

## SEM Observations on the Perithecia of *Phyllactinia corylea* Causing Powdery Mildew Disease in Mulberry

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**Surface morphology of perithecia of the powdery mildew fungus of mulberry, *Phyllactinia corylea* is described under scanning electron microscope. The perithecia have penicellate cells on the upper surface and at an average 17 acicular appendages towards the lower surface each emerging from a bulbous base. Many perithecial walls towards the base have shrunken walls. When the perithecia dry out they are pushed above the leaf surface by the acicular appendages which then bend at the base. The bending of the appendages may be attributed to the shrinkage of lower wall cells due to loss of water.**

**Key words :** Perithecia, *Phyllactinia corylea*, Mulberry, Powdery mildew, SEM

### Introduction

Powdery mildew caused by *Phyllactinia corylea* (Pers.) Karst. (Class Ascomycetes; Order Erysiphales; Family Erysiphaceae) is one of the major fungal diseases of mulberry (*Morus* spp.) in India and other mulberry growing countries (Itoi *et al.*, 1960; Philip *et al.*, 1994). The disease manifests by the characteristic white powdery patches on abaxial surface of mulberry leaves with corresponding chlorotic lesions on the adaxial surface (Kumar *et al.*, 1998, 2000). On advancement of disease, the patches, which vary in size from 0.5 to 2.5 cm or more in diameter, become irregular and quickly cover the entire lower leaf surface.

Diseases of mulberry cause much concern for the silk

producing countries, as the silkworm, *Bombyx mori* Linn. feeds only on mulberry leaves during its entire larval period. Powdery mildew causes 8-10% crop loss in the annual yield of mulberry leaf, besides deteriorating the leaf quality (ESCAP, 1990). The infection reduces the feeding qualities of mulberry leaves, and the consumption of infected leaves by silkworm, *B. mori* larvae adversely affects the larval development and cocoon characters (Noamani *et al.*, 1970; Sullia and Padma, 1987; Kumar *et al.*, 1993).

The genus *Phyllactinia* being semi-exoparasitic in nature, is distinguished from other exoparasitic powdery mildews (Homma, 1937). *P. corylea* is an obligate parasite and may reproduce by both asexual and sexual methods but sexual reproduction takes place only in temperate climatic conditions. In late summer, when the conidial production of *P. corylea* slows down and eventually ceases, young perithecia begin to make their appearance on the white mycelium in temperate conditions. The mycelium is at first white, later turns to orange, reddish brown and finally becomes black when mature.

Asexual conidial stage of *Phyllactinia corylea* on mulberry has been studied earlier at ultrastructural level (Kumar *et al.*, 1998). However, no information is available on the sexual perithecial stage of *P. corylea* on mulberry. Therefore, the present study has been carried out with a scanning electron microscope on the sexual stage of *P. corylea* infecting mulberry in temperate zone in particular.

### Materials and Methods

The samples of infected mulberry leaves (*Morus* spp.) containing asexual and sexual stages of *P. corylea* were collected from the mulberry gardens at Pampore (Jammu and Kashmir), a temperate zone in northern hilly part of

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India, and its neighbouring areas. Leaf samples were cut into 3 mm<sup>2</sup> pieces and were fixed for 2 hrs in 2.5% glutaraldehyde prepared in 0.2 M sodium cacodylate buffer (pH 7.2), washed thrice with cacodylate buffer for 15 min each and then dehydrated in a graded ethanol-acetone series, 10 min per step at room temperature (25.0 ± 2.0°C). The dehydrated materials were dried in a

critical point drier (EMS-850) using CO<sub>2</sub> as transition fluid and then mounted over to copper stubs with a double-sided sticky tape. The mounted specimens were coated with gold (20-nm thickness) in a sputter coater (EMS-550) and observations were made at 20 kV under a JEOL 100 CX-II ASID-4D scanning electron microscope.

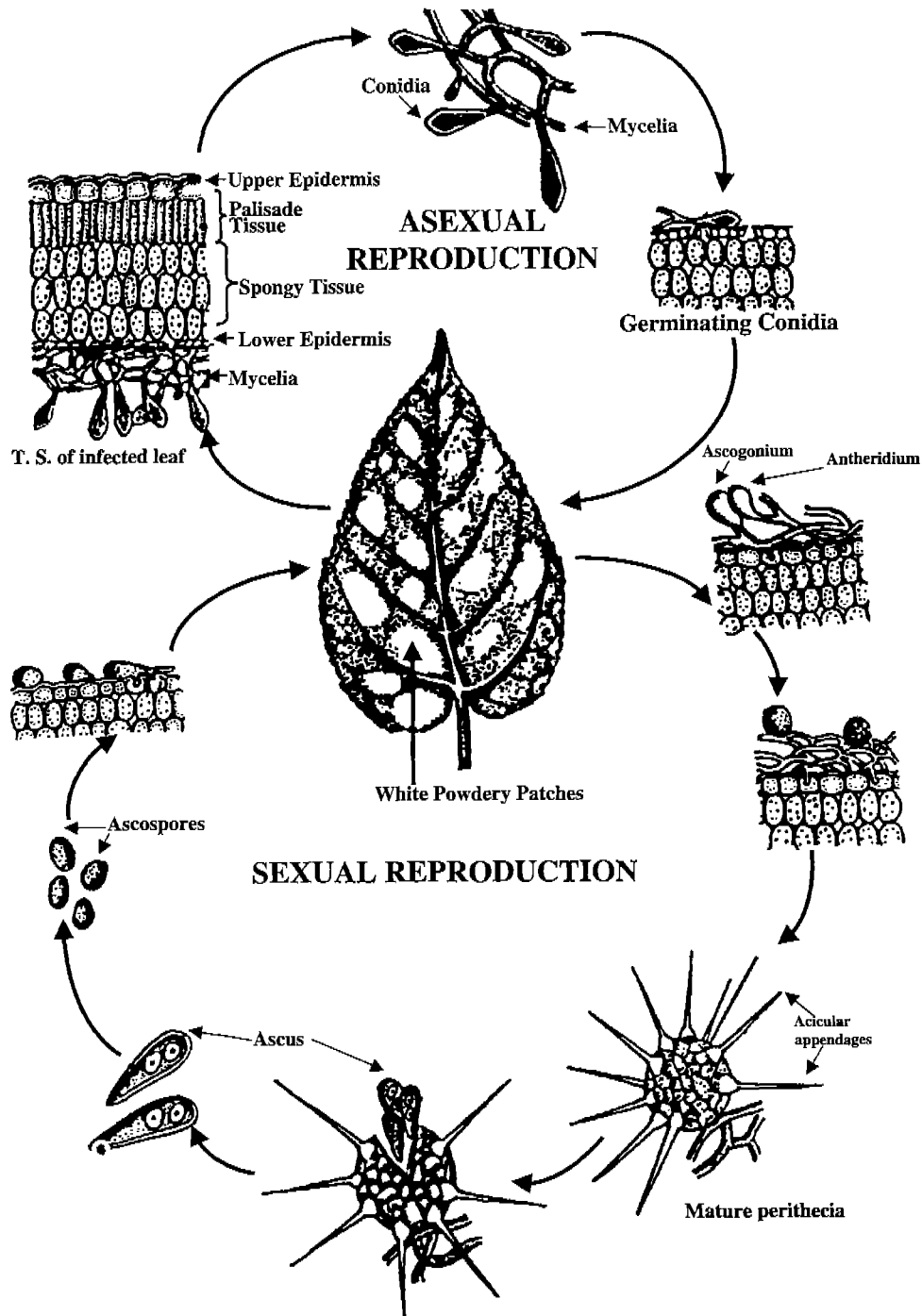
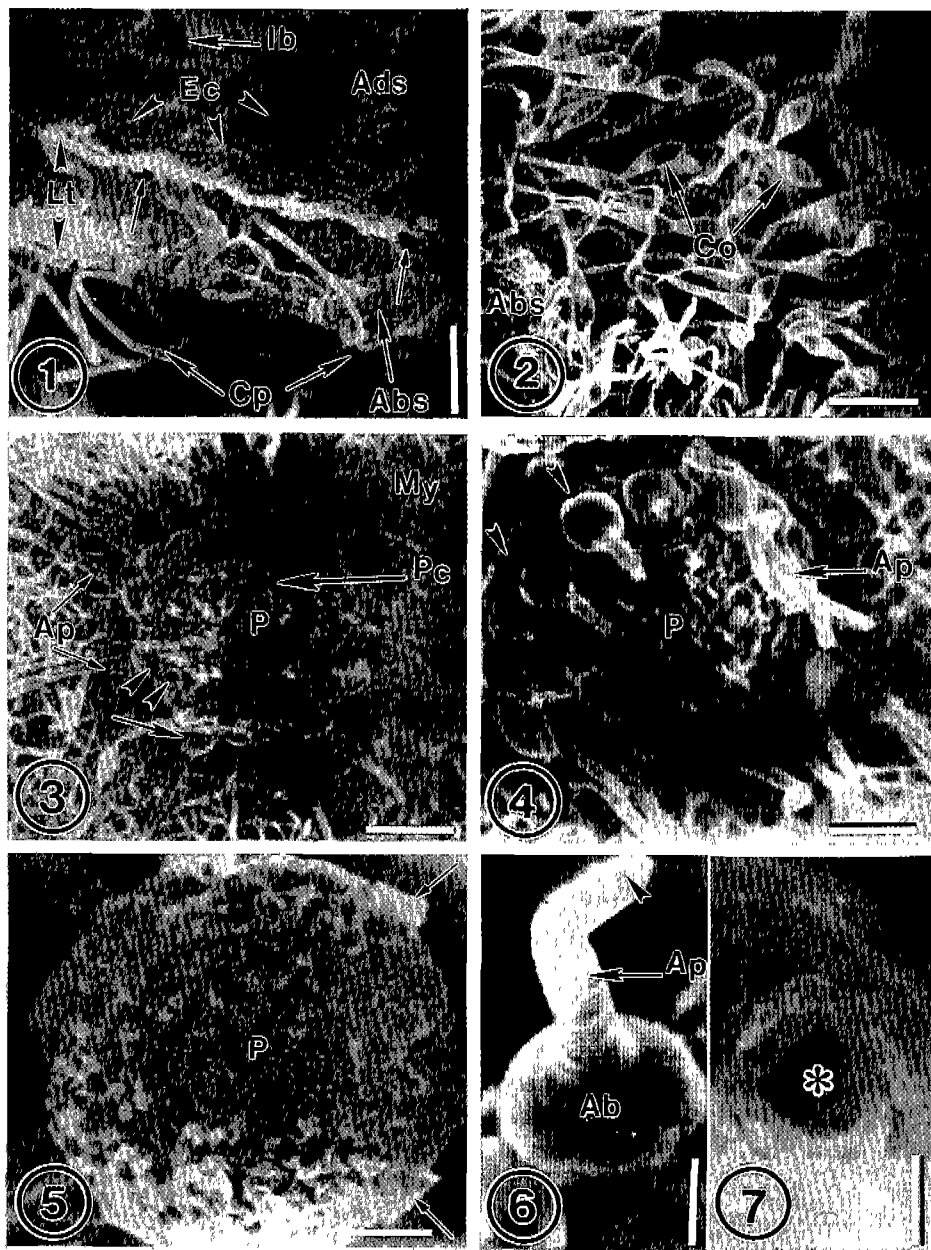


Fig. 1. Diagrammatic representation of life cycle of *Phyllactinia corylea*.



**Fig. 2.** Scanning electron microscopy of *Phyllactinia corylea*.

Panel 1. Earlier stage of conidiophores (Cp) of *Phyllactinia corylea* developed on the abaxial mulberry leaf surface (Abs). The adaxial surface shows the idioblasts (Ib) and epidermal cells (Ec). The two arrows indicate the cystolith cavities. Lt = leaf tissue. (Scale bar = 25  $\mu\text{m}$ ).

Panel 2. Abundant matured conidia (Co) of *P. corylea* on abaxial mulberry leaf surface (Abs) (Scale bar = 35  $\mu\text{m}$ ).

Panel 3. Dorsal view of a perithecium (P). Lower half of the base of an acicular appendage (Ap) of a perithecium has a cut surface (arrow), and most wall cells below the penicillate cells (Pc) have remained shrunken (arrow heads) My = Mycelium, (Scale bar = 23  $\mu\text{m}$ ).

Panel 4. An enlarged view of a pushed perithecium whose acicular appendages (Ap) bend inwardly. Note the shrunken cells (arrow heads) visible just above the acicular appendage. (Scale bar = 17  $\mu\text{m}$ ).

Panel 5. An overview of a matured perithecium which has lost its acicular appendages. Swollen bases of two acicular appendages can be seen on its periphery (arrows) (Scale bar = 15  $\mu\text{m}$ ).

Panel 6. A magnified view of an anteriorly downward bent acicular appendage (Ap) showing its broken tip (arrow head) and swollen base (Ab). (Scale bar = 4.5  $\mu\text{m}$ ).

Panel 7. The broken tip of an acicular appendage showing a hollow cavity (\*) (Scale bar = 0.2  $\mu\text{m}$ ).

## Results and Discussion

The pathogen, *P. corylea* is an ectoparasite, which reproduces asexually by means of conidia produced on the abaxial mulberry leaf surface. The fungus reproduces asexually in tropical and sub-tropical climate whereas the sexual stages are observed only in temperate climate like Jammu and Kashmir. Under temperate conditions, the disease is perpetuated by sexual reproduction and the overwintering of the fungus is mainly through perithecia. The formation of the sexual reproductive organs occurs frequently. But in regions with tropical and sub-tropical climate, the occurrence of sexual reproductive organs is not frequent (Fig. 1). In warm climates many species of Erysiphaceae never form cleistothecia and they perpetuate only by means of conidia (Yarwood, 1957). Philip (1996) reported that the formation of cleistothecia (=perithecia) is a matter of interaction of the host, parasites and environment. In tropical and sub-tropical regions the disease usually starts appearing during onset of winter in September, reaches its peak during December-January and may continue in the field up to February or March. However, the disease is perpetuated in northern region of India during December-February through perithecia formed by sexual reproduction. Philip (1996) has noticed that the incidence and intensity of powdery mildew disease vary from place to place. In hot places of India like Coimbatore (Tamil Nadu) and Palghat, now known as Palakkat (Kerala), the incidence is as low as 1.5%; while in cooler and hilly areas, where the climate is ideal for the development of the disease, the incidence goes up to 15% or more. In Mysore, the average incidence of the disease is recorded 2.7% during the peak winter season in December-January.

The fungus produces hyaline branched mycelium and perpendicular conidiophores bearing single conidium at their tips (Panels 1 and 2 of Fig. 2). The conidia of *P. corylea* have high water content and do not require free moisture for germination. The optimum temperature for development of asexual stage is  $20 \pm 2^\circ\text{C}$ . The conidia survive from season to season in infected crop debris and their dissemination is mainly through air. In the temperate climatic zones, on the advancement of infection when the entire leaf surface gets covered with brown mycelial mat, the fruiting bodies (perithecia) appear as small black spots. The periphery of the perithecia is covered with numerous colourless needle shaped appendages called acicular appendages, which help in dissemination. The long appendages of *P. corylea* bend and lift the perithecia above the leaf surface. This is probably advantageous for the dissemination of the asci and ascospores present in the perithecia. Alexopoulos and Mims (1985) emphasized that the appendages of cleistothecia of *Phyllactinia* are

rigid, spear-like with a bulbous base and a pointed tip. Further, they also reported that the probable function of appendages is anchoring the cleistothecia to the surface of leaves, especially those that bear trichomes. In the present study the average number of the acicular appendages in *P. corylea* was observed seventeen whereas Takamatsu *et al.* (1982) observed only fifteen appendages at an average in *P. moricola*, another species of *Phyllactinia*. The perithecia are crowned with immature penicillate cells and some of them on the lower side of the perithecia have shrunken walls (Panels 3 and 4 of Fig. 2). Takamatsu *et al.* (1982) reported that the shrunken immature penicillate cells at the perithecial equatorial planes ballooned and converted into the base of acicular appendages in *P. moricola*. On maturity of the perithecia, the asci are liberated during favorable conditions by the splitting of the perithecia. When the perithecia dry out, they are pushed above the substrate by appendages which then bend at the base (Itoi *et al.*, 1962). This bending is attributed to the thinner cell walls at the lower side which lose water more readily than the thicker cell walls at the upper bulbous side. During dispersal the perithecia may lose or broken their acicular appendages. Panels 4 and 5 of Fig. 2 show detached perithecia, which have lost their acicular appendages. A hollow cavity seen in Panels 6 and 7 of Fig. 2 is the cut edge of an acicular appendage. After dispersal the perithecia attach themselves to mulberry branches with the help of a paste-like substance secreted from their stigma like projections on dorsal surface (Hirata, 1951; Itoi *et al.*, 1962).

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