Zygosporium gibbum: a new and remarkable rust hyperparasite

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Zygosporium gibbum is recorded for the first time as a hyperparasite of *Coleosporium plumeriae*, the cause of *Plumeria* (frangipani) rust. *Zygosporium* species have not been previously reported as hyperparasites of rust fungi. Preliminary observations indicate that *Z. gibbum* is a potential biocontrol agent.

Key words – biocontrol – frangipani – mycoparasite – plumeria

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Introduction

Rust fungi cause extensive losses every year in many parts of the world. Chemical control of rust fungi has some limitations owing to economic, environmental and technical reasons (Moricca & Ragazzi 2008). Searches for efficient biocontrol agents for rust fungi, therefore, are of great relevance and about 84 species belonging to some 50 genera of fungi have been reported as hyperparasites of rust fungi (Kranz 1981). Some of the well known hyperparasites of rust fungi include *Cladosporium* spp., *Lecanicillium lecanii*, and *Scytalidium uredinicola*.

Plumerias (*Plumeria* family Apocynaceae; common name: frangipani) are small trees grown as ornamentals in most tropical and subtropical parts of the world. Although plumerias have been relatively free from major pests and diseases, in the last two decades, a rust disease has spread to several parts of the world where plumerias are grown often causing extensive defoliation. The plumeria rust pathogen is *Coleosporium plumeriae* Pat. (Coleosporiaceae, Pucciniales, Pucciniomycetes, Basidiomycota). *Coleosporium* is a large rust genus with more than 200 described species many of which are doubtfully distinct morphologically (Cummins & Hiratsuka 1983). Most *Coleosporium* species are macrocyclic and heteroecious with spermogonia and aecia on needles of *Pinus* and uredinia, telia and basidia on both monocots and dicots (Weeraratne & Adikaram 2006). However, spermogonial and aecial stages are not known for *Coleosporium plumeriae* (Nelson 2009).

While searching for hyperparasites of plumeria rust in Kerala State, India, we came across a remarkable hyperparasite growing on uredinial pustules of *C. plumeriae*. This observation is documented and discussed here.

Methods

All observations were made on plumeria trees (*Plumeria rubra* L.) growing in the Calicut University Campus between March and August, 2009. Conventional mycological methods, light microscopy (LM) and scanning electron microscopy (SEM) were used for observation of the pathogen and the associated hyperparasite. For scanning electron microscopy, the rust pustules fixed in 70% ethanol were carried through a graded ethanol series, transferred to 50:50 HMDS (hexamethy

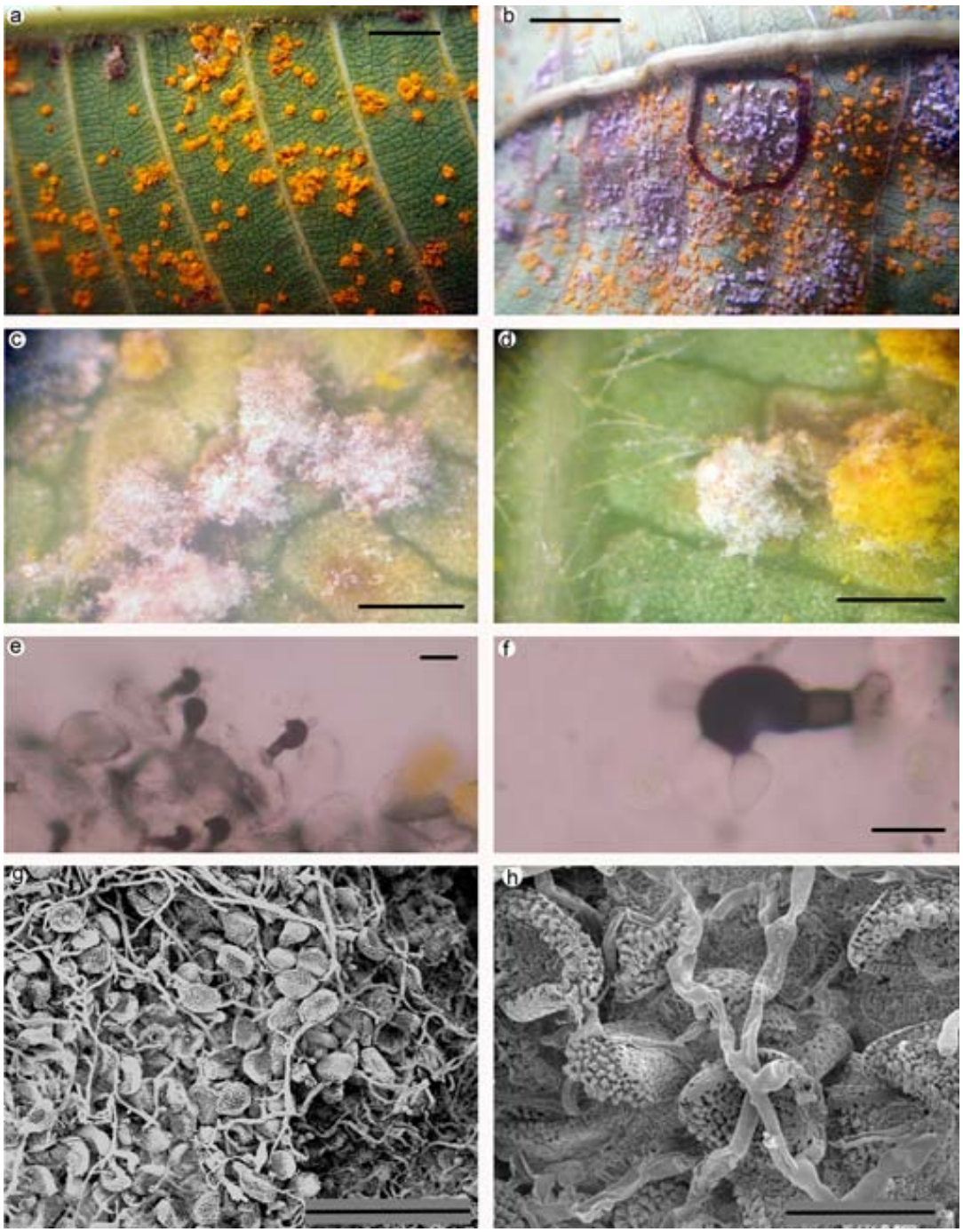

Fig. 1 a–**h** – *Zygosporium gibbum*. **a.** normal uredinial pustules of *Coleosporium plumeriae* on the lower surface of *Plumeria rubra* leaf, bar 5 = mm. **b.** lower surface of *P. rubra* leaf showing both normal uredinial pustules and those infected by the hyperparasite, bar 5 = mm. **c**, **d.** close-up views of hyperparasite infected uredinial pustules sprinkled with dark conidiophores of *Zygosporium gibbum*, bars = 2 mm. **e.** urediniospores of *Coleosporium plumeriae* supporting dark conidiophores of *Z. gibbum*, bar = 10 µm. **f.** a single conidiophore of *Z. gibbum*, bar = 10 µm. **g**, **h.** scanning electron micrographs of mycelium of *Z. gibbum* growing over urediniospores of *C. plumeriae*, bars = 100 µm for g and 25 µm for h.

disilazine): ethanol for 30 minutes, 100% HMDS for 30 minutes. The samples were removed from HMDS, air-dried, and coated with gold and observed using a Hitachi S3500N Variable Pressure SEM at 2 kilovolt. Genus level identification of the hyperparasite was based on Ellis (1971) and for species identification we followed the keys to species of *Zygosporium* given by both Ellis (1971) and Whitton et al. (2003). A voucher specimen

(CU125401) comprising a leaf of *Plumeria rubra* with uredinial pustules of *Coleosporium plumeriae* parasitised by *Zygosporium gibbum* has been deposited at the Calicut University Herbarium (CALI).

Results

On plumeria leaves heavily infected by the rust with orange uredinial pustules on the lower surface (Fig. 1a), some pustules appeared entirely or partly whitish in colour (Fig. 1b). When these discoloured pustules were examined under a stereo microscope, black dots were seen scattered over them (Fig. 1c, d). Light microscopy revealed the following features of a hyperparasite growing on the urediniospores: conidiophores with a single stalk cell, 6–10 × 3–4.5 µm, cylindrical, black in colour; vesicular cell (pseudophialide or prophialide) 10–14 × 6–8 µm, black in colour, characteristically curved and along with the stalk cell form a 'question mark' shaped structure, (Fig. 1e, f); conidiogenous cells (phialides) 3 per vesicle, 6–8×3.5–4.5 µm, ampullaceous, hyaline; conidia 4.5–6 µm diam., spherical, aseptate, hyaline, smooth to finely verruculose. Based on these features the fungus was identified as *Zygosporium gibbum* (Sacc., M. Rousseau & E. Bommer) S. Hughes (anamorphic Ascomycota). When the *Zygosporium* infected uredinial pustules were observed under a scanning electron microscope, extensive networks of hyphae of the hyperparasite were seen on the urediniospores (Fig. 1g, h). The hyperparasite was very efficient in destroying the urediniospores. The destruction of the urediniospores by the hyperparasite is visually indicated by a change of colour of the urediniospores from the normal orange colour to white. This has been further verified by the inability of the white urediniospores to germinate whereas the normal orange urediniospores from uninfected pustules germinated readily (100% germination). The hyperparasite was found to be restricted to the uredinial pustules and the leaf tissues were not invaded. *Zygosporium gibbum* was observed only during the summer months (March-May).

Discussion

Zygosporium gibbum seems to be very efficient in destroying the urediniospores of *Coleosporium plumeriae*. This destruction is accompanied by a dramatic decolourisation of the affected urediniospores and a total inability to germinate. How *Zygosporium gibbum* brings about the destruction of the urediniospore is not clear from these preliminary observations. Direct penetration of the urediniospores by the hyphae of the hyperparasite was not observed in either LM or SEM. The role of some lytic enzymes or antifungal metabolites cannot be ruled out. San-Blas et al. (1998) reported that *Zygosporium geminatum*, isolated as a contaminant in a culture of the mycelial phase of *Paracoccidioides brasiliensis*, was lethal to the latter organism. Its lytic action was due to exocellular α-1, 3- and β-1, 3-glucanases which degraded the *P. brasiliensis* cell wall. Hayakawa et al. (1968) have reported an antibiotic, zygosporin A, from *Zygosporium masonii*. Zygosporin A belongs to a class of mould metabolites called cytochalasins that have antimicrobial properties (Betina et al. 1972). Further studies are warranted to discover how *Zygosporium gibbum* attacks and destroys rust urediniospores and to see whether it can be adapted as a biocontrol agent.

Zygosporium gibbum is an anamorphic (dematiaceous hyphomycete) ascomycete whose natural habitat is primarily dead leaves. It appears to be a cosmopolitan species with records from tropical, subtropical and temperate regions (Whitton et al. 2003). This is the first record of *Zygosporium gibbum* as a hyperparasite of *Coleosporium plumeriae*. In fact, *Zygosporium* species have not been reported as hyperparasites of any rust so far. This preliminary observation indicates that *Z. gibbum* is a potential biocontrol agent and the possibility of using *Z. gibbum* for biological control of rust fungi is expected to be of interest to plant pathologists**.**

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