

8. RAMANATHAN, N. (1984). *"Studies on the downy mildew disease of grape vine caused by Plasmopara viticola (Berk. & Curt.) Berl. & de Toni".* M. Phil. Thesis. University of Jaffna, Sri Lanka.

9. RAMANATHAN, N. & SIVAPALAN, A. (1982). *The development of downy mildew disease on grape vine".* 38th Annual Sessions of the Sri Lanka Association for the Advancement of Science. December, 1982. 43 pp.

10. SOHI, H.S. & SRIDHAR, T.S. (1972). "Chemical control of downy mildew *(Plasmopara viticola* Berkely and Curtis) and anthracnose *(Gloesporium ampelophagum* Curtis) diseases of grape vine."

11. YURUT, H.A. (1976). "Control trials against grape vine downy mildew (Plasmopara viticola Berlese and de Toni) in Central Anatoha. *Zirai Mucadele Arastirma Yilligi* (1976) No: **10, 24, 54** (Tr, Cn) Inst. Plt. Prot. Ankara, Turkey.