Cey. J. Sci. (Bio. Sci.) 35 (2):157-162, 2006

BIOLOGY OF *PLUMERIA* LEAF RUST DISEASE CAUSED BY *COLEOSPORIUM PLUMERIAE*

T. P. Weeraratne and N. K. B. Adikaram*
Department of Botany, Faculty of Science, University of Peradeniya,
Peradeniya, Sri Lanka
Accepted: 13 November 2006

ABSTRACT

The leaf rust disease in *Plumeria* first appeared in Sri Lanka some time in the year 2002 and is now widespread. The disease is found in both *Plumeria rubra* and *P. obtusa* (Apocynaceae) and the infected leaves show numerous tiny, raised, orange, rusty pustules on the abaxial surface of the leaf. The adaxial surface opposite to infected sites is chlorotic reducing the available photosynthetic area of the leaf. Symptoms were absent in the stem or flowers. The causal agent was identified as *Coleosporium plumeriae.* This is the first report of *Plumeria* leaf rust in Sri Lanka. Microscopic studies indicated the presence of uredia, formed from the transversing mycelium and emerged through ruptured lower epidermis. No other fruiting structures, telium, aecium or spermatium were encountered at any stage of the disease in *Plumeria* or in *Pinus*, which was previously reported as a secondary host of *C. plumeriae.* Two other fungi, *Absidia sp*. and *Verticillium sp*., were found to colonize the rust areas of more mature leaves in succession, *Absidia sp*. appearing first. These two fungi grew as mycoparasites on *C. plumeriae* and had no direct contact with the leaf tissue. However, colonization by these two fungi resulted in necrosis around the rust infections inflicting damage to leaves. Young leaves down to about the third from the apical bud are resistant to rust infection. Young leaves contain more latex compared to mature leaves and the latex shows inhibitory action against germination of uredospores. Latex was found to possess chitinase activity on a gel diffusion assay. Latex may therefore be playing a role in the resistance of young leaves against rust infection.

Key words: Chitinase activity, *Coleosporium plumeriae*, *Plumeria* leaf rust

INTRODUCTION

Plumeria belongs to Apocynaceae which is a large family of about 300 genera with more than 1400 species, found predominantly in the tropics and sub-tropics (Dassanayake & Fosberg, 1983). *Plumeria* is an introduced plant grown as an ornamental and commonly known as '*Araliya*' or temple tree in Sri Lanka. Two species of *Plumeria* (*P. rubra* and *P. obtusa*) are found in the island, the flowers are widely used for worshiping at temples.

The genus *Coleosporium* belongs to the Family Coleosporaceae of the Order Uredinales. This family has two other genera and nearly 80 cosmopolitans including the genus *Coleosporium*. The genus has numerous described species, many of which are doubtfully distinct morphologically (Cummins, 1997). Most species are macrocyclic and thus heteroecious with spermogonia and aecia on needles of *Pinus* and uredia, telia and basidia on both monocots and dicots.

Patouillard (1902) had noticed the presence of leaf rust disease in *Plumeria alba* on Guadaloupe island in West Indies (1902, cited by Dizon *et al*., 1996, in Chung *et al*., 2006) which then spread to Central America. Later, in 1990s *Plumeria* rust was noticed in 8 species of *Plumeria* (including *P. rubra*) on South Pacific islands (Kakishima *et al*., 1995). Rust on the leaves of *Plumeria* species caused by *Coleosporium* spp. has been reported in Hawaii islands where it is grown as a common ornamental tree. The disease is often known as the 'Frangipani Rust'. To-anum *et al*. (2004) have found the disease in Thailand, the causal agent being *C. plumeriae*. A more recent report by Chung *et al*. (2006) describe the occurrence of *Plumeria* rust disease caused by *C. plumeriae* in Taiwan where the *Plumeria* trees have been imported from South Asia. The rust disease in *Plumeria* was observed in Taiwan for the first time in 2003, a little later the disease was initially noticed in Sri Lanka.

Several other *Coleosporium* spp. have been found to occur on other hosts, *C. ipomoeae* in sweet potato, *C. asterianum* on (Laundon and Rainbow, 1969) and *C. tussilaginis* in pine needles, *C. vernoniae* on *Elephantopus* spp. (Holliday, 1980). *Coleosporium* could easily be

*Corresponding aurhor's e-mail:nkba@pdn.ac.lk

transmitted to tropics and southern hemisphere with the introduction of conifers from the north as pine needle rusts are wide spread in the Northern hemisphere (Holliday, 1980).

The disease first appeared in Sri Lanka some time in the year 2002 and is now widespread and prevalent in most parts of the country in both *P. rubra* and *P. obtusa*. However, there is no information available locally on the disease and there are no previous studies conducted probably because of the lesser economic importance of *Plumeria*. The present study attempted to investigate the biology of *Plumeria* leaf rust and this is the first report of the disease in Sri Lanka.

MATERIALS AND METHODS

Study site and plant material

Plant materials of *Plumeria* were collected from the Peradeniya University premises situated at an altitude of 518-550 m and geographically located at 7º 17´ N latitudes and 80º 36´ E longitudes which has a mean annual precipitation of 2,121 mm and mean annual temperature of 24.1 ºC. This study was carried out from June 2003 - March 2004.

Both infected and healthy leaves of *Plumeria* were collected from plants belonging to *P. rubra* and *P. obtusa*. Leaf materials were collected as whole twigs, individual leaves of different maturity stages, at various stages of the study. Leaves were also used to collect latex. These materials were brought to the Plant Pathology laboratory in the Department of Botany, University of Peradeniya for examination.

Symptoms

The diameter of 100 randomly selected rust pustules, 50 from young (1 – 3 weeks old) leaves and 50 from old (4 – 6 weeks old) leaves, each of *P. rubra* and *P. obtusa* was measured, using a ruler and a Venire caliper. The growth stage at which the leaf becomes susceptible to rust infection was studied using 50 twigs each from *P. rubra* and *P. obtusa* that consisted of very young buds to mature leaves. Each leaf of the twigs was observed for symptoms and the upper most leaf that showed symptoms was noted from each twig. The twigs were further examined to determine the progression of symptoms during maturation of the leaves.

Needle leaves of *Pinus* trees growing in close proximity to infected *Plumeria* plants were also examined for the presence of any rust symptoms to ascertain the possible secondary host relationship with *Plumeria* leaf rust fungus.

Microscopic studies

Leaves with different stages of the disease were examined visually and under microscope and symptoms were noted. Scrapings were taken from infected areas and observed under the high power of light microscope. The length and breadth of 100 randomly selected uredospores each from *P. rubra* and *P. obtusa* were measured. To examine the development of the fungus within the leaf tissue, hand and microtome sections were taken across diseased sites of the leaves and examined under microscope.

Isolation of mycoparasites

Colonization of the two mycoparasitic fungi was observed on *C. plumeriae* in more mature leaves. The mycoparasites were isolated on PDA. The leaf areas with rust pustules, with mycoparasites were cut into small pieces. These were surface sterilized with 5% NaOCl for 2-3 minutes. The leaf pieces were placed on PDA plates, and the plates were incubated at room temperature (27 ºC).

Inhibitory effect of latex on uredospores

Since mature leaves (of the age of more than 5 weeks) are more susceptible to infection than younger leaves which have more latex, experiments were conducted to examine whether the latex has any effect on disease development. Fifty younger (1-3 weeks old) leaves and another 50 older (5-6 weeks old) leaves of *P. rubra* were excised separately by cutting through the petiole at mid-way using a sharp blade. The latex came out through the cut end of the younger and mature leaf petioles was collected separately into two separate measuring cylinders and the volume of latex exuded was measured. Fresh weight of the two leaf samples was recorded. Latex content (ml/g fresh weight) was determined for younger and older leaves.

The latex collected from young and old leaves was diluted ten times by adding sterile distilled water in two separate tubes. After mixing well, the water insoluble material was separated by centrifugation at 3000 rpm for 5 minutes. The supernatant containing water soluble material was collected into two clean, sterile capped-tubes.

Uredospores were scraped from rust pustules on *P. rubra* leaves and suspended in sterile distilled water in a glass tube. To wash uredispores the suspension was shaken for 10 minutes and centrifuged for another 10 minutes at 3000 rpm. The supernatant was discarded and the residue suspended in sterile distilled water was centrifuged again. Finally the residue was re-suspended in sterile distilled water and the

number of uredospores in the suspension was adjusted to $2.5x10^5$spores/ml.

Three aliquots (1 ml) of spore suspension were separately mixed with 1 ml each of diluted water-soluble fraction of (a) young leaf latex, (b) old leaf latex and (c) sterile distilled water in 3 separate tubes. The 3 tubes were shaken well and drops of each mixture were placed on four glass slides. Slides from each treatment were incubated in three separate moist chambers for 120 minutes. At the end of incubation 100 randomly selected spores in each slide were counted for germination. Percentage germination of uredospores in each was determined (American Phytopathological Society, 1943).

Chitinase activity of *P. rubra* young (1-3 weeks old) leaf latex was assayed by gel diffusion method as described by Zau *et al*. (2002). Agarose gel was used as the medium and glycol chitin was used as the substrate for chitinase enzyme. 30 ml of gel prepared by mixing 1.6% (w/v) agarose and 0.5% (w/v) glycol chitin was poured into plates with a diameter of 5.5 cm. Wells (2 mm diameter) were made and 10 µl aliquots of crude latex, heat destroyed water soluble fraction of latex, 2x diluted water soluble fraction of latex and distilled water were pipetted into individual wells. The plates were incubated at 27 °C for 16 hours. After incubation gels were stained with 20 ml of freshly prepared 0.1% (w/v) calcofluor for 10 minutes. After staining, the excess calcofluor dye was discarded and the gel plates were gently washed with distilled water overnight at 27°C in a shaker. Lytic zones in the gel were visualized by UV transillumination.

RESULTS AND DISCUSSION

Symptoms

Leaf rust of *Plumeria* species was first noticed in Sri Lanka in 2002 and is now widespread in many parts of the country. The disease appears to have been introduced to some other countries in the region about the same time, for instance to Thailand in 2004 (To-anum *et al.* 2004) and Taiwan in 2003 (Chung *et al*., 2006).

The rust symptoms on both *Plumeria* species were quite similar. The size of the old rust pustules varied slightly between the two host species. The diameter of most of the rust pustules ranged between 0.5-1.5 mm (Table 1). The pustule did not expand noticeably with maturation of the leaf, however, when there was dense infection, 2-3 pustules tended to coalesce (Fig. 1).

The leaves of *P. rubra* expanded steadily while those of *P. obtusa* remained unexpanded as buds for a longer time. This could probably be the reason for early symptom initiation seen in *P. rubra* leaves. Mycoparasites also appeared first on rust infected areas of *P. rubra*. The heavily infected leaves of *P. rubra* fell about 15 weeks after leaf development while in *P. obtusa* it took about 20 weeks.

Table 1. Average diameter of rust pustules on young (1 – 3 weeks old) and old (4 – 6 weeks old) leaves of *P. rubra* and *P. obtusa.*

Species	Stage of leaf	Average diameter of a pustule (mm)
Plumeria rubra	Young	0.6 ± 0.1
	Old	1.0 ± 0.3
Plumeria obtusa	Young	0.6 ± 0.2
	Old	1.1 ± 0.3

In *P. rubra* the two youngest leaves were most of the time in bud and free of any symptoms. In *P. obtusa* the three uppermost leaves were in bud and again no symptoms were found. In both species the rust symptoms first started to appear mostly in the 4th leaf and occasionally in the 5th leaf (Table 2). In *P. rubra* the symptoms appeared about 8 weeks after bud development while in *P. obtusa* symptoms appeared about 8-10 weeks after bud development.

Table 2. Percentage leaves of *P. rubra* and *P. obtusa* at different maturity stages showing initial stage of rust symptoms.

Leaf No.	% leaves with initial symptoms	
	Plumeria rubra	*Plumeria obtusa*
1	0 (bud stage)	0 (bud stage)
2	0 (bud stage)	0 (bud stage)
3	8	0 (bud stage)
4	76	72
5	16	28
6	0	0

In both species the rust infections took place first around the mid-rib and primary veins in small numbers. There was dense rust infection at the base of the leaf closer to the mid-rib, about 3 weeks after initiation of symptoms. The symptoms spread to the rest of the leaf, towards the apex. The apical half of the leaf had comparatively lesser number of infections than the basal half in both species. There were two other fungi associated with the disease in more mature leaves of both *Plumeria* species. These were identified as *Absidia* sp. and *Verticillium* sp. (Fig. 2). These two fungi appeared 4-5 weeks after initiation of rust symptoms in succession, *Absidia* sp. appearing first. These two fungi grew as mycoparasites on *C. plumeriae* producing white, profuse mycelium on the rust and had no direct contact with the leaf tissue. The mycoparasites never grew beyond the rust infected areas. They may be growing on uredospores utilizing the contents and spore walls as nutrients or exudates of infected tissues. The exact role of these fungi in the *Plumeria* leaf rust is, however, not clear. Colonization by these two fungi resulted in some necrosis in the leaf tissue around rust infections inflicting damage to leaves. The infected leaves fell slightly early in *P. rubra* about 7 weeks after initial infection and this took 10 – 12 weeks in *P. obtusa*.

The pustules on both *P. rubra* and *P. obtusa* had globose to ellipsoid, orange-yellow, thick walled (warty, hyaline) uredospores, the only spore type observed during the study. The average length and breadth of uredospore pathogen on *P. rubra* were 27.18 ± 4.71 µm and 20.03 ± 2.88 µm respectively. The average spore length and breadth of the pathogen on *P. obtusa* were 28.00 ± 4.93 µm and 19.42 ± 1.95 µm respectively. The average dimensions of uredospores ranged within the ranges observed for the genus *Coleosporium* in CMI descriptions (20-40 µm x 16-28 µm) (Laundon and Rainbow, 1969). These observations confirmed that the primary causal agent of *Plumeria* leaf rust is *C. plumeriae.* No rust infections were encountered in *Pinus* needles examined during the study.

Microscopic Studies

Rust infections in both *Plumeria* species were restricted to the lower surface and the upper surface corresponding to rust infected regions was chlorotic. Individual rust infections were observed as raised, localized pustules with orange, dusty spore masses.

The development and emergence of pustules were clearly observed during microscopic studies. The initial infections which were initially small, developed in to pustule bearing numerous uredospores by rupture of the lower epidermis of the leaf. Examination of the hand and microtome sections taken through the pastules, revealed that the mycelium of the fungus *C. plumeriae* was immersed in and transversed the leaf tissue. However, the mycelium is more densely arranged within spongy mesophyll cells just below the uredium. The uredium is hypophyllous. The catenulate uredospores are borne on hyphal tips of the fungal mycelium coming out of the leaf tissue (Fig. 1). The leaf tissue at the rust pustule is yellowish orange in colour. At the spot the mesophylls are yellowish orange in colour and especially the arrangement and shape of spongy mesophylls are slightly changed compared to healthy uninfected leaf sections. However, the tissue at the infected area remained intact and undisturbed.

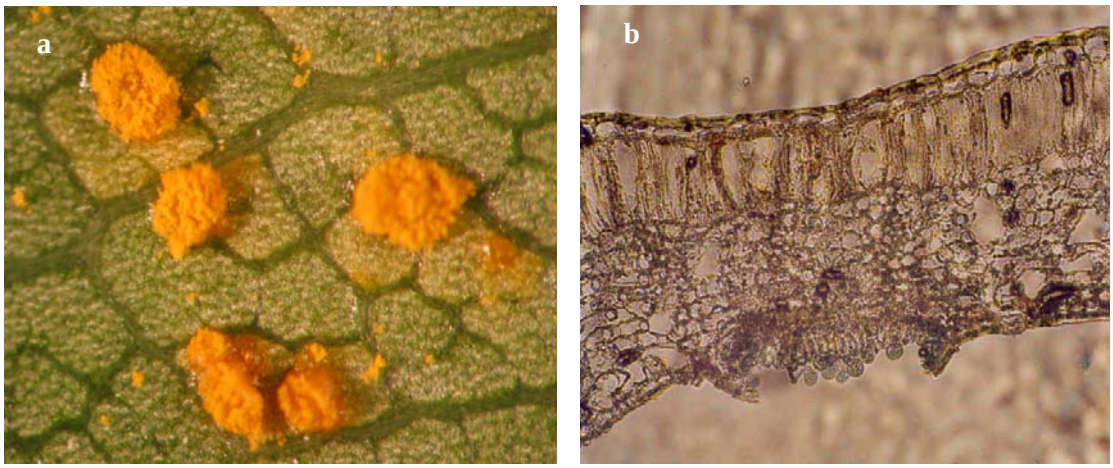


Figure 1. (a) Uredia of *C. plumeriae* on *P. rubra* leaf (under surface, x30), (b) A transverse section through a uredium (x100).

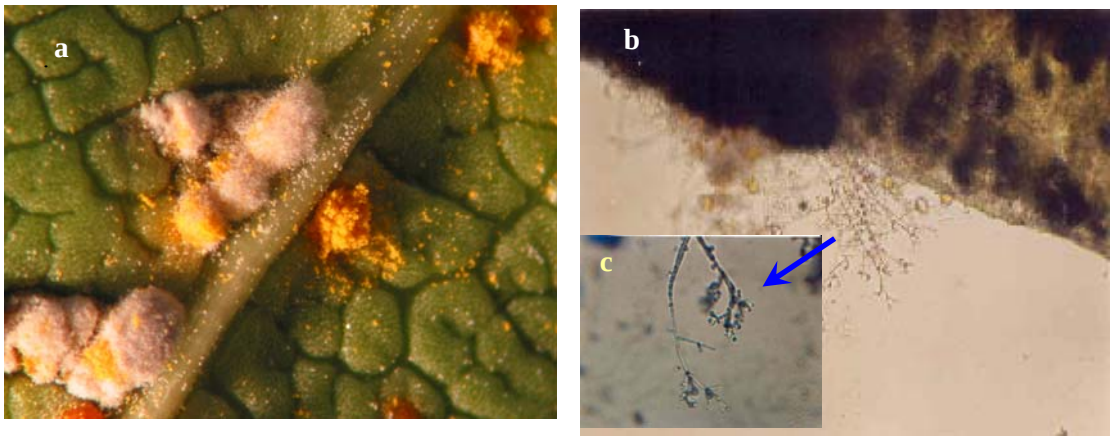
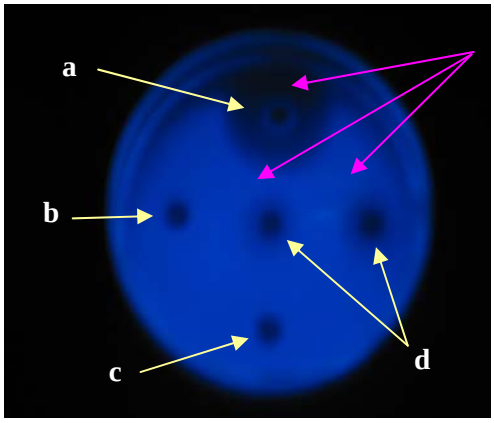


Figure 2. (a) Mycoparasite, *Verticillium sp.*, colonization on the rust fungus (x30), (b) Transverse section through rust infected area colonized by *Verticillium sp* (x100), and (c) Conidiophore of *Verticillium sp.* bearing conidia (x400).

Table 3. Amount of latex (ml/g of fresh leaf tissue) collected from young (1-3 weeks old) and old (5-6 weeks old) leaves of *P. rubra*.

Leaf type	Leaf weight (g)	Latex volume (ml)	Volume/wt (ml/g)
Young	65	2.0	0.0308
Old	165	1.0	0.0061



Clear zones due to chitinase activity

Figure 3. Gel diffusion assay plate with *P. rubra* young leaf latex, (a) Crude latex, (b) Undiluted water soluble fraction (Heat destroyed), (c) Distilled water and (d) Water soluble fraction (2 x diluted).

Chitinase activity of Plumeria leaf latex

The amount of latex collected from young leaves on fresh weight basis was about 5 times higher than that of old leaves of *P. rubra* (Table 3).

Germination of uredospores took place in water on glass slides within about 60 – 75 minutes. The latex from both *Plumeria* species had an inhibitory effect on uredospore germination compared to distilled water (control). The inhibitory effect on uredospore germination was greater in latex obtained from younger leaves compared to latex from older leaves (Table 4). Treatment of uredospores with fresh latex did not alter the time required for germination. The germ tubes elongated to a greater extent in the latex from *P. obtusa* than that from *P. rubra* within 35 minutes incubation period. During germination, emergence of one or more germ tubes was observed.

Table 4. Uredospore germination in young (1-3 weeks old) and old (5-6 weeks old) leaf latex of *P. rubra.*

Type of latex	% germination of uredospores
Young	60 ± 9.9
Old	70 ± 8.8
Distilled Water	92 ± 3.0

The *lumeria* latex was shown to exhibit chitinase activity by using a gel diffusion assay technique (Fig. 3). Chitinases that catalize the cleavage of chitin show antifungal properties against fungi containing chitin in walls. The presence of chitinase has also been reported in papaya (Adikaram *et al*., 1998) which may defend the host against fungal infection. Therefore, it is possible that the latex may play a role in the resistance of young *Plumeria* leaves against *Coleosporium* infection. As the leaf matures this ability decreases as the latex content is less which may allow the pathogen to infect leaf tissues.

REFERENCES

Adikaram, N. K. B., Karunaratne, A., Indrakeerthi, S. R. P. and Menike, P.R. (1998). Resistance of immature papaya (*Carica papaya* L.) fruit to fungal infection: An Overview. In: Disease Resistance in Fruit. Ed. Johnson, G. I., Highley, E. and Joyce, D. C. ACIAR Proceedings No. 80, 121-128.

American Phytopathological Society – standardization of fungicidal tests. (1943). The slide germination method of evaluating protectant fungicides. *Phytopathology* **33**: 627 – 632.

Chung, W. H., Abe, J. P., Yamaoka, Y., Haung, J. W. and Kakishima, M. (2006). First report of *Plumeria* rust disease caused by *Coleosporium plumeriae* in Taiwan. *Plant Pathology* **55**: 306.

Cummins, G. B. (1997). Illustrated Genera of Rust Fungi. Burgess Publishing Company, Pp. 36-37.

Dassanayake, M. D. and Fosberg, F. R. (1983). A Revised Hand Book to the Flora of Ceylon (Vol. IV). Amerind Publishing Co. Pvt. Ltd., New Delhi, Pp. 25-29.

Dizon, T. O., Virtudazo, E. and Kakishima, M. (1996). Rust of *Plumeria acuminata* Ait. and *Canna indica* L. *Philippine. Phytopathology* **32**: 118-123.

Holliday, P. (1980). Fungus Diseases of Tropical Crops. Cambridge University Press, Pp. 91.

Kakishima, M., Kobayashi, T. and Mackenzie, E. H. C. (1995). A warning against invasion of Japan by the rust fungus, *Coleosporium plumeriae*, on *Plumeria*. *Forest Pests* **44**: 08.

Laundon, G.F. and Rainbow, A.F. (1969). *Coleosporium ipomoae*. CMI Descriptions of pathogenic fungi and bacteria No. 282. Commonwealth Mycological Institute, England.

To-anum, C., Visarathanonth, N., Engkhaninum, J. and Kakishima, M. (2004). First report on *Plumeria* rust, caused by *Coleosporium plumeriae*, in Thailand. *Natural History Journal of Chulalongkorn University* **4**: 41-46.

Zau, X., Nonogaki, H. and Welbaum, G. E. (2002). A gel diffusion assay for visualization and quantification of chitinase activity. *Molecular Biotechnology* **22**: 19-23.