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Crop loss in grapes due to downy mildew infection on clusters at pre- and post bloom stages under non-epiphytotic conditions

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ABSTRACT

Severe necrosis of young clusters of grape cvs. Thompson, Tas-A-Ganesh and Sonaka was observed in many vineyards in Maharashtra resulting in about 20 to 80% mortality. The symptoms were similar to those caused by downy mildew disease, but there was no sporulation on or near the necrotic tissues. There was negligible leaf infection in these vineyards, and there was no preceding rainy period suggesting some other cause of necrosis. Investigations revealed structures resembling immature oospores in some of the infected clusters. Molecular detection using *Plasmopara viticola* specific primers confirmed that the mortality was due to downy mildew infection. Though the estimated possibility of downy mildew infection, based on the downy mildew forecasting module of Metwin 2 software was low to nil, analysis of weather parameters indicated that dew formation had led to build up of 95 to 100% RH and leaf wetness of minimum 4 to 12 h from near midnight to early morning hours. These environmental conditions supported infection, but not incubation and sporulation of *P. viticola*. Nutrient analysis showed that vines with high cluster mortality had significantly lower potassium content in petioles (1.18-1.57%) as well as in blades (0.90-1.10%), which further predisposed the vines to infection.

Key words: *Plasmopara viticola*, micro-satellite markers, cluster necrosis, *Vitis vinifera.*

INTRODUCTION

Downy mildew is a highly devastating disease of grapevines in Maharashtra affecting all commercial grape varieties. The disease is caused by *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni and occurs during warm, wet and humid weather conditions. Due to the serious economic losses under favourable environmental conditions, attempts are also ongoing to incorporate disease resistance in the susceptible cultivars (Sahijram *et al*., 10).

All green, photosynthetically active plant parts are susceptible to infection, which occurs only through the stomata and requires at least 2-3 h foliage wetness (Pearson and Goheen, 8). The fungus grows extensively within the host tissues and cells and emerges through the stomata to form the sporangiophores and sporangia. Hence, the incidence of the disease in vineyards can be easily made out by the presence of white, downy fungal growth on the infected surfaces (Fig.1a & b). Sexual reproduction is by production of oospores in the infected host material at the end of the growing season (Vercesi *et al*., 14). During 2006 and 2007, partial or complete necrosis of young clusters of Thompson Seedless and its mutants Tas-A-Ganesh and Sonaka was seen in many vineyards in Maharashtra. The problem was very serious as about 20-80% clusters were affected. Although the symptoms appeared similar to those caused by downy mildew disease, but due to the absence of sporulation on or near the necrotic tissues; almost negligible downy mildew infection on leaves; and, absence of a rainy period, it was generally presumed that the necrosis was due to toxicity of externally applied plant bio-regulators or nutrient inbalance(s). However, perusal of the vineyard spray schedules indicated that the symptoms were mainly present in vineyards where the growers had not applied systemic fungicides for control of downy mildew in the early growth stages. This indicated the possible involvement of downy mildew disease in cluster mortality and prompted us to study the problem in detail.

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MATERIALS AND METHODS

Clusters exhibiting total to partial mortality were collected from thirty vineyards of table grape cultivars Thompson Seedless, Tas-A-Ganesh or Sonaka grown on Dogridge rootstock from the Research Farm of NRC for Grapes, Pune; Khedgaon, Nasik; Palus, Sangli; and Malgaon, Miraj during November- December 2006 and 2007. The vines were trained to extended 'Y'-trellis and spaced at 6 × 10 feet. The vineyards were pruned at 5-7th node in the third or fourth week of October. At the Pune site, 25 clusters exhibiting reddish-pink discolouration on the rachis were also tagged and left on the vine for further observations.

The affected tissues from four bunches from each vineyard were observed under a binocular microscope (Leica MZ12.5) for presence of sporangiophores and sporangia of *P. viticola*. The affected tissues were also crushed, stained with either cotton blue or with 0.05% aniline blue and observed under normal or UV light in a compound microscope with epifluorescence facility (Leica DMLS model) (Navajas *et al*., 7). Similar observations were also made from green, apparently healthy tissues. Four affected bunches from each vineyard, with partial necrosis, were washed under running tap water and incubated in a humid chamber with > 95% RH and 26 ± 2^{0}C temperature for 4 days under darkness. The bunches were sprayed with sterile water as to maintain surface wetness. To rule out the possible involvement of any other pathogen(s), isolations were also done from the symptomatic tissue on either sterile potato dextrose agar (PDA) or nutrient agar (NA) following standard procedures.

Total DNA was extracted from affected bunches using modified CTAB method as described by Lodhi *et al.* (6). Total DNA extracted from leaf and bunch showing fungal sporulation and DNA from fungal spores was used as positive control, whereas total DNA from healthy leaves and bunch was used as negative control. Seven microsatellite primers specific to *P. viticola* (Delmotte *et al*., 3) were used for PCR amplification of DNA from affected, known infected as well as healthy tissues. The forward primers were fluorescently labelled. PCR reaction consisted of 10 ng DNA, 1 µM each of forward and reverse primers, 100 µM each dNTP and 0.5 U *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd.). PCR was carried out in Geneamp PCR system 9700 (Applied Biosystems, USA) using the following temperature profile : Initial denaturation at 94°C for 2 min. 35 cycles of denaturation at 94°C for 30 sec, annealing at recommended temperature for each primer for 30 sec and 72°C for 30 sec and final extension at 72°C for 2 min. PCR products were diluted 50 times and 1 µl of diluted mix was added to a mixture of 10 µl HI-DI formamide and 0.10 µl of GeneScan 500 ROX internal size standard. The mixture was denatured at 94°C for 5 min. and analyzed on ABI 3130 genetic analyzer using 36 cm capillary filled with POP7 polymer. GeneMapper ver 4.0 was used to determine the peak size using local southern method and allele call.

The nutrient contents of vines with high cluster mortality (>60% clusters infected) was also analysed and compared to the contents of healthy vines. Ten affected vines growing adjacent to a healthy vine were selected for sampling and the leaves opposite the clusters were collected. The contents of N, P, K, Na, Ca, Mg, Cu, Zn, Mn and Fe in the petioles and leaf blades were analysed by standard procedures (Tandon, 13). The samples were prepared by wet digestion procedure using diacid (sulphuric acid and perchloric acid in 9:4 ratio). Total N was analysed by Kjeldahl N determination method on Gerhardt distillation unit (model-Vapodest 30), P was estimated by ammonium heptamolybdate vanadate yellow colour method on Spectronic-20 spectrophotometer at 420 nm, and the other nutrients were analysed on atomic absorption spectrophotometer (Perkin Elmer model- Aanalyst 100). The mean nutrient content of healthy and affected vines was compared using students 't' test.

The weather data on canopy temperature, RH, rainfall, leaf wetness and solar radiation was recorded using µMetos automatic weather station at Pune. Downy mildew risk was estimated using downy mildew module of Metwin-2 software (Pessl Instruments). The model is a simulative model, which considers precipitation, actual period of leaf wetness, and temperature and humidity during the period of leaf wetness, to estimate the possibility of infection which is expressed as nil, low, medium or high. Then the temperature and humidity is considered for estimating incubation period Sporulation is expected when 100 per cent of incubation is reached. The software was validated after three years of field testing at the vineyards of NRC for Grapes (Anon., 1) and is being used successfully by growers in Maharashtra (Bhaskar and Sawant, 2).

RESULTS AND DISCUSSION

The symptoms at pre-bloom and flowering stages varied from complete necrosis of the cluster with dropping-off of the floral tissues at slight shaking (Fig. 1c) and necrosis of cluster tip or laterals (Figs. 1d, e & f). In post-berry-set stages, the symptoms varied from necrosis on the pedicle; the point of attachment of the laterals to the main rachis or of the berry to the pedicle (Fig. 1g); complete necrosis or brownish, reddish or pinkish discolouration of main rachis of clusters (Fig. 1h); and sunken brown spots on 5-11 mm size berries (Fig. 1i). The growth of clusters exhibiting reddish-pink discolouration, which were tagged and left on the vine for further observations, initially appeared unaffected but after a few weeks the portion of the bunch ahead of the discoloured area collapsed and the berries discoloured and dried out.

Sporangiophores and sporangia of *P. viticola* were not observed in any of the samples. Even after incubation in humid chamber there was no sporulation of *P. viticola*. Furthermore, there was no growth or sporulation of any other microorganism too on the affected areas and no microorganism was isolated either PDA or on NA. However, structures resembling immature oospores could be detected

Table 1. Microsatellite analysis of healthy and affected tissues of Thompson Seedless grape.

Sample	Marker	Allele 1	Allele 2	Marker	Allele 1	Allele 2
Fungal spores	PV14	121	123	PV13	221	221
Bunch with fungal sporulation	PV14	121	123	PV13	221	221
Leaf with fungal sporulation	PV14	121	123	PV13	221	221
Affected bunch 1	PV14	121	123	PV13	221	221
Affected bunch 2	PV14	121	123	PV13	221	221
Healthy bunch	PV14	NA		PV13	NA	
Healthy leaf	PV14	NA		PV13	NA	
Fungal spores	PV17	149	151	PV16	254	254
Bunch with fungal sporulation	PV17	149	151	PV16	254	254
Leaf with fungal sporulation	PV17	149	151	PV16	254	254
Affected bunch 1	PV17	149	151	PV16	254	254
Affected bunch 2	PV17	149	151	PV16	254	254
Healthy bunch	PV17	NA		PV16	NA	
Healthy leaf	PV17	NA		PV16	NA	
Fungal spores	PV31	248	248	PV39	178	178
Bunch with fungal sporulation	PV31	248	248	PV39	178	178
Leaf with fungal sporulation	PV31	248	248	PV39	178	178
Affected bunch 1	PV31	248	248	PV39	178	178
Affected bunch 2	PV31	248	248	PV39	178	178
Healthy bunch	PV31	NA		PV39	NA	
Healthy leaf	PV31	NA		PV39	NA	
Fungal spores	PV7	291	291			
Bunch with fungal sporulation	PV7	291	291			
Leaf with fungal sporulation	PV7	291	291			
Affected bunch 1	PV7	300	300			
Affected bunch 2	PV7	291	291			
Healthy bunch	PV7	NA				
Healthy leaf	PV7	NA				

in some of the samples under bright field or UV illumination (Fig. 1j).

The downy mildew specific primers amplified fragments in expected size range in affected tissues as well as downy mildew infected tissues (Table 1). Two primers *viz.,* PV14 and PV17 were heterozygous, while other five were homozygous resulting only in one allele. No polymorphism was detected for six of the seven primers. However, primer PV7 was polymorphic and two different alleles were detected among different tissues. No amplification with downy mildew specific primers was obtained in any of the healthy tissues as well as non template control.

Detection of *P. viticola* by primer sequences of seven variable microsatellite loci in the species, confirmed that downy mildew infection was responsible for cluster mortality. The high susceptibility of clusters of *Vitis vinifera* to *P. viticola* from pre-bloom to about 1 to 6 weeks post-bloom is well known (Kennelly *et al.*, 5). The clusters acquire ontogenic resistance due to the conversion of stomata to lenticels and thus infection is not observed at later crop growth stages. In this study also complete cluster necrosis at pre-bloom and bloom stages was observed while at post-berry set stages only parts of the clusters had turned necrotic or remained discoloured.

Table 2. Nutrient contents in petiole and leaf blade of healthy and affected vines of Thompson Seedless.

Nutrient		Petiole		Blade	
		Healthy	Affected	Healthy	Affected
	Range	0.73 - 1.00	0.72 - 0.95	2.51 - 2.89	2.40 - 2.86
N (%)	Mean	0.84	0.83	2.65	2.68
	t value (p=0.05)	0.26		0.49	
	Range	0.32 - 0.38	0.31 - 0.40	0.29 - 0.34	0.31 - 0.34
P (%)	Mean	0.35	0.32	0.32	0.32
	t value (p=0.05)	0.80		-0.77	
	Range	1.66 - 3.11	1.18 - 1.57	1.18 - 1.33	0.90 - 1.10
K (%)	Mean	2.12	1.35	1.27	0.98
	t value (p=0.05)	4.79*		10.56*	
	Range	0.26 - 0.33	0.32 - 0.47	0.09 - 0.15	0.12 - 0.18
Na (%)	Mean	0.30	0.40	0.12	0.16
	t value (p=0.05)	-5.48*		-4.88*	
	Range	0.67 - 0.74	0.65 - 0.75	1.07 - 1.36	1.09 - 1.27
Ca (%)	Mean	0.70	0.70	1.20	1.20
	t value (p=0.05)	0.87		1.43	
	Range	0.28 - 0.44	0.31 - 0.40	0.31 - 0.38	0.31 - 0.34
Mg (%)	Mean	0.38	0.35	0.34	0.32
	t value (p=0.05)	1.17		1.90	
	Range	20 - 60	24 - 73	232 - 327	223 - 344
Cu (%)	Mean	35.6	46.2	270.4	278.8
	t value (p=0.05)	-1.70		-0.52	
	Range	24 - 32	24 - 32	24 - 28	23 - 35
Zn (%)	Mean	28.2	29.3	25.2	26.4
	t value (p=0.05)	-0.95		-1.05	
	Range	40 - 58	42 - 86	113 - 214	97 - 202
Mn (%)	Mean	48.4	55.0	154.4	157.3
	t value (p=0.05)	-1.4		-0.18	
	Range	26 - 46	22 - 52	90 - 180	110 - 175
Fe (%)	Mean	36.0	33.8	137.0	142.8
	t value (p=0.05)	-0.69		-0.5	

*Significant at P = 1%.

Nutrient contents of vines with high cluster mortality (affected vines) and with no cluster mortality (healthy vines) indicated significant differences in only potassium and sodium contents (Table 2). In the healthy vines, the potassium content ranged from 1.66-3.11% in petioles and 1.18-1.33% in blades which was in the optimum range (1.61-2.95%) for the flowering stage (Sharma and Shikhamany, 11). Vines with high cluster mortality had significantly lower potassium content in petioles (1.18-1.57%) as well as in blades (0.90-1.10%). Sodium content exhibited inverse trend compared to potassium indicating a substitution of potassium by sodium in affected vines leading to lower potassium contents. Decrease in severity of downy mildew infections at higher levels of potassium is reported in grape (Soyer, 12**)**, which is due to increase stilbene phytoalexin levels which helps in imparting resistance (Fregoni *et al.*, 4). In 2008, also

we observed that Thompson Seedless vines showing visual symptoms of potassium deficiency had higher incidence of cluster infections than the vines with no visual deficiency symptoms. Thus, the low potassium contents of the affected vine would have further predisposed the clusters to infection.

The estimated possibility of downy mildew infection based on the downy mildew module of Metwin-2 software, was low to nil during both the years (Table 3). This was corroborated by the field situation as negligible leaf infection was observed in the vineyard. But in both years, 95-100% RH was recorded during the night for 8-15 h mainly due to dew formation (Fig. 2). Whenever leaf wetness was recorded, possibility of low-level infection was shown (Table 3). However, maximum incubation after infection was restricted to 5-80% only. As 100 per cent of incubation was never reached, sporulation did not occur. The absence of sporulation on clusters even after incubation in the humid chamber could have been due to sudden necrosis of the tissues or due to the inhibitory effect of a subsequently applied fungicide spray.

In the downy mildew epidemiology, temperature is not critical during this period in the tropical viticultural zone of Maharashtra as it is present in the favourable range (10 to 30°C), but the extent and time of rainfall / dewfall is more important as it is necessary to create suitable conditions of humidity and leaf wetness. Most of the weather stations measure leaf wetness and not cluster wetness, hence the models may show low to nil risk of the disease especially under non-epiphytotic weather conditions, which will be applicable to leaf infections but may not be true for cluster infections. The clusters can remain wet for longer duration because of their structure and compactness as compared to leaves that have a flat surface and dry out faster. Hence the leaf wetness data, recorded on a flat sensor placed within the canopy may not represent the actual situation on the clusters. Further, during the early growth period a number of agrochemicals are applied as sprays or cluster dip for pest control and quality improvement which also create foliage wetness. Moreover, when the cap stem starts cracking at the initiation of the bloom stage, free water from dew or hormonal or pesticide

Table 3. Prediction of simulative forecasting model Metwin-2 for downy mildew during 2006 and 2007.

Date	Possibility of sporangial formation	Estimated severity of downy mildew infection			Maximum incubation after infection (%)
		Low	Medium	Severe	
Year 2006					
16-26 Oct.	+	-	-	-	-
27 Oct.	-	-	-	-	-
28 Oct. - 6 Nov.	+	Yes	-	-	10
6-8 Nov.	+	Yes	-	-	20
9-15 Nov.	+	-	-	-	80
17-18 Nov.	+	Yes	-	-	-
22 Nov.	+	-	-	-	60
Year 2007					
22 Oct.-1 Nov.	+	-	-	-	-
2 Nov.	+	Yes	-	-	>10
3 Nov.	+	Yes	-	-	10,30
6 Nov.	+		-	-	70
7-14 Nov.	+		-	-	-
15-27 Nov.	-	-	-	-	-
28-30 Nov.	+	-	-	-	-
1 Dec.	+	Yes	-	-	-
2 Dec.	+	Yes	-	-	5,20
3 Dec.	+	Yes	-	-	5,15,30

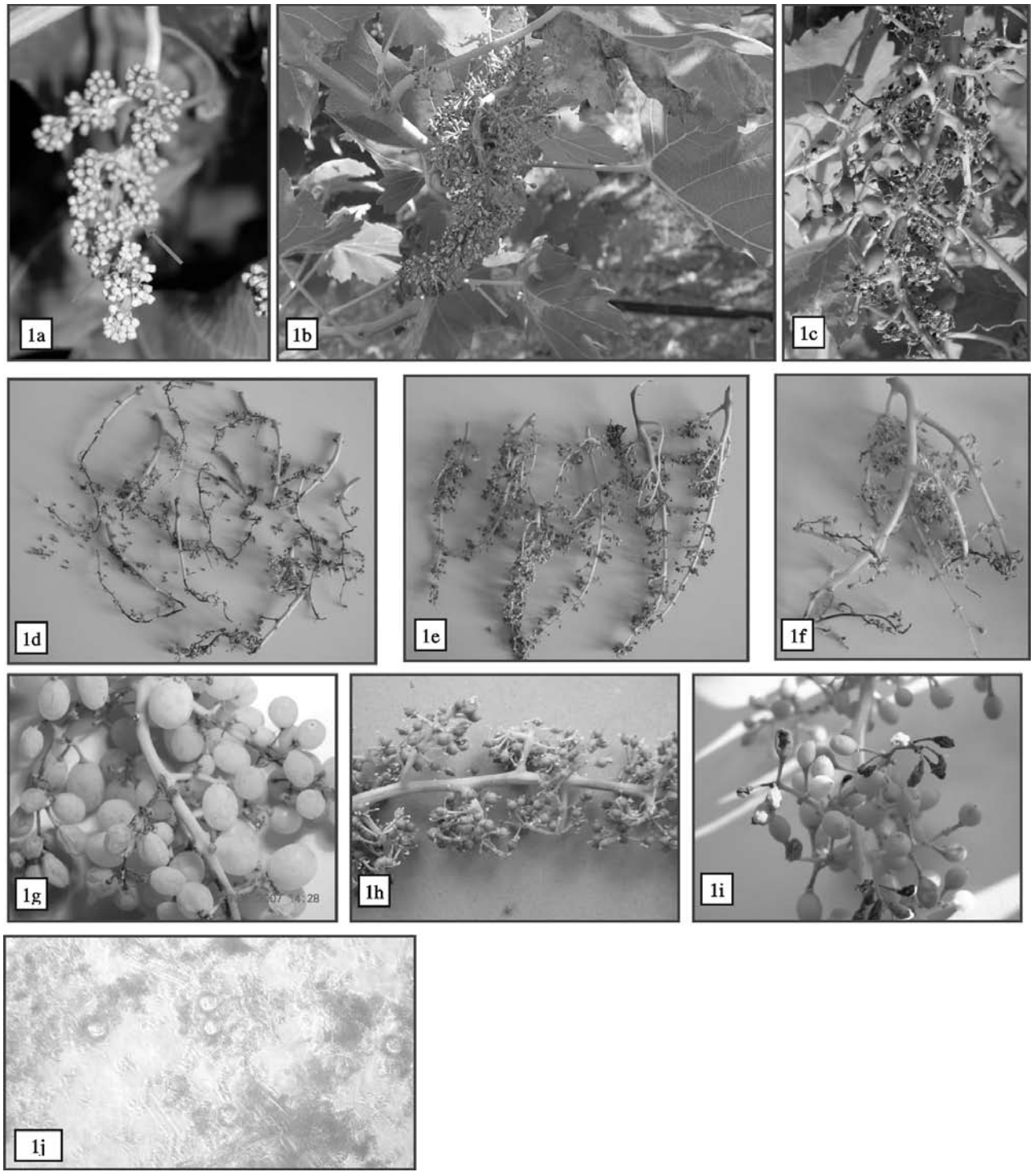
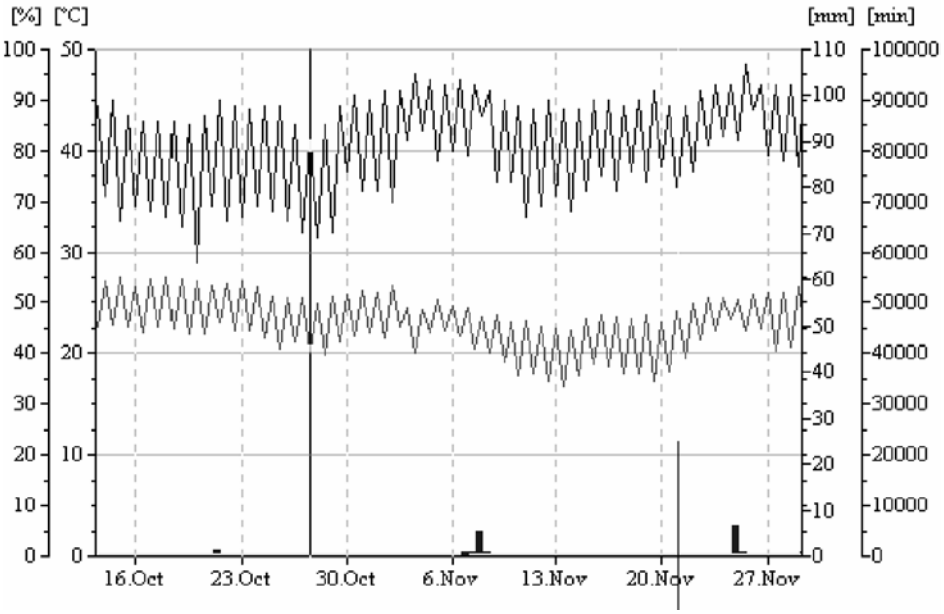
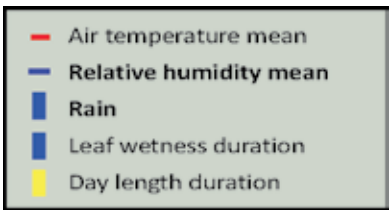
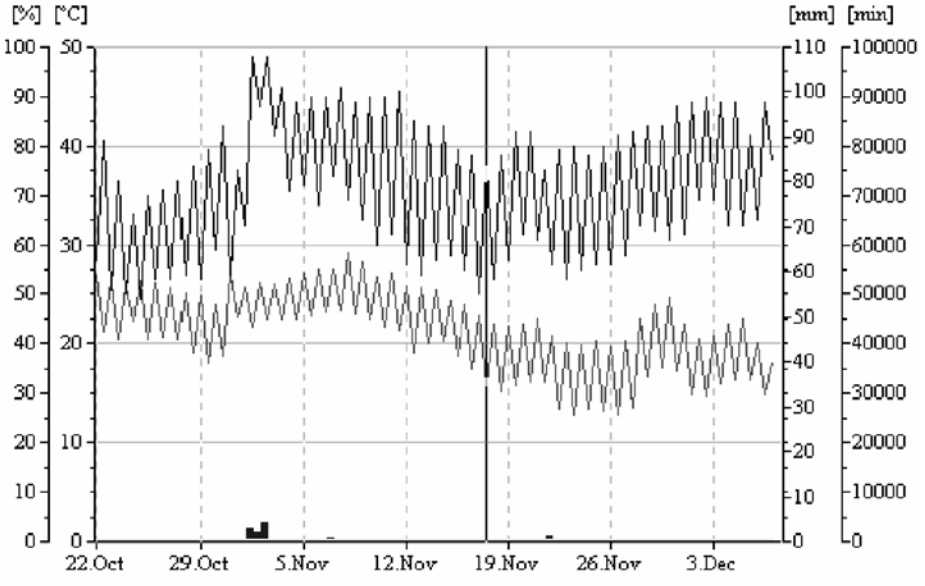


Fig. 1. Stage of downy mildew occurance in grape. (1a) Typical white downy fungal growth on clusters, (1b) Typical white downy growth of *P. viticola* on cluster. Note infection on nearby leaves, (1c) Atypical symptoms of cluster necrosis. Note absence of infection on nearby leaves, (1d) Necrosis of floral tissues, (1e & 1f) Necrosis of cluster tip and laterals, (1g) Necrosis on the pedicel, the point of attachment of the lateral to the rachis and on the berry to the pedicel, (1h) Discoloration of the rachis, (1i) Necrosis of young berries and sunken brown spots on older berries (1j) Immature oospores like structures under UV light (picture not to scale).



Weather sensors graph during fruiting season 2006





Weather sensors graph during fruiting season 2007

Fig. 2. Daily averages of temperature, RH, rainfall and leaf wetness for the susceptible period 2006 & 2007 at Pune site.

applications could have entered under the cap stem through the cracks thereby increasing the wetness duration for longer period and hence encouraged infection at pre-bloom stage. This study corroborates earlier observations that downy mildew is not an easy disease to predict or manage (Rocque, 9).

Study indicates that if high humidity (>95%) is present at night, then even in the absence of rains, the free moisture on clusters due to early morning dew and the wetness due to spray and dip applications of agrochemicals, needs to be considered for assessing the disease risk and planning of control strategies. It is also important to maintain sufficient potassium content in the vines to avoid their predisposition to downy mildew disease.

REFERENCES

1. Anonymous. 2003. *Annual Report 2002-03*. National Research Centre for Grapes, Manjri Farm P.O., Pune 412 307, India.
2. Bhaskar, M.K. and Sawant, S.D. 2004. Effect of an electronic weather station's disease forecasting on exportable grape production. **In:** *First Indian Horticultural Congress "Improving Productivity, Quality, Post-harvest Management and Trade in Horticultural Crops"* New Delhi, 6-9th November, 2004. *Book of Abstracts*, pp. 320-21.
3. Delmotte, F., Chen, W.J., Cervera, S.R., Greif, C., Papura, D., Griesse, X., Genson, G.M. and Costet, F.C. 2006. Microsatellite DNA markers for *Plasmopara viticola*, the causal agent of downy mildew of grapes. *Mol. Ecol. Notes*, **6**: 379-81.
4. Fregoni, C., Bavaresco, L., Cantu, E., Petegolli, D., Vizzon, D., Chiusa, G. and Trevisan, M. 2000. Advances in understanding stilbene (resveratrol, epsilon-viniferin)- grapevine relationships. *Acta Hort*. **526**: 467-77.
5. Kennelly, M.M., Gadoury, D.M., Wilcox, W.F., Magarey, P.A. and Seem, R.C. 2005. Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. *Phytopathology*, **95**: 1445-52.
6. Lodhi, M.A., Ye, G.N., Weeden, N.F. and Reisch, B.I. 1994. A simple and efficient protocol for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Mol. Biol. Rep.* **12**: 6-13.
7. Navajas, A.M.D., Greif, C., Poutaraud, A. and Merdinoglu, D. 2006. Two simplified fluorescent staining techniques to observe infection structures of the oomycete *Plasmopara viticola* in grapevine leaf tissues. *Micron*. **38**: 680-83.
8. Pearson, R.C. and Goheen, A.C. 1988. (Eds). *Compendium of Grape Diseases*. APS Press, USA. 93 p.
9. Rocque, B. de. La. 2001. The vagaries of downy mildew in 2001. Increased potential risk of unpredictable weather: Comments on decision making. *Phytoma, la defense des vegetaux,* **543**: 34-35.
10. Sahijram, Leela, Murthy, B.N.S. and Shikhamany, S.D. 2005. Introgression of downy mildew resistance into grape cv. Thompson Seedless through hybrid embryo *in vitro*. *Indian J. Hort*. **62**: 107-11.
11. Sharma, J. and Shikhamany, S.D. 2006. Petiole nutrient standards for Thompson Seedless vines on Dogridge rootstock. *Acta Hort.* **785**: 379-81.
12. Soyer, J.P. 1989. Influence of diverse agronomical factors on the sensitivity of grapevine to downy mildew (*Plasmopara viticola*). Influence of environmental factors on the control of grape pests, diseases and weeds. *Proc. Meeting of the EC Expert's Group*, Thessaloniki, 6-8 Oct. 1987. pp. 245-54.
13. Tandon, H.L.S. (Ed.). 2001. *Methods of Analysis of Soils, Plants, Waters and Fertilisers.* Fertiliser Development and Consultation Organisation, New Delhi, India, 144 + vi p.
14. Vercesi, A., Tornaghi, R., Sant, S., Burruano, S., Faoro, F. 1999. A cytological and ultrastructural study on the maturation and germination of oospores of *Plasmopara viticola* from overwintering vine leaves. *Mycol. Res.* **103**: 193-202.

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