Diseases and associated fungal pathogens of mulberry nurseries

V.P. GUPTA, GOVINDAIAH and H.V. RAJU

Mulberry Pathology Laboratory, Central Sericultural Research and Training Institute, Srirampura, Mysore 570 008

Abstract: Occurrence of various diseases viz., stem-canker, cutting rot, collar rot, sprout/leaf blight, die-back and root rot were observed in mulberry nurseries which affected initial establishment of mulberry by causing mortality of stem-cuttings and death of saplings. On isolation and identification, Botryodiplodia theobromae, Fusarium solani, Phoma sorghina, F. pallidoroseum and Colletotrichum gloeosporioides were identified as causal pathogens of these diseases. Amongst these, B. theobromae was found to be highly virulent causing 44.4% mortality of cuttings when inoculated to the soil, however, it caused 51.4 to 61.1% mortality when inoculated in combination with F. solani and/or P. sorghina. B. theobromae also attacked the foliage of saplings and killed 50-60% saplings by causing die-back disease.

Keywords: Mulberry, nursery, diseases, identification, virulence

Mulberry (Morus alba L.) is one of the most important crops for sericulture industry as its leaves are the sole food of silkworm (Bombyx mori L.). Being a vegetatively propagated perennial crop, its initial establishment plays a vital role in better plant growth and leaf yield. Mulberry is generally propagated through stem-cuttings which are planted either directly to the fields, or in the nurseries to raise saplings for transplantation (Dandin et al., 1988). When the cuttings are planted to the soil, their cut-ends get predisposed to soil-borne pathogens, resulting in failure of cuttings to sprout and death of saplings due to diseases. Besides, saplings, being young and tender, are also prone to air-borne pathogens which cause to foliage diseases to foliage and sometimes lead to the death of saplings.

During the survey undertaken in the year 1993 and 1994 at different locations in Karnataka and Andhra Pradesh, it was observed that several diseases attack mulberry cuttings and saplings in nurseries as well as in fields which cause 30-35% mortality of cuttings and death of saplings. The mortality is more severe (more than 50%) in case of high-yielding, poor-rooting mulberry varieties, particularly var. S-36. No systematic study has been conducted so far on the occurrence of diseases in mulberry nurseries. Therefore, keeping in view the importance and seriousness of these diseases, present investigations were undertaken to study the occurrence of various diseases in mulberry nurseries and identify their causal pathogens.

MATERIALS AND METHODS
Diseased plant materials

Diseased cuttings and saplings were collected from different mulberry nurseries and fields in
Fig. 1: Photographs showing symptoms of diseases of mulberry nurseries.
(a) Stem-canker; cuttings showing failure to sprout and death of sprout; (b) Leaf blight; shoot of a sapling showing leaf blight symptoms; (c) Collar rot; diseased saplings showing death of tissues at the collar-region (cl), and wilting of leaves; (d) Die-back; shoot of a sapling showing dying of leaves from the apex.
Karnataka and Andhra Pradesh, and critically observed for disease symptoms. Isolation of the pathogens were made from the diseased cuttings and saplings showing different types of symptoms.

**Isolation and identification of pathogens**

The fungi were isolated from diseased portions of cuttings and saplings by plating their bits on to the potato dextrose agar (PDA; pH 5.6) and incubated at $27 \pm 2^\circ C$ for 5-7 days. After isolation, all the fungal cultures were purified by single-hyphal tip inoculations and maintained on PDA slants in refrigerator (4°C). All the cultures were identified with the help of CMI Descriptions of Pathogenic Fungi, and some other standard descriptions of fungi (Booth, 1971; Barnett and Hunter, 1972; Nelson et al., 1983).

**Pathogenicity tests**

The fungi isolated from diseased cuttings were inoculated to the soil, alone as well as in combinations, before plantation of healthy cuttings; whereas the fungi isolated from diseased leaves of saplings were inoculated singly by atomizing the spore suspension on to the leaves of healthy saplings. Soil inoculation was done by adding the fungal culture (7-day-old) grown on sand-compost meal medium to the autoclaved soil (soil-sand-compost mixture; 4:1:1) in wooden seed pans (60 x 40 cm). Inoculum was mixed with soil thoroughly and adjusted to provide an initial population density of $1 \times 10^6$ CFU/g of soil. Healthy cuttings (24 cuttings/seed pan with 10 cm x 10 cm spacing) were planted in inoculated soil, and seed pans were kept in a greenhouse ($27 \pm 2^\circ C; 80\% RH$). Three seed pans were maintained for each fungal culture, alone as well as in combinations. The cuttings planted in uninoculated autoclaved soil served as control. For foliar inoculation of saplings, spore suspension ($1 \times 10^4$ conidia/ml), obtained by flooding the culture (7-day-old) with sterile distilled water, was atomized on to the leaves of healthy saplings (45-day-old) raised in earthen pots. The inoculated saplings were kept in a humid chamber ($27 \pm 2^\circ C; 100\% RH$) for 24 h, and then shifted to a greenhouse. For each fungal culture, ten saplings were inoculated. The saplings atomized with sterile distilled water served as control. Mulberry var. Kanva 2 was used as a test variety. Each set of experiment was kept under observation for two months for the development of symptoms, recording the disease incidence, and mortality of cuttings and saplings. All the experiments were repeated twice in order to confirm the results.

**RESULTS**

**Diseases and symptoms**

Various diseases viz., stem-canker, cutting rot, collar rot, sprout or leaf blight, die-back and root rot were observed affecting the initial establishment of mulberry cuttings and saplings in nurseries as well as in fields. Almost all popular mulberry varieties cultivated in South India namely, Local, Kanva 2, MR-2, S-13, S-34, S-36 and S-54 were found to be affected with these diseases.

Stem-canker disease manifests as the failure of cuttings to sprout or sudden withering, defoliation and death of sprout/saplings. On close examination of diseased cuttings, greenish-black eruptions are observed on cuttings near the soil surface which later discharge black masses of conidia. The bark becomes dead and decayed on underground portion of cuttings (Fig. 1a). Cutting rot, as name indicates, appears as rotting of whole cuttings and decaying of bark, resulting in failure of cuttings to sprout or wilting and death of sprouts. The decayed bark can easily be separable from the wood. In case of collar rot disease, the cutting just near the soil surface (collar region) shows discoloration and death of tissues which lead to the sudden withering of sprouts and leaves, and death of saplings (Fig. 1c). Sprout or leaf blight appears as blackening or burning of sprouts, buds and leaves of saplings (Fig. 1b). In case of die-back disease, wilting and dying of saplings start from top (apex) and progress downwards (Fig. 1d), whereas in case of root rot, sudden withering of whole sapling is observed and root system is rotten.
Identification of pathogens

On the basis of morphological and cultural characters, the fungi isolated from diseased cuttings were identified as *Botryodiplodia theobromae* Pat. [stem-canker], *Fusarium solani* (Martius) Sacc. [cutting rot] and *Phoma sorghina* (Sacc.) Boerema *et al.* [collar rot], whereas two fungi isolated from diseased saplings showing sprout as well as leaf blight symptoms were identified as *Fusarium pallidoroseum* (Cooke) Sacc. and *Colletotrichum gloeosporioides* (Penzig) Penzig & Sacc. *B. theobromae* was also constantly isolated from the diseased leaves of saplings showing die-back symptoms, whereas rotten roots of saplings yielded *F. solani*. Further, the identification was confirmed from International Mycological Institute, Surrey, U.K. (IMI No. 356552, 356554), and Indian Type Culture Collection at Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. The culture of *B. theobromae* was deposited in IMI herbarium vide IMI No. 356554, and other cultures were deposited with Indian Type Culture Collection vide ITCC No. 515, 544, 659.94 and 668.94.

Pathogenicity and virulence

Pathogenicity of the fungi was successfully proved on artificial inoculation. All the fungal isolates induced symptoms similar to that of those occurring under natural conditions, and were reisolated from the diseased cuttings and saplings, thus fulfilling the Koch’s postulates. Although few cuttings planted in sterilized soil (uninoculated control) also failed to sprout, these unsprouted cuttings neither showed any particular symptoms, nor yielded any pathogenic fungi on isolation. Therefore, it may be either due to the technical failure in planting or due to some physiological reasons. However, saplings in control did not show any symptoms.

The fungi varied in their virulence to cuttings

<table>
<thead>
<tr>
<th>Cuttings/saplings mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>44.4</td>
</tr>
</tbody>
</table>

**Pathogens**

*Bt* = *Botryodiplodia theobromae*  *Fs* = *Fusarium solani*  *Ps* = *Phoma sorghina*

Fig. 2: Pathogenic potential of various pathogens causing diseases in mulberry nurseries.
and saplings. The soil-borne fungi isolated from diseased cuttings were found to be more virulent causing 31.9 to 44.4% mortality of cuttings on inoculation. Of these, B. theobromae was highly virulent causing 44.4% mortality of cuttings when inoculated alone, however, when B. theobromae was inoculated in combination with F. solani and/or P. sorghina, mortality of cuttings was further increased and it was recorded 51.4 to 61.1% (Fig. 2).

B. theobromae also induced foliar infection in saplings and killed 50-60% of them by inducing die-back symptoms within a month of inoculation. F. pallidoroseum and C. gloeosporioides induced sprout/leaf blight symptoms in inoculated saplings with 60-65% disease incidence after a month of foliar inoculation, however, these fungi could not kill any of the inoculated saplings.

DISCUSSION

Present study revealed that B. theobromae is one of the most serious pathogens of mulberry nurseries. Because of its soil-as well as air-borne nature, it causes stem-canker and die-back diseases to mulberry which lead to the mortality of cuttings and death of saplings. Although B. theobromae has been reported to attack mulberry (Luke and Paul, 1982), it was hitherto not known to be a serious pathogen to mulberry plantation. B. theobromae has also been reported causing canker and die-back diseases in many other plantation crops like pear (Verma and Cheema, 1984), rose (Shukla and Choudhury, 1991) and mango (Sharma and Gupta, 1994), however, it has so far not been reported to cause die-back of mulberry saplings. Therefore, this is the first report of B. theobromae causing die-back in mulberry.

In the present study, F. solani and P. sorghina were also found to be serious pathogens of mulberry nurseries causing cutting rot and collar rot diseases, respectively which lead to the failure of cuttings to sprout, and wilting and death of saplings. F. solani, though, has been reported to cause leaf spot (Chowdhary and Raj, 1986) and root-rot diseases (Philip et al., 1995) in established mulberry plants, it has so far not been known to be a serious pathogen of mulberry nurseries. Yadav and Sukumar (1987) have reported two species of Phoma viz., P. mororum and P. exigua causing collar-rot and stem blight diseases, respectively in mulberry, however, P. sorghina has not yet been reported causing disease in mulberry. Therefore, it appears to be the first report of P. sorghina as a causal pathogen of mulberry.

F. pallidoroseum causing sprout/leaf blight in mulberry saplings has earlier been reported causing leaf blight in established mulberry plants (Govindaiah et al., 1990). However, C. gloeosporioides which has been reported causing black leaf spot disease in established mulberry plants (Philip et al., 1994), was found to be causing sprout/leaf blight disease in mulberry saplings. The variations in symptoms may be due to the plant age as saplings, being young and tender, are more susceptible to diseases; whereas in mature plants, infection may become localized in the form of black spot due the development of resistance.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. R.K. Datta, Director, C.S.R.T.I., Mysore for extending necessary facilities during the study. Thanks are also due to Drs. E. Punithalingam and D. Brayford of Biosystematic Services, IMI, Surrey, U.K., and Drs. P.N. Chowdhry and S.P. Lal of Indian Type Culture Collection, IARI, New Delhi for identification of fungi.

REFERENCES


