# *Cordana versicolor* sp. nov. (dematiaceous hyphomycete) causing leaf-spot on *Canna denudata* (*Cannaceae*) in Brazil, with observations on *Cordana musae*

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*Cordana versicolor*, a new dematiaceous anamorphic fungus, was found in Brazil associated with leaf spots on the herbaceous ornamental *Canna denudata*. It differs morphologically from related species and is the first species in the genus proven to be pathogenic to a member of the *Cannaceae*. It produces boxing-glove-shaped appressoria apically on germ tubes. A related species, *C. musae* pathogenic on banana, produces ampulliform appressoria. Taxonomic significance of such distinct structures have not been previously considered for this genus.

**Key words:** appressorium formation, taxonomy.

## Introduction

*Canna denudata* Rosc. (*Cannaceae*) is a herbaceous plant, native to Central America and the Caribbean. In Brazil it is commonly used as an ornamental, especially on fully sun-exposed sites (Lorenzi and Souza, 1999). Damaging leaf spots were found affecting *C. denudata* at three separate places in the municipality of Viçosa (state of Minas Gerais, Brazil). No description of a similar disease on this host has been reported in the literature. A dematiaceous hyphomycete was found consistently associated with such lesions, and its morphology corresponded with that of *Cordana* Preuss. This fungus was initially identified as *C. musae* (Zimmerm.) Höhn. (Nechet and Barreto, 2000), but a re-examination and more detailed observations indicated that this record was incorrect. The genus *Cordana* has been recently revised (Markovskaja, 2003) and a comparison of the fungus on *Canna denudata* showed that it did not correspond to any other known species of this genus.

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## Material and methods

Infected plant material was collected at three different sites in the municipality of Viçosa, Brazil. Description and illustrations of the fungus were made from freshly collected specimens. Representative specimens were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC). To prove pathogenicity small fragments (0.5 × 0.5 cm) of freshly collected diseased leaves were placed with their sporulating (abaxial) side on drops of water deposited on both the upper and lower surface of healthy leaves. The control consisted of similar fragments of healthy *Canna denudata* placed on healthy leaves as described above. After six hours (late afternoon) the fragments were removed and the plants were maintained under shade in the field. Half of the leaf surface areas where the fragments were placed had been previously wounded by piercing with a finely pointed needle. Four repetitions were made for each treatment. In addition, four healthy leaves of both banana and *C. denudata* plants were brush-inoculated with a conidial suspension of 1.5 × $10^4$ conidia/mL of either *Cordana musae* or of the new *Cordana* species. Several attempts were made to isolate the fungus. Leaf fragments, taken from the margin of leaf spots where the fungus was actively growing, were surface sterilized with Chlorox (0.5%) and plated on V8 agar. As an alternative, a slightly modified form of the method described by Allen and Dettmann (1990) was utilized. Small fragments of leaves showing disease symptoms were placed in Petri dishes containing wet filter paper and incubated at 25 ± 2ºC in the dark for 24 hours. Freshly produced conidia were then transferred to each of the following culture media: V8 juice (200 ml V8 juice; 1 litre of distilled water) + calcium carbonate (3 g/L); half-strength V8 agar; potato-carrot agar; a decoction of 270 g of macerated leaves of *C. denudata* filled up to 1 L, 20 g dextrose; the same with banana leaves. The plates were left at 25 ± 2ºC in the dark and observed daily.

Conidial germination and appressorium formation were observed by suspending conidia in drops of tap water deposited on a microscope slide, and left for 24 hours at room temperature in a Petri dish lined with wet filter paper. The slides were then left on a laboratory bench until the water dried, and a drop of lactophenol and coverslip were placed over the area for microscopic observations.

## Results

***Cordana versicolor*** D.J. Soares & R.W.Barreto, **sp. nov.** (Figs. 1-12)

*Etymology*: named in reference to the basal cell of the conidia being paler than the apical cell.

*Maculae* amphigenae, ovales vel ellipticae, 1-2.5 × 0.8-1 cm, in medio pallidogriseae, marginem versus atrobrunneae, interdum confluentes. *Mycelium* immersum, septatum, hyalinum vel pallide brunneum. *Conidiophora* solitaria, macronemata, cylindrica, recta vel paulo flexuosa, ad 260 µm longa, 7-14 µm lata ad basim, 3-7 µm lata in medio, 1-3-septata, laevia, brunnea, ad apicem subhyalina. *Cellulae conidiogenae* terminales, vulgo polyblasticae, denticulis cylindricis praeditae. *Conidia* late ellipsoidea, 15-25 × 10-15 µm, in medio uniseptata, versicoloria, cellula basilari pallidiore quam apicali, interdum cellula tertia subulata apicali, ad 15 µm longa, laevia.

*Lesions* on living leaves with an eye-spot pattern, oval to elliptical, 1-2.5 × 0.8-1 cm, pale grey centrally, with dark brown margins, widely distributed on the lamina, coalescing and leading to extensive or complete necrosis of the leaves. *Internal mycelium* branched, septate, hyaline to subhyaline. *Conidiophores* single, cylindrical, straight to slightly flexuous, up to 260 µm, 7-14 µm wide near the base, 3-7 µm in the middle, 1-3-septate, smooth, brown at the base, becoming paler towards the apex. *Conidiogenous cells* commonly polyblastic, terminal, with 1-5 intercalary and terminal swellings from which usually a solitary cylindrical denticle arises. *Conidia* broadly ellipsoidal, 15-25 × 10-15 µm, medianly one-septate, basal cell subhyaline, apical cell pale brown, usually with a somewhat distinct and protruding hilum, sometimes with an additional subulate apical cell (beak) up to 15 µm long, smooth; germinating always at the basal cell and forming germ tubes with boxing-glove-shaped, dark-brown appressoria, 10-25 × 8-14 µm.

*Teleomorph*: not seen.

*Habitat*: on living leaves of *Canna denudata* (*Cannaceae*).

*Known distribution*: Viçosa.

*Material examined*: BRAZIL, Minas Gerais, Viçosa, Associação Atletica Banco do Brasil, 02/05/1999, R.W. Barreto (VIC 22157; **holotype designated here**). *Paratypus*: BRAZIL, Minas Gerais, Viçosa, Cristais, 02/04/2000, R.W. Barreto (VIC 22158); *Paratypus*: BRAZIL, Minas Gerais, Viçosa, Paraíso, 23/12/2000, D.J. Soares (VIC 22798).

## Discussion

*Cordana versicolor* is morphologically similar to *C. musae*, *C. johnstonii* M.B. Ellis and *C. andinopatagonica* Gamundí & Arambarri, but it can be distinguished from these by shape, size and colour of the conidia and the host family (Table 1). It differs from *C. musae* by having larger conidia that are broadly ellipsoid, whereas *C. musae* has obovoid to pyriform conidia and grows on *Musaceae* (Ellis, 1971; Castañeda Ruiz *et al*., 1999). In addition, the germ tubes of *C. musae* end with ampulliform, 10-14 × 7-10 µm, dark grey appressoria (Figs. 13-15), whereas those of *C. versicolor* are dark brown and shaped like a boxing glove. *Cordana andinopatagonica* has obovoid to ellipsoid evenly coloured conidia and is known as a saprobe on leaves of

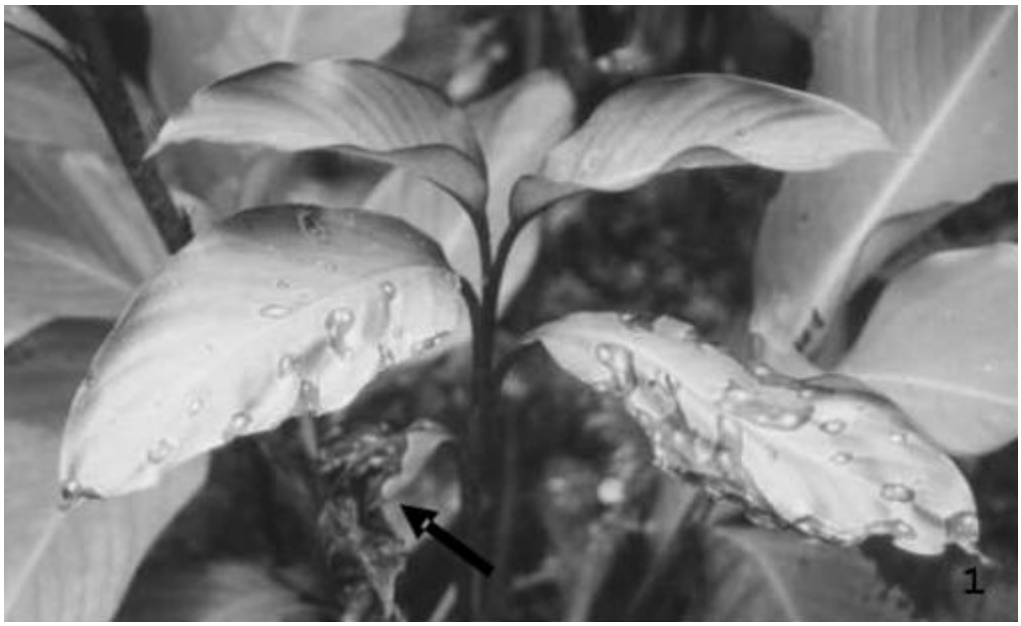
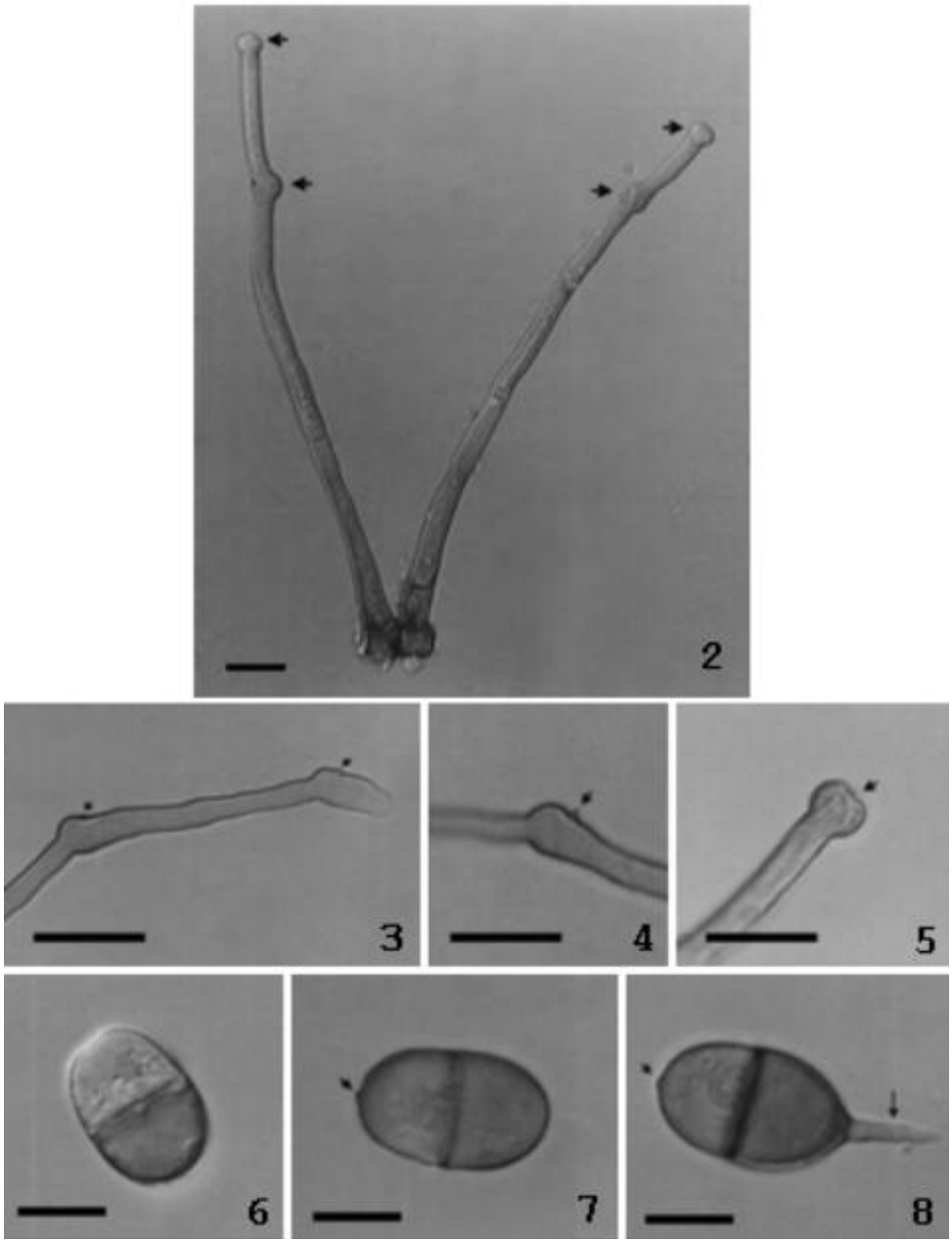


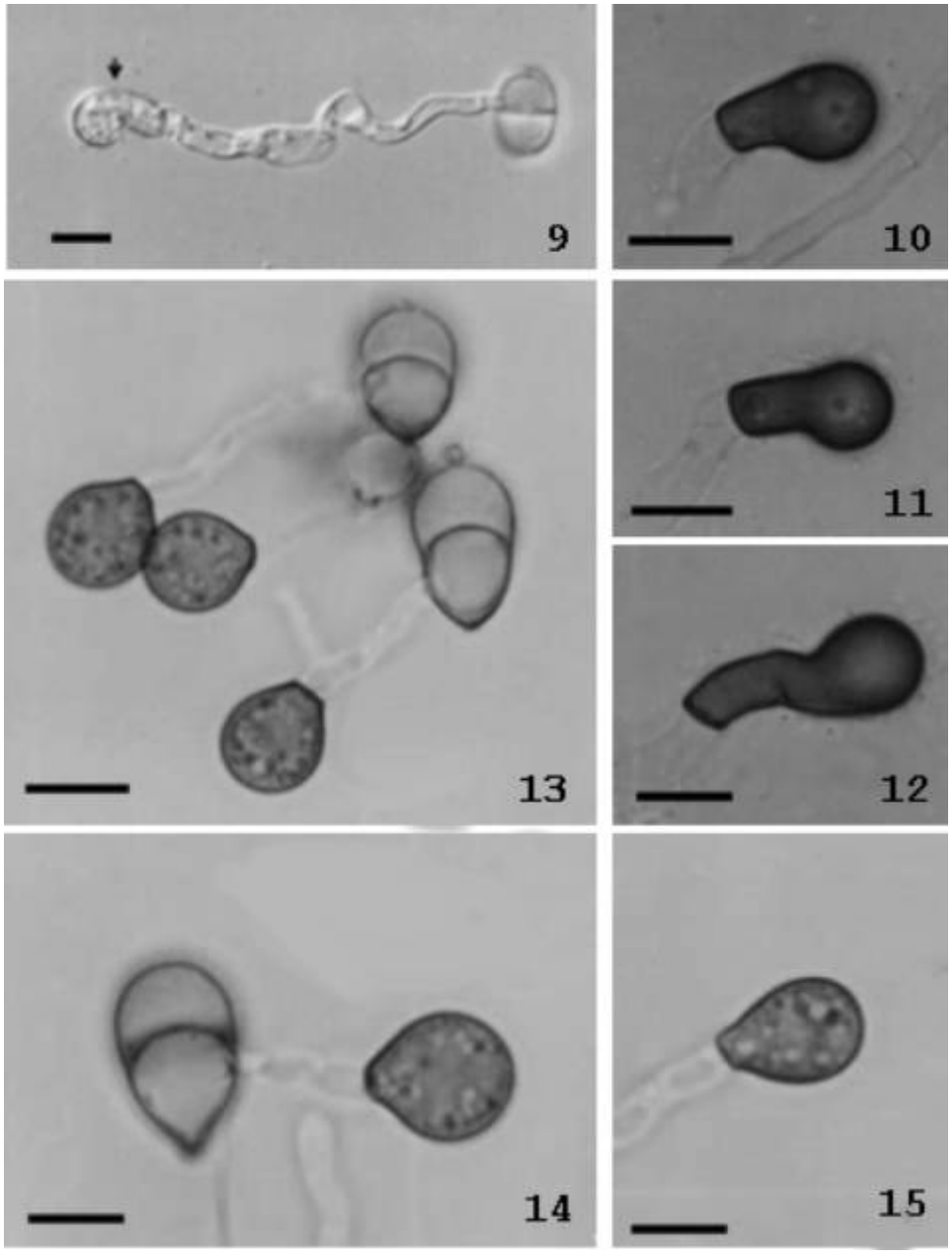

**Fig. 1.** *Canna denudata* with eye-spot lesions caused by *Cordana versicolor*. Note the complete necrosis of older leaf (arrowed).

*Nothofagus dombeyi* (Mirb.) Oerst. in Argentina (Gamundí *et al*., 1979). *Cordana johnstonii* has evenly pigmented and somewhat larger conidial cells and never forms a conidial beak (that is occasionally seen in *C. versicolor*), and only grows on plants in the genus *Musa*. Additionally, *C. versicolor* has apical and intercalary bulbous portions on the conidiophore that each bears one cylindrical denticle (Figs. 3-5), whereas conidiophores of *C. johnstonii* are not so swollen and have more then one denticle per swell (Ellis, 1976). Further observations of germinating conidia of *Cordana* spp., particularly of *C. johnstonii* would help to clarify the value of appressorium morphology as a feature for distinguishing species in this genus. Examination of *C. johnstonii* appressoria was not feasible during this study as this is an exotic species for Brazil. This structure has been largely neglected by taxonomists that studied this genus and remains undescribed for most of the species in the genus.

Pathogenicity of *C. versicolor* was proven. Typical eye-spot symptoms were observed at inoculated portions of leaves 15 to 25 days after inoculation both in injured and intact leaf portions. Inoculations by deposition of inoculum abaxially resulted in a slightly quicker development of symptoms as compared with adaxial deposition of inoculum (Fig. 16). Cross inoculations with *C. musae* and *C. versicolor* resulted in infection only of banana by *C. musae* and *Canna denudata* by *C. versicolor* (after 20 days).




**Figs. 2-8.** Light micrographs of *Cordana versicolor* (from holotype). **2.** Conidiophores with intercalary and terminal swellings (arrows). **3-5.** Close-up of the conidiophore swellings with solitary denticles (arrows). **6-8.** Conidia. Note the versicolored cells, the slightly protuberant hilum (short arrows) and subulate beak (long arrow)]. Bars = 10 μm.




**Figs. 9-15.** Germ tubes and apressorium formation of *Cordana versicolor* and *C. musae*. **9.** Germinating conidium of *C. versicolor* and young boxing-glove-shaped appressorium (arrowed). **10-12.** Close-up of mature boxing-glove-shaped appressoria of *C. versicolor*. **13-14.** Germinating conidia of *C. musae* with germ tubes arising from the basal cell and ampulliform appressoria. Bars = 10 μm.

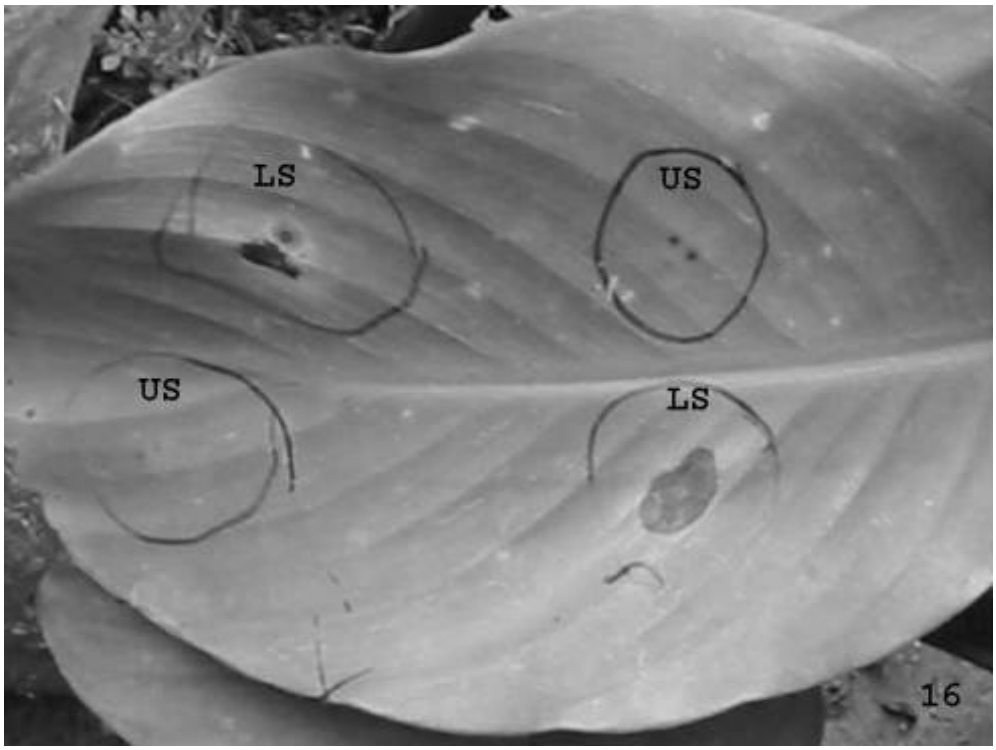



**Fig. 16.** Eye spot lesions produced on adaxial surface of *Canna denudata* leaf after inoculation with *Cordana versicolor*. Inoculation made on upper survace (US) and lower surface (LS).

**Table 1.** Comparison between *C. versicolor* and related *Cordana* species.

| Species | Conidia | | | Substrate | Appressoria | Reference |
|---|---|---|---|---|---|---|
| | Shape | Size (µm) | Colour | | | |
| *C. andinopatagonica* | Obovoid to ellipsoid | 17-19 × 10-13 | Equally subhyaline | Dead leaves of *Nothofagus dombeyi* | Undescribed | Gamundí *et al*., 1979 |
| *C. johnstonii* | Broadly ellipsoid | 20-30 × 12-18 | Equally pale brown | Parasitic on *Musa* spp. | Undescribed | Ellis, 1976; Castañeda Ruiz *et al*., 1999 |
| *C. musae* | Obovoid to pyriform | 11-18 × 7-10 | Equally subhyaline to pale brown | Parasitic on *Musa* spp. | 10-14 × 7-10 µm; dark gray | Ellis, 1971; Castañeda Ruiz *et al*., 1999; this publication |
| *C. versicolor* | Broadly ellipsoid | 15-25 × 10-15 | Basal cell subhyaline apical cell pale brown | Parasitic on *Canna denudata* | 10-25 × 8-14 µm; dark brown | This publication |

Attempts to cultivate *C. versicolor* failed. Although *C. musae* will grow in culture (Photita *et al*., 2004) and an isolate is available at CBS (W. Gams, pers. comm.), there is no published record of growth of *C. johnstonii in vitro* (Ellis, 1971; Stover, 1972; Priest, 1990; Matsushima, 1993). Other typically saprobic *Cordana* are commonly isolated from dead wood or leaves and are easily cultured. Systematic attempts at cultivating *C. johnstonii* should be attempted and described, but the information available would suggest that the genus is split into two physiological groups, one containing highly specialized pathogens and the other saprobic species. de Hoog *et al*. (1983) had already suggested the existence of two ecological groups for the accepted species in *Cordana*, but gave more importance to morphology and discharge of conidia than to possible physiological specialization in his discussion. The pathogenic status of disease-associated *Cordana* (*C. musae*, *C. johnstonii and C. versicolor*) remains open to debate. Stover (1972) and Wardlaw (1972) have referred to *C. musae* and *C. johnstonii* as 'secondary opportunistic pathogens'. However, the difficulties found in culturing *C. versicolor* suggest that these species are ecological equivalents to biotrophs, an unsuspected condition for 'opportunistic pathogens'.

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