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SURVIVAL AND VIABILITY OF OVER WINTERING OOSPORES OF *PLASMOPARA VITICOLA* CAUSING DOWNY MILDEW IN GRAPES IN KASHMIR, INDIA

Sabiha Ashraf[1*], Vaseema Yousuf[2], Shahina A. Nagoo[1], Aisha Nabi[3], Masarat Bashir[1], Saba Banday[3], Aroosa Khalil[2], K.A. Dar[1], S.A. Wani[1], H. A. Malik[4], Nasreen Fatima[4] and Z.I. Buhroo[1]

[1]College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India
[2]Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India
[3]Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India
[4]Krishi Vighyan Kendra, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India
*Corresponding author E-mail: sabiha_ashraf@rediffmail.com
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ABSTRACT

Downy mildew of grapes (*Plasmopara viticola*) is widespread and most destructive disease of grapes (*Vitis vinifera* L.) and has attained the status of a major disease of grapes under temperate conditions. The disease affects all the green parts of the vine namely leaves shoots, tendrils, inflorescence and bunches and is serious in climate with abundant rainfall, high humidity and longer periods of wetness on leaves and fruits. The pathogen perpetuates through winter in the form of oospores on fallen diseased leaves in/on floor of vineyards and in infected buds of pruned snags and intact shoots. The oospore production and their viability in soil decreased with increasing depth of placement of infected leaves Maximum oospore production in leaves located on the orchard floor is observed from the second fortnight of May to the first fortnight of June, while in buds of pruned snags, the maximum occurs from the first to the second fortnight of June. These infected leaves and buds of pruned snag lying on the ground served as the most significant overwintering sites for *P. viticola.*
Keywords **:** Downy mildew, grapes, overwintering, *Plasmopara viticola.*

Introduction

Grape (*Vitis vinifera* L.) is a refreshing fruit, rich in sugars, acid, vitamins, minerals and tannins (Radha and Mathew, 2007). It is a deciduous crop, and its natural habitat is temperate climate (Chadha, 2002). Grapes are prone to a number of fungal, bacterial and viral diseases which significantly affect their quality and production (Nimbalkar *et al.,* 2005). Amongst the diseases, downy mildew caused by an obligate parasite *Plasmopara viticola* (Berl. & Curt) Berlese & de Toni (Goeker *et al.,* 2003) is an economically important, widespread and destructive disease of *Vitis* species in viticulture areas (Koledenkova *et al.,* 2022; Bois *et al.,* 2017, Williams *et al.,* 2007). The disease affects all the green parts of the vine namely leaves shoots, tendrils, inflorescence and bunches (Kenelly *et al.,* 2007) and is serious in climate with abundant rainfall, high humidity and longer periods of wetness on leaves and fruits (Choi *et al.,* 2017). The economic significance of the damage caused by downy mildew is apparent during the actual year of the attack especially if the bunches are affected early (Valarmathi and Ladhalakshmi 2020). When control is poor and/or weather conditions are favourable and no protection against the disease is provided, it causes severe losses due to total or partial destruction of grape bunches, besides secondary influence of foliage loss (Walker and Haesbrock, 2007) and is a highly destructive disease causing up to 100% losses if the disease is not controlled during favourable weather (Toffolatti *et al.,* 2018). Oospores play a main role at the initiation of the disease and contribute to occur throughout the epidemiological season (Fedele *et al.,* 2025). The pathogen perpetuates throughout the winter on fallen diseased leaves lying on the orchard floor, infected buds of intact twigs and pruned snags in the form of

oospores (Yang *et al.,* 2023). In Jammu and Kashmir, anthracnose and powdery mildew have been reported to occur on grapes in Kashmir region, occurrence of downy mildew has been reported recently from the valley and has now assumed an alarming threat to its successful cultivation. So, considering the importance and potentiality of being an epidemic pathogen, the present investigation was undertaken to observe the effect of various factors like temperature, humidity and rainfall on development and intensity of downy mildew of grapes and to study the survival and viability of oospore in fallen diseases leaves and in buds from pruned snags/unpruned twigs.

Materials and Methods

Perpetuation Studies

The survival of pathogen in fallen diseased leaves and in buds on pruned snag and unpruned twigs of susceptible grape cultivar "Thompson Seedless" was studies during the year 2019 and 2020 in a vineyard at SKUAST-K, Shalimar.

Survival in over-wintered diseased leaves

Fallen diseased leaves of almost similar size and disease severity were collected during last week of October during both the years of experiments. The leaves were kept in mesh wire bags, each bag containing equal number of leaves and divided into three sets. One set of such leaves was kept on soil surface (Fig. Ia), and other two were buried in soil at 7.5cm and 15cm depth (Fig I b,c) in the vineyard. The fallen diseased leaves in the mesh wire bags were removed at fortnightly intervals beginning from the first week of March onwards and examined in the laboratory for the production and viability of oospores.

Estimation of Oospore Production

The method given by Ronzon-Tran and Clerjeau (1988) was adopted for estimation of oospore production in fallen diseased leaves. Twenty-five leaf discs of 1cm^2 dia. bearing downy mildew symptoms were randomly selected, ground to powder in pestle and mortar, the powder was soaked in 80ml of distilled sterile water and strained through a double layer cheese cloth. Twenty milliliters of this suspension was centrifuged at 6000 rpm for 15 minutes. After centrifugation, 15 ml of the supernatant was drawn off with a pipette. The pellet was re-suspended in 5ml sterilized water for 9 hours at 18^{o}C and the number of oospores estimated as means of ten haemocytometer readings per replication. The oospore production was computed on the basis of number per unit leaf area.

Viability of Oospores

Oospore germination method was used to study the viability of oospores in over-wintered leaves Panchbhai *et al.* (1991). From the sample used for estimation of oospore production, water was drained off and oospores re-suspended in 5ml of 2.5% Sodium hypochlorite (NaOCl) solution at 18 ^{o}C. After 24 hours incubation, NaOCl was drained off, and oospores re-suspended in 5ml of sterilized distilled water. Two drops of 50µl from each processed sample were placed on a glass slide and incubated in moist chamber at 20 ±1 ^{o}C. After 24-hour incubation, one drop of cotton blue in lactophenol was added to each drop and semi-permanent slides prepared by placing a 20 x 20 mm cover slip over each drop. The number of oospores in each drop and the number that germinated were recorded.

Survival in buds from pruned snags/unpruned twigs

The bud samples were collected separately from pruned snags, kept in mesh wire bags on vineyard floor (Fig. I d) and intact twigs, starting from first March onwards in both the years of study and observed in laboratory for production and viability of oospores. Collection dates were the same as those for leaves except that no bud samples were taken from intact twigs in April, because buds had initiated growth. Fifty randomly selected buds of similar size were excised each from intact twigs and pruned snag separately, ground to powder in pestle and mortar soaked in 80 ml sterilized distilled water and strained through a double layer cheese cloth. The number of oospores per bud was enumerated as described for leaf sample. The per cent viability of oospores in buds of intact twigs and pruned snag was estimated as per the method described for viability of oospores in over-wintered diseased eaves.

Results

Survival on overwintered diseased leaves

Diseased leaves in mesh wire bags were placed separately on ground surface as well as buried at 7.5cm and 15.0cm depth. Observations regarding the oospore production and their viability were recorded at fortnightly intervals and are presented in Table 4 and 5.

Perusal of data revealed that the oospore production in over-wintered diseased leaves continued for a comparatively longer period upto second fortnight of July in both the years (2019 and 2020) of experimentation, when kept at ground surface. Burial of diseased leaves at 15cm depth exhibited the oospore production only upto first fortnight of May during both

the years. Thereafter, leaves were decomposed. However, leaves buried at 7.5 cm depth decomposed 1-2 weeks earlier than at 15cm depth. Oospore production decreased with increase in depth of placement. The overwintered leaves on the ground surface yielded highest number of oospore production when compared to that of buried leaves.

The average number of oospore cm^{-2} diseased leaf area increased upto first fortnight of June in the year 2019 and 2020, with maximum number of 976 and 1198 oospores, respectively. However, by the second fortnight of July, the number gradually declined to 176 and 220 oospores, respectively. The leaves buried at 7.5 cm depth yielded maximum number of oospore (170 and 221cm^{-2} leaf area) in the first fortnight of March during both the years of study. However, the leaves buried at 15 cm depth yielded comparatively lesser number of oospores with a maximum of 158 and 134 oospores cm^{-2} leaf area, produced during the same period. Their production showed gradual decline in the later periods (Fig. 2).

The viability of oospore exhibited a sharp decline with the increase in depth of placement. Highest oospore viability was recorded in over-wintered diseased leaves at ground surface and comparatively least at 15 cm depth. The highest oospore viability of 61.0 and 73.0 per cent in leaves on ground surface was recorded in the first and second fortnight of June, 2019 and 2020, respectively. Oospore from buried leaves exhibited a sharp decline in their viability with increasing depth and duration of burial. At 7.5 cm depth the viability declined from 22.0 to 19.3 per cent and 25.3 to 21.6 per cent during the first fortnight of March to second fortnight to April in both the years (2019-20) of experimentation respectively. However, during the year 2020, maximum oospore viability of 31.0 per cent was recorded in second fortnight of March and thereafter declined to 21.6 per cent in second fortnight of April. Whereas, at 15 cm depth viability declined from 18.6 to 10.3 per cent and 20.6 to 15.0 per cent from first fortnight of March to first fortnight to May 2019 and 2020, respectively.

Survival on buds from pruned snags and intact twigs

Perpetuation of the test fungus in grape buds was studied by examining the bud samples from pruned snag on ground surface and intact twigs for the production and viability of *P. viticola* oospores at fortnightly intervals commencing from the first week of March. The data recorded is presents in Table 6 and 7. The data revealed that the buds collected from pruned snags continuously produced oospores during whole period of study in both the years (2019 and 2020). However, in case of intact twigs, observations beyond the first fortnight of April could not be taken because all the buds had sprouted. The average number of oospores per bud from pruned snag increased up to second fortnight of June, both in 2019 and 2020, with a maximum number of 491 and 532 oospores per bud respectively. The number then gradually declined to 160 and 216 oospores per bud, respectively, till last observation recorded in first fortnight of August. The oospore production in buds from intact twigs showed an increase till their sprouting during both the years. In first week of March 100 and 150 oospores per bud were recorded during 2019 and 2020, respectively. A maximum number of 200 and 261 oospores per bud were recorded in the first fortnight of April during 2019 and 2020, respectively.

Initial viability of oospore produced in buds from pruned snag in first week of March was 24.5 and 28.0 per cent during 2019 and 2020, respectively. The viability of oospore gradually increased with time till second fortnight of June during the year 2019 and 2020, respectively, which then showed a gradual decrease to 27.6 and 21.5 per cent till last observation. Initial oospore viability in buds from intact twigs was 43.5 and 49.0 per cent, respectively. The viability showed a gradual increase till buds sprouted i.e. first fortnight of April with a maximum viability of 200 and 261 per cent during both the years of experimentation.

Discussion

The present study has established that the pathogen *(P. viticola)* perpetuated in the form of oospores throughout the winter on diseased leaves and buds of pruned snags lying on orchard floor besides its perpetuation in buds of intact twigs. These findings are supported by Yang *et al.*. 2023;, Killigrew and Sivasithamparam (2005), and Rouzet and Jacquin (2003).

However, diseased leaves and buds of pruned snags on the orchard floor were observed to be the most important source of primary infection of downy mildew of grapes. Carisse (2016) postulated that when high numbers of leaves are infected in the fall, higher levels of primary inoculum (oospores) are present in the following spring. These findings are supported by Rumbou and Gobbin (2005); Gobbin *et al.* (2005), Vercesi *et al.* 2010, who reported fallen diseased leaves on ground surface as most important overwintering site of the pathogen.

The diseased leaves on the ground surface exhibited maximum oospore production between first and second fortnight of June coinciding with the

susceptible phonological stage of grape tree. The leaves buried in soil at two depths (7.5 and 15cm) exhibited viable conidia only upto second fortnight of April and first fortnight of May, respectively. Thereafter leaves at both the depths decomposed and pathogen could not be isolated. However, leaves at 7.5cm decompose 1-2 weeks earlier than 15cm depth. This could be attributed to greater aerobic respiration at 7.5cm depth, which favoured quick decomposition of leaves. The proportion of oospores and their viability decreased with the increase in depth of placement in soil. Similar, observations have been reported by Tombisana and Singh (1995). The decrease in viability of oospores with increasing depth may be attributed to microbial action on nutrient coating of oospores (Meyers and Cook 1972) resulting in loss of their viability.

The buds of intact (unpruned) twigs exhibited oospore production only upto second fortnight of March beyond which all the buds had initiated growth. The oospores found in these buds though viable may not be of much epidemiological significance because of unfavourable temperature for infection prevailing at the bud break phenophase of grape vines (mid-March to mid-April) in the valley. These observations are in accordance with the observations made by Shahzad (2003).

Although disposal of pruned snags from the orchard has been recommended for the management of various grape diseases, no work has been reported on the role of infected pruned snags, lying on orchard floor, in perpetuation of downy mildew pathogen (*P. viticola*). The buds on pruned snags lying on the orchard floor exhibited viable oospores upto first fortnight of August with maximum production recorded between first and second fortnight of June. These oospores may be of much epidemiological significance because temperature during the month of June remains generally favourable for primary infection. These observations are supported by the findings of Nishna and Mizuhaka (1981) who reported cut mulberry branches infected with Dogare blight (*Diporthe nomurai*) lying on the ground floor are most important source of primary infection. The production of viable oospores in overwintered diseased leaves and buds of pruned snag on orchard floor increased from the month of April to July. This suggests that in spring, under favourable weather conditions, lesions on overwintered leaves and buds of pruned snag resume sporulation and the inoculum may build up to the levels sufficient to initiate primary infection (June). The environmental conditions in the early summer thus determine the extent of epidemic development because *P. viticola* is polycyclic pathogen with apparent infection rates. The present observations are in agreement with the observations made by Ronzon-Tran and Clerjeau (1988)

Conclusion

The pathogen perpetuates both on overwintered diseased leaves and in buds of infected shoots as oospores and serve as a source of primary inoculum for the next cropping season.

Table 1 : Production and viability of *Plasmopara viticola* (Berl. &Curt) Berlese & de Toni oospores on overwintered diseased leaves of grapes (cv. Thompson seedless) during the year 2019

Period of observation		Number of oospores cm^{-2}leaf area*			Viability (%)		
Month	Fortnight	Ground surface	7.5cm depth	15 cm depth	Ground surface	7.5cm depth	15 cm depth
March	I	263	176	158	28.0	22.0	18.6
	II	397	141	133	30.3	27.6	21.3
April	I	486	88	62	35.0	25.0	16.0
	II	622	56	44	41.7	19.3	13.0
May	I	755	-	35	48.6	-	10.3
	II	931	-	-	57.7	-	-
June	I	976	-	-	61.0	-	-
	II	710	-	-	55.7	-	-
July	I	442	-	-	51.3	-	-
	II	176	-	-	42.0	-	-
August	I	-	-	-	-	-	-

* Mean of three replications each comprising of 25 leaf discs of $1cm^2$ surface area

- Indicates completely decomposed leaves from which oospore production could not be assessed

Table 2 : Production and viability of *Plasmopara viticola* oospores on overwintered diseased leaves of grapes (cv. Thompson seedless) leaves during the year 2020

Period of observation		Number of oospores cm^{-2} leaf area*			Viability (%)		
Month	Fortnight	Ground surface	7.5cm depth	15 cm depth	Ground surface	7.5cm depth	15 cm depth
March	I	308	221	134	30.6	25.3	20.6
	II	442	177	130	37.0	31.0	25.7
April	I	532	132	84	42.3	28.3	22.0
	II	797	72	65	45.3	21.6	18.6
May	I	886	-	40	54.0	-	15.0
	II	1098	-	-	63.0	-	-
June	I	1198	-	-	70.7	-	-
	II	842	-	-	73.0	-	-
July	I	530	-	-	62.6	-	-
	II	220	-	-	51.7	-	-
August	I	-	-	-	-	-	-

* Mean of three replications each comprising of 25 leaf discs of $1cm^2$ surface area
- indicates completely decomposed leaves from which oospore production could not be assessed

Table 3 : Production and viability of *Plasmopara viticola* oospores on overwintered grapes (cv. Thompson seedless) buds during the year 2019

Period of observation		Number of oospores/bud*		Viability (%)	
Month	Fortnight	Pruned snag	Intact twig	Pruned snag	Intact twig
March	I	50	100	24.5	43.0
	II	111	161	29.0	51.6
April	I	150	200	31.6	59.3
	II	165	--	37.0	--
May	I	216	--	35.5	--
	II	321	--	42.3	--
June	I	387	--	43.7	--
	II	491	--	46.3	--
July	I	370	--	39.0	--
	II	220	--	31.5	--
August	I	160	--	27.6	--

*Mean of three replications of 50 buds each
-- Indicates sprouted buds

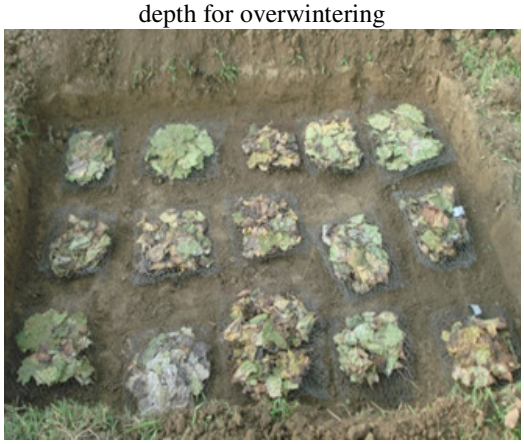
Table 4 : Production and viability of *Plasmopara viticola* oospores on overwintered grapes (cv. Thompson seedless) buds during the year 2020

Period of observation		Number of oospores/bud*		Viability (%)	
Month	Fortnight	Pruned snag	Intact twig	Pruned snag	Intact twig
March	I	111	150	28.0	49.0
	II	161	220	22.5	55.3
April	I	200	261	38.0	64.2
	II	228	--	41.6	--
May	I	270	--	46.3	--
	II	385	--	40.0	--
June	I	415	--	49.5	--
	II	532	--	52.7	--
July	I	436	--	47.3	--
	II	331	--	36.0	--
August	I	216	--	21.5	--

*Mean of three replications of 50 buds each
-- Indicates sprouted buds

a. Overwintering of diseased grape leaves on orchard floor

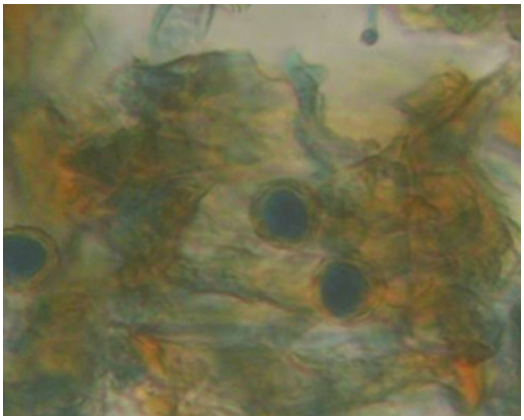
b. Burial of diseased leaves at 7.5cm depth for overwintering



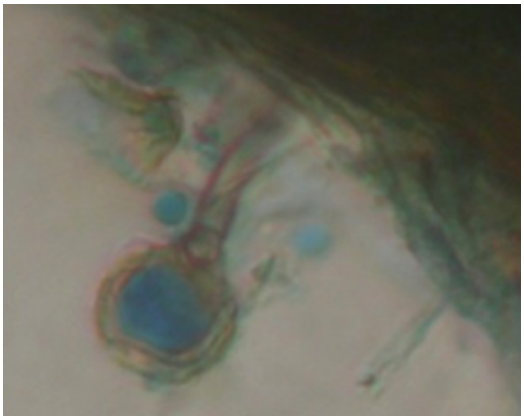
c. Diseased grape leaves at 15cm depth for overwintering

d. Pruned snags on orchard floor for overwintering

Fig. 1: Perpetuation Studies of the fungus



Oospores from infected host tissue



Germinating oospores

Fig. 2: Oospores of *Plasmopara viticola*

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Competing Interest

The author declares that there is no competing interest in the publication of this manuscript

Ethical statement

This research did not involve any human and/or animal participants

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