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Grape Downy Mildew in India. III. Effect of Infection on Phenolics, Sap Concentration, Organic Acids and Amino Acids

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Total phenolics content was greater in downy mildew infected grape leaves than in healthy leaves. Polyphenoloxidase activity was roduced in infected leaves, the maximum reduction being in grade 3 infection. The sap in healthy leaves of all ages was acidic. The minimum pH was found in viridian green leaves and maximum in red leaves. The total organic acid content in infected leaves increased with disease intensity. Among the organic acids indentified, citric acid decreased in infected leaves.

The effect of infection of grape leaves of different ages by *Plasmopara viticola* (B. & C.) Berl. and Det. on plant pigments were reported earlier (Srinivasan and Jeyarajan, 1975, 1976). The results of further investigations on phenolics, sap concentgation, organic acids and amino acids in infected leaves are presented in this paper.

MATERIALS AND METHODS

The estimations were carried out in Anab-e-Shahi grape leaves of five different ages as mentioned earlier, (Srinivasan and Jeyarajan, 1976). The healthy leaves were collected from vines which were given frequent sprays with Bordeaux mixture. The total phenolics were estimated by the method of Bray and Thorpe (1954) and expressed as catechol equivalents. Polyphenoloxidase activity was determined by the method of Matta and Dimond (1963) and expressed as enzyme units, one unit being definite μ moles of pyrogallol used per ml of enzyme source per minute. The leaves were macerated in distilled water foilowed by filtration and then the PH was recorded by a PH meter (Williard *et al.*, 1951) Ascorbic acid content was estimated by the colorimetric method (Anon., 1960) and organic acid and amino acid contents by partition paper chromatography (Block *et al.*, 1955).

RESULTS AND DISCUSSION

1. **Total phenolics:** Phenolics contents, were estimated in healthy and infected leaves. The results are presented in Table I. The data revealed that in healthy leaves the peak concentration of phenolics occurred in the youngest (red) leaves. In infected leaves of all colours the content increased significantly over healthy leaves. But in older leaves showing downy growth (viridian green, agathia green, emerald green) the differences in phenolic content between three grades of infec-

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TABLE. 1. Phenolics, polyphenoloxidase activity, ascorbic acid content and pH of green leaf

Leaf colour	Nature of leaf	*Total phenolics* (µg/g)	Polyphenoloxidase activity		Ascorbic acid		pH of healthy leaves
			Units	*Diseased* Healthy (per cent	mg/g	*Diseased* Healthy (per cent)	
Red	H	690.5	145		0.50		6.62
Jade green	H	480.5	86		1.35		6.37
Viridian green	H	460.3	164		2.56		6.17
	1	463.4	152	92.7	1.50	58.6	
	2	469.6	139	84.8	1.00	39.1	
	3	475.7	121	73.8	0.85	33.2	
Agathia green	H	490.5	142		2.83		6.31
	1	493.3	134	94.4	1.60	56.5	
	2	497.5	133	85.9	1.18	41.7	
	3	504.1	106	74.7	1.03	36.4	
Emerald green	H	549-3	113		2.80		6.44
	1	551.0	106	93.8	1.55	55.4	
	2	554.3	92	81.4	1.10	39.3	
	3	558.3	70	61.9	1.00	35.7	
t (Healthy vs diseased)		*4.5	*5.1		*17.0		CD = 0.2 (P = 0.05

Note : * Significant at P = 0.05 H - Healthy. 1, 2, 3 - Grades of infection
Young leaves (red, jade green) did not show downy growth.

tions were not significant. The mean total phenolics was highest in emerald green and lowest in viridian green leaves. The mean content in the three infection grades was highest in leaves showing infection grade 3 and lowest in infection grade 1.

2. Polyphenol oxidase activity: The activity of polyphenol oxidase in different coloured leaves and grades of infection was estimated to find out whether infection caused any derangements in the activity of this enzyme. The results are presented in Table I. It was found that in healthy leaves the peak activity of polyphenol-oxidase occurred in viridian green leaves and lowest activity in jade green leaves. The activity of this enzyme in infected leaves was significantly less than in healthy leaves. In leaves of each colour, the higher the disease intensity the lower the activity of this enzyme. The greatest per cent of reduction in activity namely 38.1 was noted in emerald green leaves showing infection grade 3 and least per cent increase in agathia green leaves showing infection grade 1. When infected leaves of the three colours were compared the maximum reduction in activity was noticed in emerald green leaves which had only 79.1 per cent of activity found in healthy leaves. The least reduction to 83.8 per cent was observed in viridian green leaves. The mean activity of this enzyme was highest in viridian green and lowest in emerald green leaves. When the mean activities in different

grades of infection were compared, the maximum and minimum were found in leaves showing grades 1 and 3 respectively.

Peroxidase is an active competitor for polyphenoloxidase as both have phenols as substrates. Increase in peroxidase activity in infected grape leaves (Srinivasan, 1974) might have rapdily oxidised phenolics and thus reduced the polyphenoloxidase activity for want of substrates. Since phenolics in general are considered to offer resistance to diseases, the higher content of phenolics noted in red leaves than in other leaves may also possibly be responsible for the longer incubation period in red leaves. (Srinivasan and Jeyarajan, 1976). Inoculated red leaves developed downy mildew growth after 18 days when they had turned jade green. The incubation period was just 10 days when jade green and older leaves were inoculated (Srinivasan, 1974).

3. **Sap concentration**: The PH of healthy leaves of different ages was estimated with a view to finding out if it can be a cause for the absence of downy growth in young leaves The results are presented in Table I. The PH was maximum in the youngest (red) leaves and minimum in viridian green leaves. But the sap was always on the acidic side in healthy leaves of all colours.

4. **Ascorbic acid content**: With a view to finding out whether the content of ascorbic acid was affected due to infection, it was estimated in leaves of different colours in healthy as well as infected leaves showing different grades of infection. The results are presented in Table I. It was found that ascorbic acid content increased gradually with the age of leaves. In infected leaves its content was always significantly less than in comparable healthy leaves. However, the contents in three infection grades in leaves of the same colour, were on par. The greatest and lowest per cent reductions were noticed in viridian green leaves showing grades 3 and 1 respectively.

5. **Organic Acids Content**: In order to find out the changes in organic acids brought about by downy mildew infection, the organic acids were estimated in leaves of different colours showing different infection grades. The results are presented in Table II. It was found that in healthy leaves the highest and lowest contents of organic acids were found in viridian green and red leaves respectively. Tartaric acid was absent in red leaves but present in leaves of all other colours. There was no qualitative difference between healthy aud infected leaves. The total organic acid content in diseased leaves was significantly higher than in healthy leaves. Such an increase was more in leaves showing higher infection grades. The content of citric acid in infected leaves of all the three colours was reduced by 33 5 to 49 per cent as compared to healthy leaves In higher infection grades, the reduction was more. However, the content of malic acid and unidentified organic acid was higher in infected leaves than in healthy leaves. The mean total organic acids content was significantly higher in

infected viridian green leaves than comparable healthy leaves.

The minimum PH noted in viridian green leaves coincided with maximum total organic acids and maximum disease per cent. Ravaz (1930) stated that the immunity of American varieties and their hybrids was due to high concentration of their sap at the start and the progressive dilution as the season advanced. But Kale and Rives (1931) found no significant difference in cell sap concentration between highly resistant and highly susceptible varieties. The optimum PH requirement of the pathogen for growth could not be found out since it is an obligate parasite. However, red leaves in which the incubation period was longer than older leaves, had the highest pH as against the lowest pH in the most infected viridian green leaves. Resistance of plant tissues to pathogens has been attributed to specific organic acids only in a few cases. In the present study, citric acid decreased in infected leaves. It is possible that it was utilised by the pathogen for its growth.

6. Free amino acids content : With a view to finding out the changes in free amino acids brought about by downy mildew infection, the free amino acids were estimated in different colours of healthy and dieased leaves showing different grades of infection. The results are presented in Table III. In healthy leaves the highest content of amino acids was found in jade green leaves. There was no qualitative difference in amino acid content between leaves of different colours as well as healthy and infected leaves. The contents of lysine, arginine, cysteine and asparagine increassd several folds in jade green, viridian green, agathia green and emerald green leaves

TABLE II. Organic acid content in healthy and downy mildew infected grape leaves (ug/g)

Leaf colours	Nature of leaf	Citric acid	Malic acid	Tartaric acid	Succinic acid	Unidentified acid(Rf value= 0.6)	Total
Red	H	625	385	—	732	—	1742
Jade green	H	610	907	367	1208	675	3767
Viridian green	H	632	955	415	1276	705	3982
	1	420	1200	430	1290	810	4150
	2	400	1230	450	1330	800	4210
	3	390	1250	440	1350	800	4230
Agathia green	H	608	919	372	1250	675	3815
	1	370	1145	390	1270	795	3970
	2	360	1175	405	1350	735	4025
	3	345	1205	390	1320	725	4055
Emerald green	H	500	875	162	1190	670	3597
	1	300	1150	370	1200	730	3750
	2	270	1165	385	1220	725	3765
	3	255	1200	360	1275	710	3800

Note: * Significant at P = 0.05 H = Healthy 1, 2, 3 = Grades of infection
Young leaves (red, jade green) did not show downy growth.

TABLE III. Amino acid content in healthy and downy mildew infected grape leaves (ug/g)

Leaf colours	Nature of leaf	Lysine	Arginine	Cysteine	Asparagine	Aspartic acid	Glycine	Glutamic acid	Serine	Alanine	Trypto-phane	Valine	Total
Red	H	8	6	3	26	30	12	16	18	20	32	23	294
Jade green	H	5	10	5	38	45	18	15	27	28	26	30	357
Viridian green	H	18	13	14	52	47	22	13	38	32	25	33	307
	1	21	15	18	45	38	20	10	30	27	22	30	276
	2	23	16	20	38	34	18	8	26	25	20	28	256
	3	26	16	22	35	30	18	5	22	20	17	27	238
Agathia green	H	20	18	20	60	44	17	10	42	37	20	31	319
	1	25	22	23	50	36	12	8	32	31	15	27	281
	2	29	26	27	47	32	10	6	28	28	12	23	269
	3	32	27	29	43	29	7	6	26	25	10	20	254
Emerald green	H	21	19	23	66	36	25	11	35	33	16	26	311
	1	25	24	27	51	30	23	8	27	28	13	24	280
	2	28	26	31	45	21	22	6	25	25	10	20	266
	3	31	26	34	40	25	20	6	23	20	8	17	250
								t (Healthy vs diseased) * 2.8					

Note : * Significant at P = 05
H. Healthy. 1, 2, 3 - grades of infection
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over that found in the youngest (red) leaves. The contents of aspartic acids, glycine and valine were maximum in viridian green leaves. The total amino acids in diseased leaves was significantly less than in healthy leaves and such a reduction was more in leaves showing higher infection grades. In infection leaves, the contents of lysine, arginine and cysteine increased by 23 to 60 per cent over healthy leaves. However, there were reductions in contents of asparagine, aspartic acid, glycine, glutamic acid, serine, alanine, tryptophane and valine in dieased leaves ranging from 9.1 to 61.5 per cent. The maximum reductions in the contents of amino acids were noted in viridian green leaves showing infection grade 3.

It is possible that certain amino acids which were appreciably reduced in infected leaves were preferably utilised by the pathogen to meet its metabolic requirements. In this instance also the lack of information on the specific nutritional requirements of the obligate parasite stands in the way of coming to a conclusion about the reasons for changes in the content of amino acids.

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