PATHOLOGICAL STUDIES OF *GLŒOSPORIUM PSIDII* CAUSING DIE-BACK OF GUAVAS

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Introduction

Allahabad has the proud privilege of being the best guava growing centre in the state. For the last few years a very high death rate of branches and wilting of guava plants has been observed in certain orchards at Allahabad. It is fast becoming a menace to orchard owners. The trouble is not confined to Allahabad but has been observed at other places also.

A thorough survey of Allahabad guava orchards was undertaken and severe losses were observed during the rainy season when the growth of the new twigs was considerably checked due to this trouble. Hardly any plant was free from this disease.

Die-back is a common disease on many plants. Waterston (1941) reported *Glœosporium psidii*, the cause of 'leaf spot' disease of guava. There is no other record of the Die-back organism of guava.

It has been shown by Baker and Wardlaw (1937) and Baker (1938) that certain fungi may remain inactive in the tissues of the host for considerable time before the formation of lesions gives a visible clue of their presence. This type of infection was termed by them as 'Latent Infection'. It was further demonstrated that the infections occur early in the development of fruit and that they were confined to the upper layers of the rind irrespective of the age of the fruit at the time of infection. The lesions become visible only when a certain stage of ripening has been reached. Simmonds (1941) has described latent infection associated with species of *Glœosporium* and *Colletotrichum* in immature banana fruits infected with *Glœosporium musarum*. It remained in latent state for about 5¼ months after which the characteristic anthracnose lesions developed and the fungus became active.

There has been practically no work on *Glœosporium psidii* isolated from guavas. It was, therefore, decided to undertake a detailed physiological and pathological study of *Glœosporium* isolated from the stems of guava trees.

Material and Method

The material for isolation was collected from the trees of *Psidium gujava*. The methods used for isolating the fungus were the same as described by Tandon (1952). It invariably gave *Glœosporium psidii*. Single spore cultures were prepared by dilution method and they formed the parent cultures.

In order to test the pathogenicity of the isolates a large number of artificial inoculations were carried out on the injured and uninjured plants. The stem and leaf inoculations were made on the new young growth near the top of a twig, or at the growing point as well as at older regions of the plants. To avoid infection and drying of the spores, moist cotton pads were placed on them. In case of fruits, inoculations were made by the method described by Granger and Horne (1924). They were also inoculated through injured and uninjured regions by placing the inoculum at calyx and stem ends and the injured surface of the fruits as described by Tandon (1948). Controls were maintained in each case.

Symptoms

The most characteristic symptom of the disease is that the plant begins to die backwards from the top of a branch. Young shoots, leaves and fruits, while they are still tender, are readily attacked. The greenish colour of the growing tip is changed into dark brown colour which later on develops into black necrotic areas extending backwards and causing the die-back. The disease becomes more noticeable after a period of incubation in the infected buds and twigs. The brown spots, formed previously, change into silvery grey and ultimately develop a pronounced demarcating ring or girdle at the junction of the diseased and healthy part. This ring may either be very distinct or it may gradually diffuse into healthy green part of the shoot.

The fungus makes its track from the infected twigs to the petioles and ultimately the young leaves are attacked. In severe cases of infection they are entirely killed but ordinarily they may have dead areas on the margins or tips with distortion of the remaining portions. These may droop down or fall on account of a slight jerk of the gentle breeze. The dried twigs altogether devoid of leaves are ultimately left (Plate II, Fig. 1).

In moist weather when disease is more vigorous acervuli of the fungus may be seen as black dots scattered throughout the dead parts of the twigs, and if they are kept in moist chambers they ooze out the pinkish masses of spores.

OCCURRENCE AND EXTENT OF DAMAGE

All sizes of trees from those located in the nursery beds to the old trees growing in the orchard are subjected to attack. In Minto Park, Allahabad (Govt. Garden), the guava orchards were severely infected in 1952. Several trees died out completely without bearing a single fruit while others were nearly half dead (Plate II, Fig. 2). The ripened fruits hanging in these infected trees were clearly showing the pinkish masses of acervuli causing the rot on the outer skin of the fruit.

A regular fortnightly survey was carried out for one year (June 1952 to July 1953) in different orchards at Allahabad and the amount of damage during different periods was carefully recorded.

In June when there was slight rain, only the old twigs showed the symptoms of die-back. New shoots with tender stems and leaves were appearing and they showed no apparent symptom of any disease. In July it was often cloudy, the temperature was lower and the atmosphere was very humid. The growth of the new shoots was vigorous and no evident symptom of the disease could be noticed in young shoots. In the first fortnight of August, the disease caused the death of small tender growing twigs. From August to October, the infected twigs withered and shrivelled at their tips upto a distance of one to several inches. This greatly reduced the younger growth and prevented blooming in severe cases. The disease appears in epidemic form from August to September only and under favourable conditions it may reach the main trunk. If due to any reason the infection does not prove very severe only the growth of the first sprout is checked, while other subsequent sprouts do not show the sign of arrested growth of the plant. When the infection reaches a node or some point of bifurcation of the two twigs, it again shows the sign of dormancy for a short or even for a fairly long period.

The die-back of guava trees becomes more prominent with the advent of time. It can also be noticed in December and later months after which the older leaves and twigs remain immune from further attack. Whenever the weather conditions are almost on the two extremes and the atmosphere is less humid (*viz.*, from January to June) the further progress of the disease is greatly inhibited. There are no new tender shoots and leaves on the trees for the parasite to attack. The older ones escape damage as these are sufficiently hard and woody. The percentage infection in the months of July, August, September, October, November and December was 5, 13, 25, 17, 8 and 4 respectively.

Observations confirmed that hot and dry weather of April to June, and cold season of January to March prevents the organism from starting new infection during these months.

Pathogenicity

Pathogenicity tests on leaves, twigs and fruits were conducted both in the laboratory and garden during the rainy season as well as in the summer months.

Twigs and Stalks.—During the rainy season the inoculated twigs began to change their greenish colour to brown after 15–20 days. The intensity of infection continued to increase and the young twigs exhibited dry conditions upto a length of 1–1½ inches. It was further noticed that the fungus placed inside the injured areas succeeded in infecting the twigs while the inoculations made without injury gave negative results. In summer even injured regions failed to show any infection without application of moist cotton pads and even under such conditions the percentage infection was low. The necessity of applying the cotton pads was not felt in the rainy season when the infection extends towards the trunk more rapidly than in summer months. The twigs which were inoculated soon after the drizzling or when the rain started and continued for a week or more showed withering within a week. The damage was more severe, if the clouds were present. Under such conditions the new growth of the current season was largely attacked. It, therefore, confirmed that humidity played an important part in the growth of the organism. Successful re-isolations of *Glœosporium psidii* were made from most of the natural or artificially infected parts. The old branches which were sufficiently hard and woody resisted the entrance of the casual organism as the old stalks of the trees did not develop any infection.

Leaf Infection.—The inoculations made on the upper and the lower surfaces of the leaves showed the symptoms of the disease which appeared after one week on the lower surface of the leaf only. The young leaves which were infected showed dead areas on the margins and the tips with distortion of the remaining portions. The speed of the disease from tip to the base of the leaf was sufficiently rapid in some cases. Inoculations on older leaves invariably failed to develop any disease.

Flower Infection.—Small flowers of guavas and their opened buds were sprayed with the atomizer containing a suspension of the spores of *Glœosporium psidii.* Unopened buds showed infection within two or three days and failed to develop further. The petals turned brown and the buds

fell off without opening. Flowers also showed the same symptoms and they ultimately dropped off before fertilization.

Attempts were also made to observe the effects of a drop of spore suspension on the stigmatic surface of each pistil. The flowers selected for this purpose were the unopened buds which were carefully opened with the help of fine needles. Few out of these opened buds were not inoculated and were used as controls; while the spore suspension was placed with the help of sterilized eye dropper on the stigmatic surface of others. It was observed that the stigmas inoculated with spore suspension developed dark brown colour after 24 hours and in many cases the entire flower including the stalk dropped off within 3–4 days. The infected ovary was occasionally left hanging on the tree and subsequently it dried into dark brown colour and fell on the ground after a few weeks. In still fewer cases, the fertilized infected ovary persisted to grow into a fruit which ultimately changed into a dry hard stony structure with dark brown or black brown colour (Plate II, Fig. 3). These were developed due to infection at early stages. The unsprayed flowers (Control) developed normal fruits.

Fruit Infection.—Mere contact of the diseased and the healthy fruit was sufficient to develop a rot in ripened fruits but it never developed into a mummy. The rot is usually slow unless the fruit is ripe. At all subsequent stages, the organism could cause a rot of mature fruit near the point of infection. Pink to dark brown circular patches of acervuli were produced on the rotten areas. These small patches increased in diameter and they covered a large surface of the fruit within two days after which cottony mycelium appeared at the surface of the fruit provided they were kept in moist chambers. It was, however, noticed that if the infected fruits were allowed to remain on the tree or in the laboratory they were usually deformed and they became worthless as they gradually became hard dry and shrunken.

Besides artificially developed hard and stony fruits other small dried blackish brown or sometimes black fruits of guavas (commonly known as mummies) were frequently observed hanging on the diseased guava plants. An attempt was made to find out whether they were developed, as a result of infection of flowers or of fruits at different stages of their development. For this purpose guava fruits of different ages were inoculated on the tree in their natural condition. The inoculations were started soon after the fertilized ovary became rounded. In one set of experiments the raw fruits of approximately one inch in diameter were inoculated by Granger and Horne's method (1924). In another set only the spore suspension was sprayed with the atomizer on the fruit of similar size. At this stage the young

fruits were as hard as stone and were rich green in colour. It was observed that all fruits on which spores were sprayed showed no infection under any condition at any stage. The fruits which were inoculated by Granger and Horne's method also did not show any trouble in early stages but infection was observed when those fruits reached maturity. The organism survived in latent stage inside the rind for about 2½–3 months. When the fruits ripened it resumed its activity to produce typical rot on the outer surface of the fruit. It was also observed that the inoculum placed in the subcuticular region of injured raw guavas developed a mass of hyphæ which remained as such till the fruits ripened. This indicated that during the latent period the fungus survived in the form of hyphal structure. Numerous appressoria were formed near the subcuticular surface.

The absence of active parasitism in the green guava fruits even though the fungus grows on its extract indicates that the hard tissue prevents the fungus from growing. It remains dormant till the tissues become quite soft. It was noticed that when the organism was grown on guava extract medium, it sporulated more or less with the same rapidity as on the ripened guava extract medium; while in inoculation experiments, the fungus did not develop any symptom of disease till the fruits were fully matured. It is very likely that due to very hard nature of the host (unripe guava) the fungus finds it difficult to develop on it. Blackman and Welsford (1916) showed that the rupture of the cuticle and subsequent penetration was due to mechanical pressure exerted by the penetration hyphæ. Dey (1933) also reported mechanical penetration in the infection of beans by *Colletotrichum lindemuthianum* as well as on lemon leaves infected by *Colletotrichum glœosporioides*. The pressure needed for penetrating raw guavas does not develop in the present case but on ripening the cellular mass is loosened and hence the intercellular development of the organism becomes possible. The rapid growth of the parasite starts after a long dormant period. The spores penetrate through the soft cuticle with the help of fine infection thread which afterwards develops a thick mass of mycelium adjacent to the epidermal cells and the characteristic acervuli are produced on the outer skin.

Cross Inoculations

Cross inoculations of *Glœosporium* isolated from *Citrus aurantifolia*, *Citrus Karna*, *Citrus medica* and *Psidium gujava* were tried and it was found that *Glœosporium psidii* could effect guava plants alone and other organisms failed to infect except the host from which they were isolated.

CONTROL

The disease was most active when the humidity was high and new branches appeared. It was also more serious on trees growing close together or in shade.

An attempt was, therefore, made to control the die-back or the development of mummies. Some proprietory chemicals, *viz.*, Zerlate, Parazate, Diathene Z-78 were tried but none of them could check the disease.

Finely powdered sulphur, lime sulphur 15%, 25%, peronox ·22%, ·33% and bordeaux mixture 2:2:50, 3:3:50 and 5:5:50 were applied either on the diseased plants directly or after removing the dead twigs. The spraying or dusting was carried out after the rains and three applications were given at an interval of three weeks each. It was observed that they were successful in reducing the losses. The application of fungicides was more effective when it was applied after removing the dead branches.

Out of all these fungicides used, bordeaux mixture 3:3:50 was more effective and was closely followed by peronox. The percentage losses on lime sulphur 15%, 25%, sulphur, peronox ·22%, ·33%, and bordeaux mixture 2:2:50, 3:3:50, 5:5:50 were 50, 50, 28·5, 10, 12, 12·5, 6·6, and 13·5 respectively. The losses were about 15% higher when dead twigs were not removed.

SUMMARY

1. *Glœosporium psidii* was isolated from die-back of guava trees. A detailed study of the symptoms, the amount and extent of damage has been recorded. It was found that severity of the disease increased from middle of August to beginning of October.

2. The pathogenicity of the organism was established and it has been found that it can readily attack the young tender shoots, injured leaves, flowers and buds. It failed to attack mature twigs and leaves.

3. It has been established that mummies are produced if buds and flowers are infected at an early stage.

4. The fungus can live in dormant condition for about 2½–3 months. It subsequently resumes its activity and develops the rot when the fruits ripen.

5. None of the fungicides so far tried could control the disease fully but the application of 3:3:50 bordeaux mixture and ·22% or ·33% peronox gave encouraging results.

FIG. 1

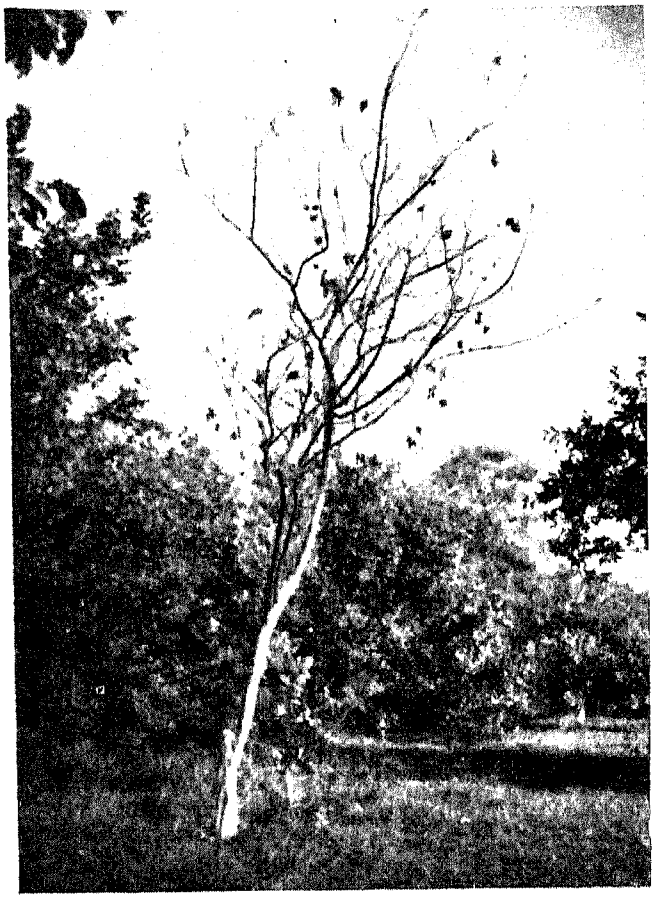

FIG. 2

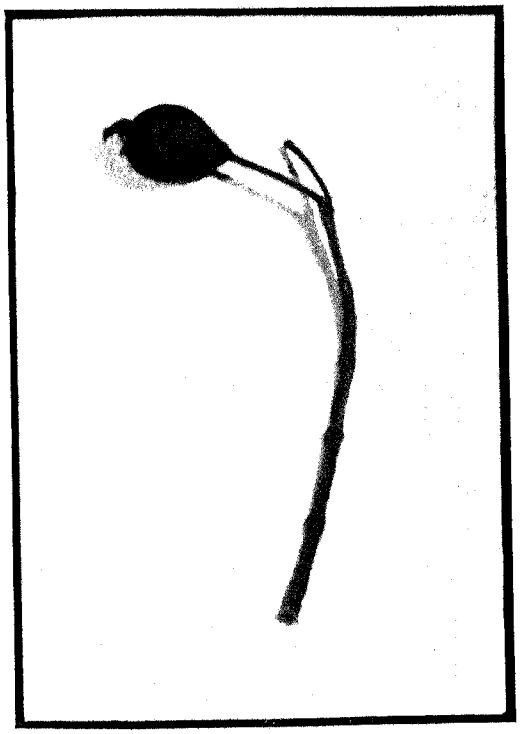

FIG. 3

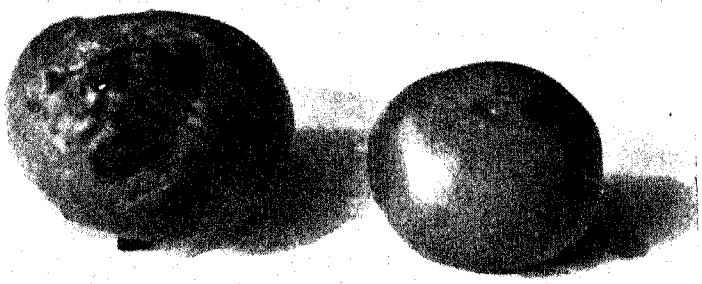

FIG. 4

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Explanation of Plate

Fig. 1. Symptoms incited by *G. psidii* on guava stems.

Fig. 2. Showing severe infection due to which the plant is killed. A few mummies are seen.

Fig 3. Showing the infected ovary which developed into a mummy.

Fig. 4. Showing ripened fruits (towards left) inoculated which *Glæosporium psidii* and a healthy fruit used as a control (towards right).