Mycopathologia (2008) 166:87–91
DOI 10.1007/s11046-008-9120-5

Pseudocercospora cryptostegiae-madagascariensis sp. nov. on *Cryptostegia madagascariensis*, an Exotic Vine Involved in Major Biological Invasions in Northeast Brazil

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Received: 14 February 2008 / Accepted: 3 April 2008 / Published online: 18 April 2008

Abstract *Cryptostegia madagascariensis* is a plant native from Madagascar, belonging to the Apocynaceae that is invading the native vegetation in Northeast Brazil and threatening the unique riverine formations dominated by the carnauba palm. Individuals of *C. madagascariensis* cultivated in the campus of the Universidade Federal de Viçosa, state of Minas Gerais, Brazil, showing leaf spot symptoms of unknown etiology were observed. Two fungal species were found associated to such leaf spots: *Colletotrichum gloeosporioides* and a new species of *Pseudocercospora*. The latter was named *Pseudocercospora cryptostegiae-madagascariensis* and described herein. The discovery of those two fungal pathogens on *C. madagascariensis* coincide with the recognition that the weedy vine that is involved in the infestations in the Northeast of Brazil is not *Cryptostegia grandiflora* as formerly reported. These mycological findings are of greater importance now since *C. madagascariensis* is likely to become a target for biological control together with its former status in Brazil of an ornamental plant of limited relevance.

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Keywords Apocynaceae · Biological control · *Cryptostegia* · Plant disease

Introduction

Cryptostegia madagascariensis Bojer ex Decne. is a plant native from Madagascar, belonging to the Apocynaceae. In Brazil it has been used occasionally as an ornamental because of its showy pink to violet inflorescences [1]. *C. madagascariensis* is closely related to *Cryptostegia grandiflora* R. Br. (rubber-vine), the only other species in the genus [2]. The latter is well known for the devastating invasions of natural ecosystems it promoted in Northern Australia [3, 4]. It has been recognized as the biggest threat to tropical ecosystems in Australia by McFadyen and Harvey [5]. Invasions of this weed threatening the natural vegetation are also known to be happening in the Caribbean—Dutch Antilles and Curaçao—Tomley and Evans [6]. Fortunately, *C. grandiflora* is also now known for the highly successful classical biological control program conducted against it in Australia. This program became one of the landmarks for the discipline of classical weed biocontrol. It involved the introduction of two natural enemies from Madagascar into Australia: the insect *Euclasta whalleyi* (Popescu-Gorj & Constantinescu) McFadyen et al. [7] and the rust fungus *Maravalia cryptostegiae* (Cummins) Y. Ono [6]. Until recently there were no confirmed records of *C. madagascariensis* behaving as an

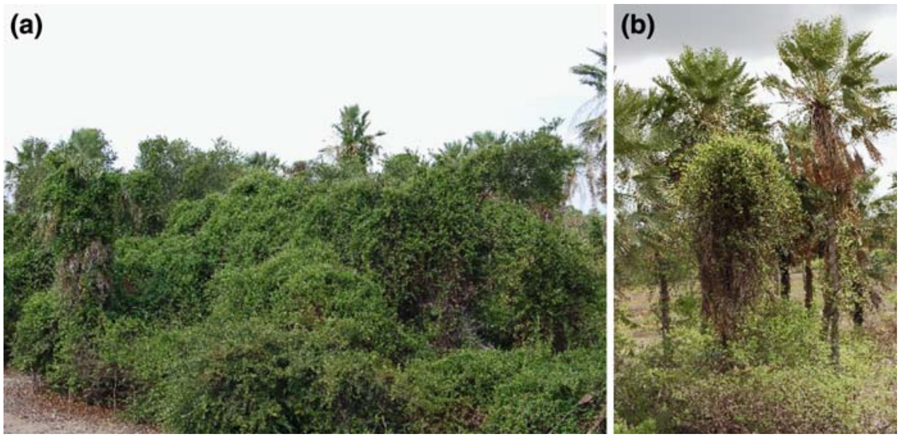

Fig. 1 (**a**) A stretch of riverine vegetation near Limoeiro do Norte (state of Ceará) severely infested with *Cryptostegia madagascariensis* (left) and (**b**) Carnauba palms smothered by rubber vine (right). Note dead individual (centrally) which had its crown totally wrapped by the vines

ecosystem invader. *C. grandiflora* was listed [8], among other exotic plants species, as invading humid locations in the semi-arid regions of Brazilian Northeast. Recently, in February 2007, a visit by two scientists (R.W. Barreto, Universidade Federal de Viçosa, Brazil, and H.C. Evans, CAB International, UK) to selected areas indicated by Herrera and Major (personal communication) for observation of infestations in the state of Ceará (along the basins of the rivers Choró and Jaguaribe) confirmed a widespread and destructive infestation of the riverine vegetation (Fig. 1) posing serious threats to a number of endemic species, particularly the palm *Copernicia prunifera* (Mill.) H. E. Moore. This palm is the sole source of carnauba wax [9], a prime matter of major importance for the food, cosmetics, pharmaceutical, computer, and other industries and is sustainably extracted from spontaneous palm stands by the local population. The vines growing in the vicinity of such palms climb the palm and wrap the unfolded leaves at the crown and smother the highly heliophilous plants leading to their death (Fig. 1). The extension of the infestations in Ceará and other neighboring states is presently being accessed but there are indications that infestations are already widespread and severe in many areas of Northeast Brazil. One surprising outcome of this visit was the conclusion that such infestations were not being caused by *C. grandiflora* as originally reported but instead by *C. madagascariensis*, as suspected from the field observations and confirmed by examination by the botanist Dr. Milene F. Vieira (Departamento de Botânica, Universidade Federal de Viçosa) of samples brought from Ceará. During the brief survey in Ceará *C. madagascariensis* plants were noted to be always healthy. No sign of insect attack of disease symptoms were observed throughout the range of locations that were visited. Tackling such infestations is difficult even for protecting the palm stands that are commercially explored. Mechanical removal of the vines is both uneconomical and dangerous due to the toxic nature of the milky sap of the vine, whereas the use of herbicides is both too costly for the poor populations of the region and ecologically undesirable in such natural or semi-natural vegetation formations. Ironically, prior to that, in 2005, a casual observation was made in the campus of the Universidade Federal de Viçosa (state of Minas Gerais, Brazil) of few individuals of *C. madagascariensis* being grown in a gardening research area showing leaf spots involving two species of fungi. Considering that there were no records of diseases on this host in Brazil, a study was initiated aimed at simply elucidating the etiology of such leaf spots. This study now became of an increased relevance because of the novel status of *C. madagascariensis* in Brazil as a weed and possible target for a classical biocontrol program instead of a minor ornamental (a use that should be now discouraged, if not forbidden).

Materials and Methods

Samples of diseased leaves of *C. madagascariensis* were collected and examined while still fresh under a dissecting microscope for the observation of fungal structures. Selected specimens were dried in a plant press and deposited in the local herbarium (Herbarium VIC).

Spores were collected from sporulating lesions and aseptically transferred with a sterile fine pointed needle onto PDA plates and later transferred to tubes containing PCA. Koch's postulates were fulfilled by depositing pure culture plugs, obtained from actively growing cultures on PDA, onto healthy leaves of plants in the field. After inoculation a humid chamber was prepared by placing a cotton plug soaked in water surrounding each branch close to the inoculated leaves. The branch was wrapped in a plastic bags wetted inside. After 48 h the plastic bag was removed. Controls consisted of branches containing only healthy leaves onto which sterile PDA medium plugs were deposited. Inoculated parts were observed daily until the appearance of disease symptoms. Whenever symptoms similar to those originally observed in the field were noted, reisolation was attempted either by direct transfer of fungal structures onto plates containing PDA or by surface sterilization of necrotic tissues (3 min in chlorox) and plating these on to PDA.

Results and Discussion

Taxonomy

C. gloeosporioides (Penz.) Penz. & Sacc. (Figs. 2 and 3)

Lesions on living leaves, subcircular, well delimited, infected tissue initially dark brown surrounded by a pale brown halo, becoming paler centrally and darker in the peryphery with age, 1–5 mm diam.

Material examined: VIC 29388 ex *Cryptostegia madagascariensis*, Viçosa, state of Minas Gerais, Brazil, 22nd Nov 2005, Janaina L. Silva.

The specimen of *C. gloeosporioides* observed on *C. madagascariensis* had very densely packed setae in their acervuli and under the conditions that it was collected it had very limited sporulation which led initially to some doubts about its correct identity. Nevertheless, in culture the fungus produced abundant typical conidia and in slide cultures appressoria having the typical shape for the species.

This is the first report of *C. gloeosporioides* associated to leaf spots on *C. madagascariensis* worldwide. H. C. Evans (personal communication) found leaf spots on *Cryptostegia* spp. during his surveys for natural enemies of *C. grandiflora* in its native range in Madagascar and according to him a series of fungi were implicated, including *Colletotrichum* and a cercosporoid fungus. Unfortunately this information was never published.

P. cryptostegiae-madagascariensis sp. nov.: J. L. Silva, R. W. Barreto & O. L. Pereira (Figs. 2 and 3)

Mycobank 511185.
Differt a *P. cryptostegiae* stromatibus 42.5–102.5 μm diam. et conidiis 2–3 μm latis.
Etymol: from the host name.

Lesions on living leaves, circular, virtually undistinguishable from those caused by *C. gloeosporioides*, 1.5–6.0 mm diam. *Intenal mycelium* indistinct. *External mycelium* absent. *Stromata* abaxial, subepidermal, erumpent, subsphaerical, 42.5–102,5 μm diam., composed of dark brown textura angularis. *Conidiophores* emerging from the stromata, subcylindrical, straight, curved or sinuose, 5.0–15.0 × 1.5–3.5 μm, 0–1

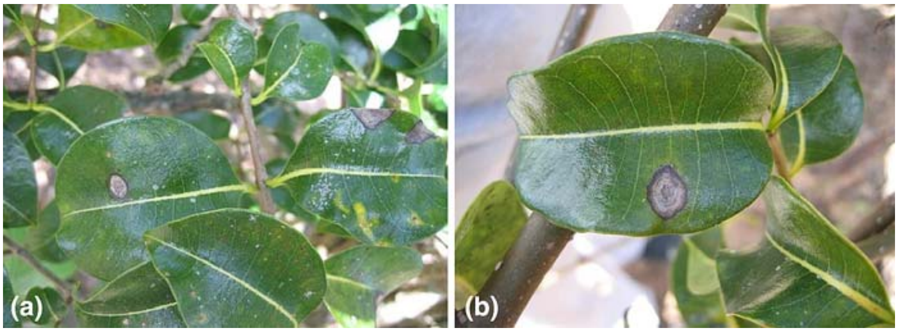

Fig. 2 Leaf spot symptoms on *Cryptostegia madagascariensis*. (**a**) Caused by *Colletotrichum gloeosporioides* (left) and (b) caused by *Pseudocercospora cryptostegiae-madagascariensis* (right)

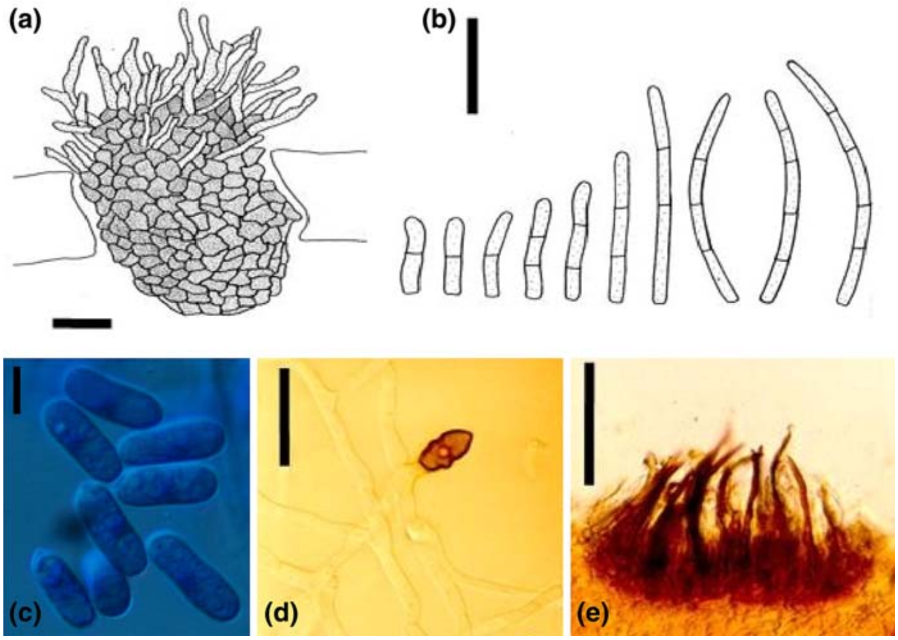

Fig. 3 (**a**) and (**b**) *Pseudocercospora cryptostegiae-madagascariensis* on *Cryptostegia madagascariensis*. (**a**) Stroma with conidiophores and conidia; (**b**) conidia; (**c**), (**d**), and (**e**) *Colletotrichum gloeosporioides* on *C. madagascariensis*. (**c**) Conidia, (**d**) well developed apressoria, (**e**) section through an acervulus packed with setae. Scale bars (**a**) = 30 μm, (**b**) = 10 μm, (**c**) = 5 μm, (**d**) = 20 μm, and (**e**) = 60 μm

septate, unbranched, pale brown, smooth. *Conidiogenous cells* terminal, holoblastic, cylindrical with rounded apex, 1.0–2.0 × 4.0–5.0 μm, subhyaline, smooth. *Conidiogenous loci* flat, 1.5–2.0 μm, unthickened, not darkened. *Conidia* isolate, holoblastic, cylindrical, straight to curved, 10–69 × 2–3 μm, apex rounded, base truncate, 1–4 septate, subhyaline, hilum unthickened, not darkened, eguttulate, smooth.

Holotype: VIC 29389 ex *Cryptostegia madascariensis*, Viçosa, state of Minas Gerais, Brazil, 29th Nov 2005, Janaina L. Silva.

There is only one species of *Pseudocercospora* recorded in association with the host-genus *Cryptostegia*. That is *P. cryptostegiae* (W. Yamamoto) Deighton [10, 11]. *P. cryptostegiae-madagascariensis* differs from *P. cryptostegiae* by having well developed stromata, which is absent in *P. cryptostegiae* and narrower conidia. These are 4–7 μm wide in *P. cryptostegiae* as compared to 2–3 μm in the new species. Conidiophores are branched and multiseptate in *P. cryptostegiae* contrarily to 0–1 septate and unbranched in *P. cryptostegiae-madagascariensis*.

Pathology

Both *C. gloeosporioides* and *P. cryptostegiae-madagascariensis* were proven to be pathogenic to *C. madagascariensis*. Inoculations resulted in typical leaf-spot symptoms 8 days after inoculation and each fungus was observed associated to inoculated leaves and reisolated whereas control leaves remained healthy.

Although *P. cryptostegiae-madagascariensis* was demonstrated to be pathogenic, this fungus is slow growing in culture and does not sporulate. Its use as a biocontrol agent would be restricted to a possible introduction of the isolate collected in Minas Gerais into Northeastern Brazil. Conversely, the mycoherbicide approach might be considered for *C. gloeosporioides.* There are many examples of studies involving the use of strains of this fungal species as mycoherbicides, including two products that actually became available commercially: COLLEGO and BIOMAL [12, 13]. The mycoherbicide approach may, nevertheless, be inadequate for dry climates such as those of Northeast Brazil, where the invasions by *C. madagascariensis* are taking place. Fungi in general, and *C. gloeosporioides* in particular, are often highly dependent on high humidity conditions for sporulation, dispersal, and infection.

During his surveys is Madagascar, H. C. Evans (personal communication) never regarded either *C. gloeosporioides* or the cercosporoid fungus (possibly *P. cryptostegiae* or *P. cryptostegiae-madagascariensis*) as sufficiently damaging to their hosts to deserve further consideration for use as classical biological control agents. The preference was early concentrated on the rust fungus *M. cryptostegiae* which causes a very damaging disease on both hosts, sporulates abundantly allowing for the accumulation of inoculum to be used in studies and during the introduction stage. This early choice proved to be wise but a first attempt of introducing the fungus in Australia resulted in failure due to the choice of an incorrect strain. At the time it was not known that there are separate strains of *M. cryptostegiae* adapted to each *Cryptostegia* species. Although both strains produced equivalent levels of disease in the greenhouse, when the strain obtained from *C. madagascariensis* was introduced into Australia, its impact to *C. grandiflora* was minimal in the field. Eventually a strain from *C. grandiflora* was selected and introduced into Australia in 1995, resulting in fast and high levels of weed control [6]. Considering that previous experience and the similar climatic conditions of Northern Australia and the Brazilian Northeast, it is expected that attention in Brazil should be focused on the use of the rubber vine rust strain that exists in Madagascar attacking *C. madagascariensis*. Cooperation with CAB International scientists would allow for piggybacking on the previous project and hence avoiding the process of searching for new rust strains for introduction and submitting these to host-range tests. It appears that collections of *M. cryptostegiae* strains are still kept in storage in CAB International collection.

Acknowledgments The authors thank Drs. I. Major and O. Herrera for their cooperation during the field work in Ceará, Dr. H. C. Evans for sharing his experience and thoughts on the topic and taking part in the field survey in Ceará. J. L. da Silva acknowledges financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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