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# Grape downy mildew in India
# I. Foliar, floral and fruit infections

by

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## Der falsche Rebenmehltau in Indien

## I. Befall von Blättern, Blüten und Beeren

Zusammenfassung. — Bei der Rebensorte Anab-e-Shahi wurden Auftreten und Intensität des Befalls durch *Plasmopara viticola* an sechs durch ihre Färbung gekennzeichneten Blattstadien verfolgt. Rote und grün-orange gefärbte Blätter zeigten keine Krankheitssymptome. Agathia- und chromgrüne Blätter erkrankten am häufigsten. Der Übergang von Parasitierungsgrad 1 nach 2 dauerte länger als von 2 nach 3 (1 = bis zu 25%, 2 = 26—50%, 3 = über 50% der Blattfläche von Myzel bedeckt). Bei den roten und grün-orange gefärbten Blättern betrug die Inkubationszeit 18 bzw. 14 Tage, bei älteren Blättern dagegen nur noch 10 Tage. Die Weite der Spaltöffnungen war bei allen Blattstadien größer als der Durchmesser des Sporenkeimschlauches. Nach künstlicher Infektion wurden sowohl Infloreszenzen wie Trauben mit 2,0 mm und 6,5 mm großen Beeren befallen, während ältere Trauben mit 12,5 mm großen sowie mit reifen Beeren nicht erkrankten. Infizierte Blüten verrieselten vollständig; bei einer Größe von 2,0 mm fielen 53,1% der Beeren ab.

## Introduction

In Tamil Nadu (India), grape is an important fruit crop occupying about 1,360 hectares with an annual production of 13,440 tons. Downy mildew *(Plasmopara viticola* (B. and C.) Berlese and de Toni) causes great damage to vines under moist and warm conditions and is prevalent in an endemic form. Cursory observations indicated that young leaves and mature fruits were generally free from downy mildew. The present investigation compares the stages of leaf, flower, and fruit development at which infection occurs.

## Materials and Methods

The disease incidence was recorded in "Anab-e-Shahi" leaves of six different ages as revealed by their colours namely red, green with orange, jade-green, viridian-green, agathia-green, and emerald-green (Anonymous 1938) starting from the growing tip of the shoot. The disease intensity was rated according to the following three grades:

Grade 1: up to 25 per cent of the leaf area covered with mycelium.
Grade 2: 26 to 50 per cent of the leaf area covered with mycelium.
Grade 3: over 50 per cent of the leaf area covered with mycelium.

The number of leaves in each infection grade was calculated. The percentage of diseased leaves was calculated by using the following formula:

$$\text{Percent diseased leaves} = \frac{\Sigma n v}{3 N} \times 100$$

where n was the number of leaves in each grade, v was the infection grade and N was the total number of leaves (TOWNSEND and HEUBERGER 1943).

Ten leaves from the tip of each of 25 marked shoots were observed at 4-day intervals from August to October for leaf colour, disease appearance and infection grade. The number of days taken for passage to the next colour was calculated.

For artificial inoculation the sporangia were collected at 2 a. m. and a suspension containing about 200,000 sporangia per ml was prepared in sterile water using a haemocytometer. The inoculum was applied with a hand sprayer to leaves and inflorescences, which were enclosed for 48 hours in polyethylene bags moistened inside.

The penetration of germ tubes into grape leaves was studied by the method of SHIPTON and BROWN (1962). Detached fresh leaves of each colour were surface-sterilized with 0.1 per cent mercuric chloride, washed repeatedly in sterile water, sprayed with the inoculum and incubated in moist chambers for 48 hours in darkness. The leaves were then cut into 20 mm squares and immersed immediately in 10—15 ml of alcoholic cotton blue. They were boiled for 90 seconds and then allowed to remain in the stain for 48 hours at room temperature. After rinsing in water, they were placed in chloral hydrate solution for 30—50 minutes, mounted in 50 per cent glycerine and examined under oil immersion lens for the zoosporial germination and germ tube entry. The length and maximum width of 20 stomatal openings and number of stomata in 20 microscopic fields were measured with an ocular micrometer. From the area of a microscopic field the number of stomata per $mm^2$ was calculated. The width of germ tubes from zoospores was also measured.

Table 1

Downy mildew incidence in relation to leaf colour
Auftreten der *Plasmopara* in Beziehung zur Blattfarbe

| Leaf colours | Total leaves | Healthy leaves | Diseased leaves | | | Disease per cent |
|---|---|---|---|---|---|---|
| | | | Gr. 1 | Gr. 2 | Gr. 3 | |
| Red | 360 | 360 | 0 | 0 | 0 | 0 |
| Green with orange | 102 | 102 | 0 | 0 | 0 | 0 |
| Jade-green | 447 | 266 | 122 | 28 | 31 | 49.9 |
| Viridian-green | 445 | 34 | 173 | 104 | 134 | 63.5 |
| Agathia-green | 463 | 46 | 210 | 125 | 82 | 56.4 |
| Emerald-green | 693 | 112 | 345 | 154 | 82 | 51.5 |

## Results

### 1. Downy mildew incidence in relation to leaf colour and age

To determine the leaf colour at which maximum mycelium growth occurred, the incidence and intensity of the disease were assessed in all leaves present on 100 shoots selected at random during August (Table 1).

Young leaves (red and green with orange) did not show any symptom of the disease. Jade-green leaves, 24 days old, were the youngest to show symptoms. The maximum percentage of infection was noticed in viridian-green leaves.

In order to determine the rate of disease development in leaves of different colours, the number of days taken to reach the next infection grade was observed in jade-green, viridian-green, agathia-green, and emerald-green coloured leaves (Table 2). In agathia-green leaves, the infection reached grade 3 within the shortest period of 11 days after the appearance of downy mildew. The disease spread was slow in emerald-green leaves. In leaves of all colours, the time taken for infection to reach grade 3 from grade 2 was shorter than the time taken for development to grade 2 from 1. Even when disease development was slow, the infection progressed to grade 3 within 18 days after infection, making the leaf useless to the plant. The most rapid rate of disease development occurred in agathia-green and viridian-green leaves.

Table 2

Downy mildew development in relation to leaf colour
*Plasmopara*-Entwicklung in Beziehung zur Blattfarbe

| Leaf colour | Minimum age in days | Days taken for conversion from grade to grade | | Days taken for reaching grade 3 after disease appearance |
|---|---|---|---|---|
| | | 1—2 | 2—3 | |
| Red | 14 | — | — | — |
| Green with orange | 18 | — | — | — |
| Jade-green | 24 | 9 | 7 | 16 |
| Viridian-green | 34 | 7 | 6 | 13 |
| Agathia-green | 48 | 6 | 5 | 11 |
| Emerald-green | 65 | 10 | 8 | 18 |

C.D. (P = 0.05) between colours = 1
C.D. (P = 0.05) between grades = 1

Table 3

Incubation period in relation to colour of inoculated leaves
Inkubationsperiode in Beziehung zur Farbe der beimpften Blätter

| Colour of inoculated leaves | Days after inoculation to | | Colour of inoculated leaves when symptoms appear |
|---|---|---|---|
| | yellow discolouration | downy mildew | |
| Red | 15 | 18 | Jade-green |
| Green with orange | 11 | 14 | Jade-green |
| Jade-green | 8 | 10 | Viridian-green |
| Viridian-green | 8 | 10 | Viridian-green |
| Agathia-green | 8 | 10 | Agathia-green |
| Emerald-green | 8 | 10 | Emerald-green |

## 2. Incubation period in relation to leaf colour

Since the symptoms were observed in jade-green and older leaves, the incubation period in leaves of all colours was studied. Five leaves in each colour class were inoculated. The data are presented in Table 3.

The data revealed that all the five leaves inoculated in each colour were infected, but downy mildew did not appear in red and green with orange-coloured leaves. However, in inoculated leaves of these two colours, symptoms appeared after they turned jade-green. The inoculated jade-green leaves developed downy mildew when they became viridian-green. But the inoculated viridian-green, agathia-green, and emerald-green leaves developed downy mildew before changing to the next colour. The longest incubation period of 18 days was noticed when red leaves were inoculated. The incubation period was shortest in jade-green, viridian-green, agathia-green and emerald-green leaves.

In inoculated red and green with orange coloured leaves, downy mildew was noticed three days after yellow discolouration appeared on the upper surface of leaves. But in leaves of other colours, downy mildew was noticed within two days of appearance of yellow discolouration.

## 3. Penetration of leaves of different colours

Since the pathogen enters the leaf through stomata, the number of stomata per unit area, length and width of stomatal pore in leaves of different colours were measured (Table 4).

Table 4

Stomatal measurements in leaves of different colours
Messungen an den Spaltöffnungen bei Blättern unterschiedlicher Färbung

| Serial No. | Leaf colours | Pore length (μm) | Pore width (μm) | Stomata /mm² |
|---|---|---|---|---|
| 1 | Red | 18.2 | 4.6 | 143 |
| 2 | Green with orange | 20.0 | 4.8 | 160 |
| 3 | Jade-green | 21.5 | 5.2 | 177 |
| 4 | Viridian-green | 18.2 | 5.5 | 185 |
| 5 | Agathia-green | 17.8 | 5.7 | 210 |
| 6 | Emerald-green | 15.0 | 5.9 | 227 |
| | C.D. (P = 0.05) | 3.5 | NS | 32 |

"r" between number and pore length of stomata —0.952

Conclusions: Stomatal pore length: 3 2 4 1 5 6

Stomata/mm²: 6 5 4 3 2 1

The stomatal pore lengths in red, green with orange, jade-green and viridian-green leaves were equal and significantly greater than in agathia and emerald-green leaves. But the differences in stomatal width in leaves of various colours were not statistically significant. With advancing leaf age, the number of stomata per unit increased. There was a negative correlation between number and pore length of stomata. The germ tube from encysted zoospores had a maximum width of 1.1 μm

while the minimum and average widths were 0.6 and 0.8 μm respectively. In leaves of all colours, the stomatal pore was much broader than the width of the germ tube of the pathogen. The germination of zoospores and growth of germ tubes towards stomata was observed in leaves of all colours.

4. Effect of infection on inflorescence and different stages of berry development

Observation on 100 inflorescences before anthesis indicated that 95 per cent were infected. In order to find out which stage in the development of berry is vulnerable to infection, artificial inoculations were made in the cultivar Muscat Hamburg in the following five stages: inflorescence before opening of florets, berries of mustard seed size (2.0 mm diam.), pea size (6.5 mm), neem *(Melia azadirachta)* size (12.5 mm) and mature berries. The number of flower buds or berries, respectively, in each bunch was counted before inoculation. The number of flowers (berries) showing mycelium was recorded after 12 days. Subsequently, the flowers (berries) retained on the bunches were counted at 2-day intervals. The observations were continued till the fruits matured. From the final count the percentage of fruits that had fallen was calculated in each treatment. The data are presented in Tables 5 and 6.

It was found that the inflorescences containing fully developed florets as well as bunches with mustard-sized and pea-sized berries were completely infected. But all the bunches with neem-sized berries and mature berries remained uninfected.

Complete shedding of flowers and mustard seed-sized berries in inoculated inflorescences and bunches respectively, indicated that they were vulnerable to infection. But the cumulative drop was only 53.1 per cent in inoculated bunches with pea-sized berries. The flowers and mustard seed-sized berries started to drop just two days after the appearance of downy mildew, whereas in bunches with pea-sized berries, shedding began only after six days. The initial losses were almost equal in flowers and pea-sized berries. The mean cumulative percentage of drop was maximum in flowers followed by mustard and pea-sized berries. The drop of infected flowers and berries in different days after the appearance of downy mildew was noticed throughout the period under observation.

Table 5

Effect of downy mildew infection on inflorescence and developing bunches
Einfluß der *Plasmopara*-Infektion auf Infloreszenzen und sich entwickelnde Trauben

| Serial No. | Stage at which inoculated | No. of inflorescences or bunches, resp. | | Mean percentage of infected flowers or berries, resp. |
|---|---|---|---|---|
| | | Inoculated | Infected | |
| 1 | Inflorescence | 6 | 6 | 82.9 |
| 2 | Mustard-sized berry | 6 | 6 | 79.6 |
| 3 | Pea-sized berry | 6 | 6 | 49.8 |
| 4 | Neem-sized berry | 6 | 0 | 0 |
| 5 | Mature berries | 6 | 0 | 0 |

C.D. (P = 0.05) = 23.1

Conclusions 1 2 3

Table 6

Effect of inoculation with *Plasmopara viticola* on flowers and berries
Einfluß der Beimpfung mit *Plasmopara viticola* auf Blüten und Beeren

| Serial No. | Days after inoculation | Cumulative percentage of drop | | |
|---|---|---|---|---|
| | | Flowers (F) | Mustard-sized berries (M) | Pea-sized berries (P) |
| 1 | 12 | 0 | 0 | 0 |
| 2 | 14 | 13.1 | 4.4 | 0 |
| 3 | 16 | 20.7 | 14.5 | 0 |
| 4 | 18 | 35.2 | 23.6 | 11.5 |
| 5 | 20 | 46.4 | 35.4 | 18.5 |
| 6 | 22 | 56.7 | 48.9 | 25.0 |
| 7 | 24 | 68.0 | 63.8 | 32.1 |
| 8 | 26 | 75.3 | 69.3 | 37.4 |
| 9 | 28 | 80.9 | 75.3 | 42.7 |
| 10 | 30 | 85.9 | 79.5 | 47.1 |
| 11 | 32 | 88.1 | 82.7 | 49.8 |
| 12 | 34 | 90.0 | 85.9 | 51.7 |
| 13 | 36 | 90.0 | 86.3 | 52.8 |
| 14 | 38 | 90.0 | 90.0 | 53.1 |

C.D. ($P = 0.05$) between stages = 0.7
C.D. ($P = 0.05$) between days = 1.4
C.D. ($P = 0.05$) between stages and days = 2.5

Conclusions: stages: F M P

days: 14 13 12 11 10 9 8 7 6 5 4 3 2

## Discussion

The observations indicated variation in incidence as well as intensity of the disease symptoms in leaves of different ages. The information available in the literature is very meagre with regard to the minimum age of leaves at which downy mildew symptoms develop. In Germany, Kroemer (1921) pointed out that the youngest leaves resisted infection, contrary to our results. The reason why very young leaves with red or green with orange colour did not show symptoms can be surmised from the fact that the incubation period in red leaves was 18 days. Before the incubation period was over, the red leaves changed into jade-green colour. The fact that inoculated young leaves (red and green with orange) developed symptoms clearly indicates that they did not resist zoospore germination, penetration or establishment of the disease. This conclusion is also supported by the microscopic observation in which the zoospores sprayed on these young leaves were found to have germinated and the germ tubes were growing towards the stomata. The pore width of stomata was broader than the width of germ tube.

The incubation period of this disease varied from 4—5 days in Yugoslavia (Milisavljevic 1949) to 5—18 days in central Europe (Müller and Sleumer 1934). It is reported to be influenced by variety (Gregory 1912) and meteorological condi-

tions like temperature, relative humidity and rainfall (FOLTYN 1961). RAFAILA *et al.* (1968) indicated that the incubation period was influenced to a great extent by the age of the tissue. The present observation also indicated a longer incubation period in inoculated red and green with orange leaves than in older leaves. The incubation period under South Indian conditions is longer than that reported from other countries.

During the development of the disease the number of days taken to pass from grade 1 to 2 was always higher than the days taken for passing to grade 3 from 2. In view of the fact that in leaves showing grape-1 infection the area of infected leaf is less than in grade 2, the pathogen has the added advantage of a larger amount of mycelial growth for more rapid spread to grade 3.

In agathia-green and emerald-green leaves the number of stomata per unit area was greater than in leaves of other colours. This can be expected to provide a greater number of loci for infection. This is revealed by the fact that these two coloured leaves had a very high percentage of infected leaves. Similarly, viridian-green leaves which were on a par with agathia-green leaves in respect of number of stomata per unit area also recorded a higher percentage of infected leaves than younger ones.

Upon artificial inoculation, developing berries of mustard and pea seed size alone picked up infection, whereas the older fruits did not. This indicates that the maximum damage directly caused to fruit development by this disease under South Indian conditions occured only in the early stages of fruit development. The destruction of young clusters and resistance of mature berries to this disease have been pointed out by SINGH *et al.* (1963).

## Summary

The incidence and intensity of downy mildew on "Anab-e-Shahi" grapevine were recorded on leaves of six ages as indicated by colours. Red and green with orange leaves did not show symptoms of the disease. The rate of disease development was highest in agathia-green and viridian-green leaves. The time taken for reaching grade 3 from grade 2 was shorter than the time taken for development to grade 2 from grade 1. The incubation periods were 18 and 14 days in red and green with orange leaves, respectively. But in older leaves it was only 10 days. In leaves of all colours the stomatal pore was broader than the width of the germ tube of the pathogen. Upon artificial inoculation inflorescences as well as clusters with mustard seed-sized (2.00 mm) and pea-sized (6.5 mm) berries became infected whereas older bunches with neem-sized (12.5 mm) and mature berries were not infected. Complete shed of infected flowers and mustard seed-sized berries and a drop of 53.1 per cent of pea-sized berries were noticed.

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