CHAPTER 8
SUMMARY AND CONCLUSIONS

Mulberry (*Morus alba* L.) is a perennial plant cultivated for its foliage, which forms the sole feed for the silkworm (*Bombyx mori* L.). Mulberry like any other crop plant is susceptible to many diseases, the major crop losses being caused by fungal pathogens. The leaf rust disease is one of the serious diseases occurring during August through March. Feeding the rust infected leaves to the silk worms leads to deterioration of cocoons, thus affecting the quality and quantity of silk produced.

**Morphology and Taxonomic status**

The rust was found to manifest itself on the lower (adaxial) surface of leaves in the form of yellowish to reddish brown blisters grouped together. Each pustule was provided with a central ostiole, through which creamish masses of spores were seen coming out. The uredinium was ostiolate and was provided with the peripheral thin cellular peridium, each cell measuring 18(21) x 5(7) μm. The urediniospores were subglobose, oval to pyriform in shape and measured 15(24)-18(30) x 12-15(18) μm and the surface was echinulate to verrucose. The urediniospores were provided with four equational germ pores. The germ tube prior to entering the leaves produced characteristic appressorium from which infection hypha emerged. The telial stage for the rust on mulberry was not found, and the rust is believed to be prevailing in its anamorphic state only.

The review of literature reveals that this rust is known to the Indian workers in the field either as *Aecidium mori* Barclay (1980) or as *Cerotelium fici* (Cast) Arthur (1949, 1952). Taxonomic studies carried out showed that both these names were misnomers and based on the fact that the structures formed on mulberry were uredinia (not aecia) that were peridiate and ostiolate, the new combination *Peridiopsora mori* (Barclay) Prasad *et al.*, was coined to accommodate the fungus.

**Host Range**

Attempts were made to find out the existence of alternate or collateral hosts if any which may serve as reservoirs of the pathogen. The plants including weeds growing in and around the mulberry fields were screened for susceptibility.
to *Peridiopsora mori*. Of the 174 plants species screened including weeds distributed among 47 families growing in the vicinity of the mulberry garden, none of the plants showed any symptom nor stages of *Peridiopsora mori* infection through natural or artificial inoculation methods.

Only on 46 plants species distributed among 18 families, the urediniospores of *P. mori* germinated *in vitro* and produced germ tubes. On the leaf of *Ficus racemosa* L. 6.2% of urediniospores of mulberry rust germinated with a germ tube length of 30 μm, but on other leaves the germination of urediniospores was comparatively lesser than on *F. racemosa*. Other than germ tubes neither appressoria nor infection papillae were formed. It can be concluded that *Peridiopsora mori* is a host specific biotroph. As far as the present studies go, the rust has no alternate or collateral host and therefore there is no role of nonmulberry hosts in rust epidemics.

**Inoculum and spore dispersal**

The cycle of epidemics depends on the ability of the causal organism to disperse into atmosphere. The pathogen spores are generally dispersed by agents like wind, rain, soil, insects and human beings. Unlike the other phases in a fungal pathogenic life cycle, the dispersal phase is determined by physical constraints.

To assess the occurrence of spores in the air at various time intervals and heights in mulberry gardens, air sampling devices have been used. The samplers used in this study were sticky slide and rotorod. The vertical profile and concentration of urediniospores of mulberry rust at different durations of the day were studied with reference to meteorological data.

The sampling was begun in February 1990 and continued till March 1992 covering two consecutive rust epidemic seasons. The impaction of urediniospores commenced during August 1990 and increased gradually and considerably during September to December 1990 with climax during December 1990. Later from January 1991 the impaction of urediniospores decreased gradually with the least number of urediniospores during March 1991. A similar trend was observed from August 1991 to March 1992, with climax of spore impaction during December 1991. The spore impaction pattern indicates that the inception of rust disease of
mulberry occurs during August/September with gradual increase in severity reaching climax in December and decline from January.

In the rotorod sampler more number of spores impacted at 0.75 meter height during September and October (1990 and 1991) and January, February (1991 and 1992). The spore load was high at 2 meters height above the ground level during November and December (1990 and 1991). The spore concentration gradually decreased from January onwards reaching zero level during late March/April. The number of spores impacted on to sampler indicates the concentration of spores in atmosphere and the probable intensity of rust disease on mulberry which commences during August, reaching peak during December and declines from January onwards. The diurnal fluctuation in spore impaction during different months depends on meteorological factors viz., humidity and temperature. The diurnal peaks of spore impaction during different time of the sampling day in a month depended on weather conditions. The results indicated that the urediniospores were disseminated in two peaks during a diurnal 24h cycle i.e., during morning 08±1h and in the afternoon 16 ± 1h from September to January.

Influence of different physico-chemical factors on urediniospore germination

The effects of different chemicals such as carbon sources (carbohydrates), nitrogen sources (aminoacids and nitrogen salts), vitamins and growth promoters on the germination of rust spores were studied. The finding helps in understanding the host specificity of mulberry rust. The study also helps in elucidating the epidemiology and infection process of mulberry rust.

In the freshly collected distilled water there was neither germination nor appressorium formation by urediniospores but in distilled water that was stored for 2 days a few spores germinated. In tap water the germination was better than in distilled water, as tap water contained calcium and magnesium ions. The optimal temperature range for urediniospore germination was 20-22ºC. The provision of glucose as substrate enhanced germination considerably showing that exogenous energy is necessary for optimal spore germination.

The germ tube produced was a stout structure containing dense, granular, refractile protoplast. Under dark conditions it developed regular appressorium
after 12h of incubation at 20-22°C. The protoplasm advanced into the appressorium through a connective and later a septum developed separating the germ tube from appressorium. A papilla was produced from the apex of the appressorium which acted as infection hypha. Development of secondary vesicle from the apex of the tube was also observed. The protoplasm of spore moved into germ tube and appressorium with an increase of vacuole size in the cytoplasm. The appressorium soon gave rise to short hypha. Although four germ pores are present in an urediniospore only one germ tube was produced by majority of urediniospores and rarely two germ tubes were produced. Sometimes instead of a normal infection hypha branched finger-like infection hyphae developed. Rarely the germ tubes developed short lateral branches. Some times multiple appressoria were developed on a single germ tube.

The urediniospores developed and released on the days with a temperature range of 27-29°C showed 20 ± 5% germination. Most of the urediniospores produced germ tubes within 12h of incubation. Appressorium formation was found to be 45% at pH 6.8. The post germination structures for penetration developed between 18-24h leaving behind a degenerating germ tube and empty spore.

The final percentage of germination was lower among the urediniospores collected from older pustules. It was also observed that spores produced in the pustules on first-formed mature leaves (9th-12th leaf position from shoot apex) had higher germination potential than those spores produced in pustules of older leaves (15th-17th leaf position from shoot apex).

The monosaccharides promoted higher percentage of urediniospore germination than disaccharides and polysaccharides. The hexoses were the most effective in inducing germination, D-Glucose being the most preferred carbon source. D-Glucose elicited 46.7% germination at 48h of incubation. The disaccharides were the next in preference as carbon sources.

A greater extent of germination percentage of urediniospores and length of the germ tubes were observed in aromatic and aliphatic aminoacid substrates. The aromatic aminoacids and aliphatic aminoacids, at 10 μg ml⁻¹ stimulated germination of urediniospores to a greater extent, than acidic aminoacids, basic aminoacids, iminoacids and sulphur containing aminoacids. Comparison of germination percentage at 10 μg ml⁻¹ and 20 μg ml⁻¹ of aminoacids shows that the percentage of germination was less at the higher concentration.
In nitrogen salt liquid substrates at 10 \( \mu g \) ml\(^{-1}\) and 20 \( \mu g \) ml\(^{-1}\), there was greater germination percentage of urediniospores at 20 \( \mu g \) ml\(^{-1}\) than at 10 \( \mu g \) ml\(^{-1}\) after 24h incubation.

The vitamins influenced germination better at 20 \( \mu g \) ml\(^{-1}\) rather than at 10 \( \mu g \) ml\(^{-1}\). D-Biotin was the vitamin that induced maximum germination. The least effective germination at 10 \( \mu g \) ml\(^{-1}\) was in Ascorbic acid. Next to Biotin in efficacy were mesoinositol, folic acid and pyridoxin hydrochloride.

At 24h of incubation, 10 \( \mu g \) ml\(^{-1}\) Kinetin produced 5.9% spore germination whereas, 20 \( \mu g \) ml\(^{-1}\) produced 9.1% germination. Kinetin induced maximum germination percentage of 9.4 and 10.3 at 10 \( \mu g \) ml\(^{-1}\) and 20 \( \mu g \) ml\(^{-1}\) respectively at 48h of incubation. Other growth promoters studied did not cause any significant promotion in germination percentage.

**Host pathogen interactions**

Fungal pathogens invade host cells with a variety of specialized infection structures. Developmentally the appressorium is the first and most important infection structure formed. Appressorium is a morphological differentiation of hyphal apex that gives rise to outgrowths which invade the host.

The infection process of the rust was initiated by urediniospores with the germination of spores on the leaves. The germinating spores produced various types of infection structures. Maximum number of infection structures developed with a temperature range of 20-22°C and relative humidity at 83 ± 8 %. With the above conditions, elongation of germ tube ceased (12-15\( \mu m \) length) differentiation of infection structures viz., appressoria and infection hyphae was initiated. Continuous supply of light during the incubation period reduced the pathogenisis and frequency of appressoria formation. The appressoria formation was less on mature leaves.

The infection hypha could penetrate through cuticle of either surface or through stomata. The germ tube enlarged at the tip to form the appressorium. A short hypha from the appressorium penetrated between the guard cells and sometimes enlarged as a substomatal vesicle, from which further infection hyphae grew out. Sometimes stomatal penetration without appressorium formation was also observed. Direct penetration occurred through the epidermal cell or rarely between the two epidermal cells. At the point of penetration of the cuticle, the
infection papilla was very slender. As it traversed the depth of epidermal cells the infection hypha enlarged. Usually infection hypha continued to grow deeper into host tissue towards the vascular bundles. The hyphae grew inter and intracellularly as the arrangement of host tissue would permit.

The orientation of germ tube towards stoma was crucial for stomatal penetration. As penetration took place near stoma, the infection hypha grew paradermally between epidermis and underlying mesophyll cells. During the process of penetration and spreading pale orange pigmented contents, passed into the infection hypha which became cut off from the appressorium by a thin cross wall. Haustorium formation occurred within 72h of incubation.

The urediniospores germinated and penetrated the leaf on both abaxial and adaxial surfaces of 10-17 days old leaf i.e., 4-7th leaf position from shoot apex. During penetration the appressorium flattened out against the epidermis and penetration structure developed into epidermal cell. The opening on epidermis appeared as a small brown spot surrounded by a characteristic dark zone with the penetration hypha in host epidermal cell. The hyphal growth in epidermal cell was limited and the hypha grew along the inner wall of the cell for a short distance, it then passed through the host cell wall into intracellular and intercellular spaces below. It was also observed that the urediniospore germ tubes became oriented almost perpendicular to the venation of leaf which is the prime indication of fungal response to host leachates. The exudate formed around the germ tube of germinated urediniospore serves to attach the germ tube to substratum.

Spread of pathogen within the host

Forty eight hours after inoculation, the infection hypha penetrated into the epidermal cell, branched and enlarged. The hypha grew between the mesophyll and adaxial epidermal cells. At 96h the branched hyphae moved down into the mesophyll layer. At 144h after inoculation the bidirectional growth of the mycelium still persisted. By 168h the development of pustule began and the structure assumed a regular oval shape towards the lower epidermis. Meanwhile between 96 - 120h the mycelium spread rapidly in all directions. During the early stages i.e., 9th and 10th day after inoculation, the pustules appeared as pale yellow flecks on the adaxial surface of leaf. The yellow colour appearance may be due to the pigment within the mycelium which might have been incorporated into
urediniospores during sporulation. On the 9th day after inoculation the response of mesophyll cells to the infection was clearly visible. The palisade cells in and around the site of penetration began to develop yellowish brown coloration. Grouping of uredinial structures was found around 10 - 11 days in the spongy mesophyll cells and brown mesophyll cells could be observed surrounding uredinia. By 10th - 12th day the pale yellow appearance of the pustules became less pronounced and reddish brown colour started developing. Uredinia erupted during 13th - 14th day by the rupturing of peridial wall. Angular brown patches appeared on the lower surface of leaves at the same time. Widespread eruption of uredinia occurred during 15th - 16th day.

As the infection progressed the mycelium invaded the palisade and spongy parenchyma cells, the plastids became irregular, more often clumped and pale leaving a homogeneous uniformly unstained mass. The leaf thickness got reduced because of infection. The infection resulted in reduction of number of plastids both in palisade and spongy cells. The haustorium invaded palisade and spongy parenchyma cells leading to hypertrophy of cells. The neighbouring cells around the infected cells were shrunken and in post infection stage the cell wall of host started to disintegrate. Due to this, eventhough hypertrophy with giant cell formation occurred, there was no increase in the thickness of mesophyll. Inside the palisade and spongy cells the haustoria enlarged and occupied most of the space in them. The hyphae colonized through both the upper and lower epidermi and took yellow to reddish brown colour in severely infected areas. Probably the pathogen in the infected host cells produced some phenolic substance, hence the lesion appeared yellow to reddish brown. The mulberry rust fungus is systemic as even before the visual symptoms appeared, there was abundant mycelial growth extending several μm length from the infection site.

During the early stage of establishment of the pathogen in the mesophyll, the host cells gave the appearance of functioning with enhanced metabolic activity resulting in hypertrophy. It was also observed that the healthy cells of mesophyll appeared to be plasmolyzed, while the hypertrophied infected cells remained normal indicating an altered physiological condition in the host mesophyll.

Prior to formation of rust pustules on the adaxial surface of the leaf hyphal aggregation occurred near substomatal cavity after 9th day of inoculation. Subsequently between 10th - 12th day the urediniospores were differentiated. By 13th day rupture of the lower epidermis and peridium occurred with the maturity
of urediniospores. Number of urediniospores were found in uredinosorus as subsessile, oval to globular structures. The epidermal cells were intact and turgid till their rupture, probably due to pressure exerted by the spores. When large number of hyphae were in a leaf tissue, the pustules formed were minute with very few spores and this might be due to limitation of available nutrients. When the mycelia were scattered, the uredinia produced many spores (5-12). After the formation of primary pustule, the secondary pustules appeared around the margin of primary pustule. The formation of uredinia is restricted to adaxial surface of leaf regardless of the initial site of infection.

The opened uredinia liberated the urediniospores within 48 - 72h and new uredinia continued to develop upto 5th week. After penetration and infection of leaves the urediniospores continued to be produced upto 3 weeks. The inoculum production potential in mulberry rust was appreciable. After the first formed urediniospores were well developed, the sorus still contained the spore forming initials in the center indicating basipetal arrangement. The mycelial mat of mulberry rust was reddish brown; it radiated from original infection point and produced many sori as the hyphae reached lower epidermis.

Histochemical changes

The mulberry leaf infected by rust fungus is heterogeneous i.e., it consists of both infected and uninfected tissues, the relative proportions of these two tissues change with the progress of infection. The heterogeneity may also result from changes occurring within the individual pustules. In rust infected leaf, rapid loss of chlorophyll occurred as the infection progressed. The loss of chlorophyll was highest in leaves with higher pustule density and leaves turned yellow, papery and withered off much earlier than the healthy ones. At the flecking stage, the concentration of chlorophyll both within and between the pustules was lower than in the healthy leaves. At sporulation stage, the chlorophyll declined to a much greater extent. Approximately 20% of total area of pustule was occupied by sporulating centre. The quantity of carotenoids within the pustule appeared to be greater than in uninfected regions of leaf.

Insoluble polysaccharides: The healthy palisade and spongy parenchyma cells of mulberry leaf were rich in carbohydrates and hence stained dark magenta with PAS. But with penetration and spread of mycelium in upper epidermis and palisade tissue, there was colour change from magenta to brickred. As the fungal
development progressed the cells adjacent to rust infected palisade and spongy cells lost their stainability with PAS. But the hypertrophied palisade and spongy cells with mycelium of rust, stained deep magenta, indicating the rich presence of polysaccharides. As the infection progressed towards the lower epidermis most of the infected palisade and spongy parenchyma cells along with their adjacent cells lost their normal shape and stainability for PAS. Further there was decrease in size of mesophyll cells and disintegration of cell borders at post infection stages. Deep staining with PAS was observed at the site of formation of urediniosorus, indicating the presence of polysaccharide. The developing urediniospores were greenish to brick red in colour but the well developed urediniospores stained deep red to dark magenta with PAS indicating that the mature spores were rich in polysaccharides, which could be used during their germination.

Starch: The healthy palisade and spongy parenchyma cells of mulberry leaf were rich in starch and hence stained bluish black uniformly with Iodine-potassium iodide solution. As the infection of rust progressed the starch content in the host cells got depleted. Starch was found to accumulate in hypertrophic mesophyll cells of leaf. There was no accumulation of starch in the urediniospores.

Proteins: The healthy palisade and spongy parenchyma cells of mulberry leaf were rich in proteins and hence stained blue uniformly with mercuric-bromophenol blue i.e., MBB. With the onset of infection the presence of proteins was diffused in the infected cells. But in the spongy parenchyma cells the proteinoplastids still retained their shape as distortion was very little in the initial stage of infection. The developing hyphae and spores stained blue with MBB indicating the presence of proteins in them. The well developed hyphae, and hypertrophied cells of mesophyll stained pale blue with yellow tinge indicating the presence of very little protein. The well developed urediniospores stained deep blue, with MBB indicating the presence of proteins. It was evident that the mulberry leaves infected by rust contained less proteins and sugars making them less nutritive to silk worms.

Nucleic acids: The healthy palisade and spongy parenchyma cells of mulberry leaf were rich in nucleic acids, hence stained blue green to purplish green with Toluidine blue. The rust infected epidermal cells stained blue to purple showing rich RNA content. The hypertrophied cells of mesophyll stained deep blue to purplish green indicating the presence of both RNA and DNA. The cells of the mesophyll neighbouring the hypertrophied cells stained pale blue to green.
indicating the presence of traces of RNA and DNA. The hyphae and well-developed urediniospores stained deep blue to purplish green indicating richness in nucleic acids.

The hypertrophied cells of infected mulberry mesophyll stained deep purplish red by methyl green-pyronin indicating the rich presence of RNA. As the disease was severe the neighbouring host cells of hypertrophic cells lost their capacity to stain with MGP with a clear indication that RNA and other cell metabolites might have accumulated in hypertrophied cells indicating the presence of pathogen at its peak metabolic activity. The urediniospores were also stained deep purplish red with MGP indicating the richness of RNA in them.

Effect of agronomical practices on severity of rust

Irrigation is one of the principal means of increasing mulberry leaf biomass. The effect of irrigation on disease development depends on the interaction between conditions created by irrigation, weather factors, nature of the pathogen, crop variety and spacing.

Spacing: Different spacings of planting are followed in mulberry field under irrigated condition. The severity of the rust on mulberry was found to be high in narrow spaced and densely planted fields.

Pruning: Usually wider spacings are followed to raise mulberry plantation under nonirrigated condition with leaf harvest. The annual basal pruning is made during June/July in an year. The April base pruned plants showed the lesser number of pustules per cm² leaf area than that of July base pruned plants.

Chemical fertilizer application

Rust disease severity was found to be highest in the plants supplied with recommended dosage of nitrogen (60 kg N/hectare/crop) in irrigated condition. In plants supplied with half level of nitrogen (30 kg N/hectare/crop) the disease was less severe. A similar trend in disease severity was observed under nonirrigated condition too.
Shoot harvest

In traditional areas like Anekal, Kolar, Malur and Sidlaghatta, row system with a closer spacing of 45 x 15 cm is being practiced for growing mulberry and shoot harvest is being practiced. At present a modified spacing of 60 x 23 cm is practiced under irrigated conditions. The harvesting of shoots is being carried out in 60 x 60 cm and 120 x 60 cm spacings under irrigated conditions.

Rust disease severity was highest in narrow spaced plantation 60 x 23 cm followed by 60 x 60 and 120 x 60 with shoot harvest.

Mulching: Experiments were carried out to find out the effect of different mulching systems on the severity of the rust after the middle cut at an height of 45 ± 2 cm was carried out. In all the mulches tried viz., green manure crop mulching with 8 cm thickness beneath the soil surface (M 1), soil surface covered by green manure crop with 8 cm thickness (M 2), M 1 + M 2 (M 3) and black polyvinyl sheet covering on the surface of the soil (M 4), the severity of rust was less than in control. The severity was least in the plants with the soil surface covered by black polyvinyl sheet.

Intercropping: Intercropping in mulberry garden is practiced to achieve higher gross returns through maximum utilization of land in an unit area. The rust disease severity under different densities of subsidiary crops which included both legumes and fodder crops in between mulberry being the main crop revealed that the severity of rust was high in pure stand of mulberry crop and was less when soybean was intercropped. The increase in soybean density, correlated with decrease of rust severity on mulberry.

The trend of disease severity with reference to intercropping was the same with other intercrops i.e., green gram, maize and ragi.

Screening of mulberry varieties for disease resistance

Development of disease resistant varieties by breeding is an important goal in plant improvement programmes. With this in view 76 mulberry varieties were screened for disease resistance.
Of the 76 varieties screened 30 varieties showed less disease susceptibility than the check variety M-5. The remaining 46 mulberry varieties showed higher susceptibility than the check. Among the 30 varieties only 4 varieties namely English black, KGL-1, Goshooerami and Calabresa showed moderate resistance to rust.

The varieties Kukupila, Echihei, S-30, Mundargi, PKS 1-2, Z-1, F-1, Z R-2-10 showed lesser area of infection irrespective of having more number of lesions than M-5 indicating hyper-sensitivity.

The expression of rust severity varied during August to December. A few varieties expressed severity early and a few late.

The varieties KGL-1, OPH-1, Haveri-II, Kukupila, KGL-2, PKS 1-2, S-30, Kamiso-402, S-54 and Serpentina showed lesser area of infection and severity against the check variety M-5 during October.

The varieties RFS-135, KGL-3, Haveri-I, PKS 1-9, Z R 2-10, Goshooerami, PKS 1-12, Calabresa, Dehradun and Japan-II showed severe rust symptom during later part of October to early November.

The varieties Mundargi, E-1, E-4 and Kaliakutahi showed severe rust symptom during November.

Kairoichinose showed rust severity from later part of October to early December months. The varieties Mizusuwa, Ichinose, Ichihiei and Z-1 showed severe rust symptoms during December.

The varieties E-5, Kanzan, Japan-I, Kukusoo-27 and Schinichinose expressed the severity of rust during later part of November and early December.

The varieties Kajali and Dharwar expressed rust severity during December.

English black, Mizusuwa, Ichinose, Ichihiei and Z-1 showed delay in symptom expression with severity during December.

The variance in rust severity among different varieties and the observed differences between the years can be considered as a measure of temporal variation due to weather factors. The varieties namely English black, Mizusuwa, Ichinose, Ichihiei, Z-1, KGL-1, KGL-2, Haveri-I and II, Dehradun and Mundargi can be used for breeding to evolve rust resistant varieties of mulberry.
CONCLUSIONS

Rust is a biotroph on mulberry foliage, appears from August onwards as reddish brown pustules on adaxial surface. The pustules are uredinia covered by peridium enclosing urediniospores. The characters of mulberry rust tallies with the form genus *Peridiopsora* but not with *Aecidium* or *Cerotelium* hence the rust of mulberry is placed under *Peridiopsora mori* (Barclay) Prasad *et al.*

Mulberry rust does not have any alternate or collateral host.

The inoculum (urediniospores) are wind dispersed with diurnal peaks at a temperature range of 22-28°C and RH 70-82%. More urediniospores are present in the air during November and December indicating the epidemic state of rust.

The urediniospores germinate producing germ tube, appressorium, secondary vesicle, multiple appressoria and infection papilla at an optimum temperature of 20-22°C and 83 ± 8% RH in 2000 μg ml⁻¹ of D-Glucose substrate. More urediniospores germinated in monosaccharides (2000 μg ml⁻¹), aromatic aminoacid substrates (DL-B-Phenyl alanine at 10 μg ml⁻¹), nitrogen salts (Calcium nitrate at 20 μg ml⁻¹), vitamins (D-Biotin at 20 μg ml⁻¹) and growth promoters (Kinetin at 20 μg ml⁻¹). Eventhough a urediniospore has four germ pores, usually one germ tube is produced and occasionally two. Very rarely the germ tube shows lateral branching.

The infection papilla penetrate the leaf at both abaxial and adaxial surfaces. Direct penetration occurred through epidermal cell, rarely between the two epidermal cells and the stomata.

The mycelium of the pathogen spreads in the host mesophyll as intercellular in the initial stages and becomes intracellular leading to the formation of hypertrophic mesophyll cells with the disintegration of adjacent palisade and spongy parenchyma cells. Well developed uredinia in reddish brown pustules are produced by 12-14th day after inoculation.

With the establishment of pathogen the mycelium, hyphae, hypertrophied cells and urediniospores are rich in polysaccharides and nucleic acids. The well developed hyphae and the hypertrophied cells of the mesophyll contains less proteins than developing hyphae and the well developed urediniospores.
The severity of the rust on mulberry is high in narrow spaced and densely planted fields. The severity of the rust is less in April base pruned plants rather than July base pruned plants.

Rust severity is more in plants supplied with recommended dosage of nitrogen than in plants supplied with half the dose of recommended nitrogen in both irrigated and non-irrigated conditions.

Rust severity is comparatively lesser in shoot harvested plants than in leaf harvested plants.

Rust severity can be reduced by mulching with leguminous green manure plants and covering soil surface by black polyvinyl sheets.

The rust severity can be reduced by intercropping with legumes.

Varieties of mulberry - English black, Mizusuwa, Ichinose, Ichiihei and Z-1 show delay in expression of severity. The varieties Kukupila, S-30, Mundargi, Ichiihei, PKS 1-2, Z-1, Z.R 2-10 show lesser total area of infection inspite of having more number of lesions than M-5. Hence the above varieties can be used in breeding programme to evolve rust resistant varieties.