

## **STEM BLEEDING OF ACAI PALM PLANTS, CAUSED BY *Ceratocystis paradoxa*, IN SOUTHERN BAHIA**

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The growing interest for sub-products from acai palm has increased, lately, throughout Brazil. Even with the increasing growth of planted area few diseases have been reported for this crop so far, as for example, anthracnose. In 2015, during phytosanitary inspections carried out in some farms of the municipalities of Ilheus and Camacan, Bahia State (Brazil), a disease that, apparently, had not been previously described in the crop was observed. A characteristic and not so usual symptomatology, ending up in high plant mortality, was frequently observed in the affected areas. Aiming to investigate the disease causal agent, samples collected from infected plants were brought to the Plant Pathology Laboratory at the Cacao Research Center, for pathogen isolation in PDA medium. For the identification of the isolated fungus, preparations of the morphological structures were examined under an optical microscope. The predominantly isolated fungus, in all the attempts, was *Ceratocystis paradoxa* (anamorph *Thielaviopsis paradoxa*). The pathogenicity tests carried out on 12-years-old acai plants, under field conditions, showed that both phases of the fungus were pathogenic to the inoculated plants, with no difference, in terms of symptomatology. The reproduction of the disease symptoms on inoculated plants and the re-isolation of the pathogen in PDA medium, have proved that *Ceratocystis paradoxa* (= *T. paradoxa*) is the causal agent of the disease observed on the acai palm plants.

**Key words:** *Euterpe oleracea*, *Thielaviopsis paradoxa*, canker, stem rot.

**Sangramento do caule do açaizeiro, causado por *Ceratocystis paradoxa*, no sul da Bahia (Brasil).** O interesse crescente por produtos originários do açaizeiro tem aumentado, consistentemente, em todo o Brasil. Mesmo com o aumento na área cultivada, em todo o país, poucas doenças foram registradas na cultura, até o momento, entre elas a antracnose. Em 2015, em inspeções fitossanitárias realizadas em algumas fazendas dos municípios de Ilhéus e Camacan, Estado da Bahia, foi observada uma doença que aparentemente ainda não havia sido descrita na cultura. Plantas afetadas mostravam uma sintomatologia muito característica e não tão usual, culminando com uma elevada taxa de mortalidade. Com o objetivo de averiguar as causas da doença, amostras de tecidos coletadas em plantas afetadas, foram trazidas para o Laboratório de Fitopatologia do Centro de Pesquisas do Cacau (CEPEC), visando o isolamento do patógeno em meio de BDA. Na identificação dos fungos isolados, preparações contendo estruturas morfológicas características foram examinadas ao microscópio ótico. Os fungos predominantemente obtidos em todos os isolamentos foram *Thielaviopsis paradoxa* e seu teleomorfo, *Ceratocystis paradoxa*. Testes de patogenicidade realizados em plantas de açaí de 12 anos de idade, em condições de campo, mostraram que o fungo foi patogênico às plantas, não sendo observadas quaisquer diferenças, em termos sintomatológicos, entre as formas anamórfica e teleomórfica. A reprodução dos sintomas nas plantas inoculadas e o reisolamento do patógeno em BDA demonstraram ser o fungo *Ceratocystis paradoxa* (*T. paradoxa*) o agente causal da doença.

**Palavras-chave:** *Euterpe oleracea*, *Thielaviopsis paradoxa*, cancro, podridão do caule.

## Introduction

The acai palm (*Euterpe oleracea* Mart.), an economically important plant in the northern region of Brazil, is responsible for the production of several appreciated and consumed sub-products in Brazil. The berry pulp is raw material for the manufacture of ice creams, popsicles, jellies, beverages and energetic foods. There are also great prospects of their use in the industries of natural dyes (Nazaré et al., 1996) and isotonic drinks. In domestic cooking they are employed in the preparation of cakes, pies, custards, puddings and mousses. The plant also stands out as the main source for the palm heart (palmetto) extraction (Oliveira et al., 2000).

The number of species in the genus is not clearly defined, and has been subject of constant revisions. In the early works, in addition to the acai palm, 48 species distributed throughout the South and Central America were recognized (Glassman, 1972). However, after Uhl & Dransfield (1986) and Henderson & Galeano (1996) reviews, the number of accepted species was reduced to 28 and 7, respectively.

Among the native species found in Brazil, the most important ones, according to the industrial point of view, are *E. oleracea*, *E. edulis* and *E. precatória*. The first one, known in Brazil as 'palmito', was used for many years as the main source of raw material for the palm heart industry. Currently, its exploitation decreased due to the exhaustion of the species in its natural habitat. The second species, besides fruit production, stands out as the main source for palm heart extraction, especially in Para and Amapa States (Brazil). *Euterpe precatória*, although to a lesser extent, has similar uses to the previous species (Khan, 1986; Villachica et al., 1996; Oliveira et al., 2000).

The acai palm can be grown in areas with temperatures above 18° C and rainfall ranging usually between 2000 and 2500 mm, throughout the year. The Southern Region of Bahia fits perfectly into such conditions, and it is considered as an ideal region for its cultivation.

The plant shows an important feature: it develops in clumps, producing many tillers, which well-managed provide continuous crops for palm heart. There is no need to eliminate the whole plant, since it regenerates after each harvesting. In the Amazon Region the acai

palm produces fruits throughout the year, particularly from January to May and September to December (Oliveira et al., 2000).

Besides occurring in other South American countries such as Guyana, French Guiana, Suriname, Venezuela (Calzavara, 1972; Roosmalen, 1985; Calvacante, 1991), Colombia (Balick, 1986 b), Panama, Ecuador and Trinidad and Tobago (Henderson & Galeno, 1996), in Brazil it can be found thriving spontaneously in the States of Amapa, Maranhao, Para (Calzavara 1972; Cavalcante, 1991), Tocantins (Balick, 1986 a) and Mato Grosso (Macedo, 1995). The largest areas occupied by the plant, however, are localized in the Eastern Brazilian Amazon, precisely in the estuary of the Amazon River, considered its center of origin, where native populations of up to 1,000,000 ha can be found (Calzavara, 1972).

In spite of its increasing cultivation in several Brazilian States, few diseases have been reported in the crop. So far, apparently only anthracnose, a disease caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., and two leaf spots caused by *Curvularia* sp. and *Drechslera* sp. have been cited. These diseases, however, in special anthracnose, assume economic importance only in the nursery phase, where losses up to 70% have been reported (Bovi et al., 1988; Oliveira et al., 2002).

A kind of disorder observed on the acai palm in the State of Para, probably of physiological origin, is the crack of the stem. It is characterized by the appearance of one or more longitudinal slits, up to 70 cm long, in the stipe. Although such symptom have not been linked to any specific disease, the slits can become gateways for weak pathogens or even saprophytes, that colonize wounded tissues causing decay and collapse of the stem.

A disease showing similar symptoms was observed in some farms of the municipalities of Ilheus and Camacan, Bahia State (Brazil), in 2015, causing, additionally, canker, ringing, rotting, bleeding, collapse of the stem and great plant mortality, especially during the fruit harvesting.

The aim of this work was to investigate, under field and laboratory conditions, the disease causal agent, which could become in a very short time, a matter of great economic importance in Southern Bahia and other producing areas, taking into account the increase of its acreage and the current disease severity.

## Materials and Methods

During the months of July and August, 2015, samples of infected tissues were collected in some farms of the municipalities of Ilheus and Camacan from açai palm plants showing symptoms of a disease, which, apparently, had not been described yet. The samples were brought to the Plant Pathology Laboratory, at the Cacao Research Center (CEPEC), in Ilheus, Bahia State, for the isolation, identification and tests of pathogenicity, aiming to investigate the disease etiology. The assays were carried out under laboratory and field conditions.

The pathogen isolation was carried out after the samples were washed under running tap water and the surface sterilized with 1% sodium hypochlorite. Small pieces of tissue taken from the transition zones between diseased and healthy tissues, as well as from areas showing symptoms of discoloration were sterilized superficially with 1% sodium hypochlorite, washed in sterile distilled water, dried on filter paper, plated on potato dextrose agar medium (PDA), and incubated in a BOD at 25° C. Five-millimeter-diameter mycelial disks taken from the advancing margins of 8 to 10-day-old colonies grown on PDA, using a sterilized cork borer, were transferred to the center of Petri dishes containing the same medium. After sealed with Parafilm® the agar plates were incubated at 25°C for the further assays.

Species belonging to the genera *Fusarium*, *Lasiodiplodia*, *Colletotrichum*, as well as, *Thielaviopsis* and its teleomorph, *Ceratocystis*, were consistently isolated from all the plants showing the disease symptoms. Among the isolated fungi, one species of *Thielaviopsis*, in most cases, and one of its teleomorph, *Ceratocystis*, were the most prevalent ones. This fact, along with the characteristic disease symptoms and the results of the direct examinations under a stereomicroscope Olympus SZ-CTV (Olympus Optical Co LTD., Tokyo, Japan) and a light microscope Leica DMLS (Leica Microsystems, Wetzlar, Germany), prompted us to use only this fungus in all the subsequent studies.

Attempting to identify the disease causal agent, preparations of the fungus fruiting bodies, taken from colonies grown on PDA or scraped from the sporulated plant materials, were mounted in drops of lactophenol, on glass slides, and examined under the Leica

microscope. The images of the morphological structures were taken with a Samsung digital color camera model CCD SAC-410ND (Samsung Electronics America Inc., NJ, USA) coupled to the light microscope and connected to a computer. The identification of the pathogen was based on morphological descriptions of the fungus (Morgan-Jones, 1967).

In inoculation trials, a total of ten 12-year-old açai plants, growing in CEPEC area were selected for inoculation with isolates of both species. Holes of 07-mm-diameter and 15 to 20-mm-deep were made on the stem with either a cork borer or an electrical drill at a height of 1.3 m above ground. Seven-millimeter-diameter mycelial discs taken from advancing margin of 8 to 10-day-old colonies growing on PDA medium, were inserted into each hole of five out of 10 açai palm plants. The remaining five plants, without inoculation, were kept as controls. The inoculation sites were then covered with a cotton ball soaked with sterile water fixed to the place with a polyethylene tape. Periodically, the inoculated plants were inspected to detect any kind of disease symptom. Samples were then collected from infected plants for further analysis and re-isolation of the pathogen on PDA.

## Results and Discussion

The açai palm plants from where the samples were taken in order to determine the causal agent of a disease observed in some farms of Ilheus and Camacan municipalities, showed a very characteristic and interesting symptomatology.

Initially, infected plants showed small dark spots on the bark, as result of the infection of mechanically wounded areas, small cracks or even galleries produced by insects on the stem (Figure 1). Despite no such symptoms were reported before in the crop, they are quite similar to the ones already described on other palm plants, especially, coconut (*Cocos nucifera* L.), in several countries, including Brazil (Garofalo & McMillan, 2004; Warwick & Passos, 2009; Elliott, 2015).

With the progress of the disease some symptoms apparently not previously reported on any other host of the Areaceae family, such as canker, ringing (constriction) associated to small cracks (Figures 2a, b, c, f), in addition to bleeding of a reddish brown liquid running down the trunk from the infected areas were



Figure 1. Symptoms of stem bleeding and ringing disease of acai palm (*Euterpe oleracea* Mart.): premature death of the lowest leaves, which remain hanging down in the canopy (a); dark spots on the stem bark as result of wounds infection, small cracks (b, c, d), or galleries made by insects (arrows) (e, f).



Figure 2. Symptoms of stem bleeding and ringing disease of açai palm (*Euterpe oleracea* Mart.): canker (a, b), ringing associated to small cracks (a, b, c) and bleeding of a reddish brown liquid running down the stem (c, f). Cross sections of naturally infected plants (d, e), longitudinal sections (g, h) of inoculated plants showing decaying (g) and symptoms of discoloration (h).

consistently observed (Figure 2c). With the evolution of the disease symptoms, collapse of infected plants was observed, particularly, during bunch harvesting. The fall of the trees was caused by the bending and breakage of the stem, especially at the constriction points, as a consequence of the weight of field workers.

After the bark removal to expose underlying tissues in stem longitudinal sections, additionally to decaying of localized areas (Figure 2g), a reddish brown discoloration symptom on infected tissues was observed (Figure 2h). Such symptoms were not restricted to inoculation points, but spread around in every direction, especially, up and down of the stem (Figures 2g, h). Furthermore, in stipe cross sections, besides decaying of localized areas (Figure 2d), the discoloration symptoms were also present in infected tissue, especially, in regions of higher vascularization (Figure 2e). A few days after the infected plant materials had been taken to the plant pathology laboratory, a massive fungus sporulation was observed when exposed to humid chamber, attested by a dark stain on the infected tissues.

Although, *T. paradoxa* (De Seynes) Hohn was the predominantly isolated fungus from all the infected acai plants collected at the different sites, its teleomorph, *C. paradoxa* (Dade) C. Moreau, was also present in the isolations. Single-spore derived cultures were deposited in a Phytopathogenic fungi Collection of CEPEC. The fungus identification was based not only on examination of morphological structures developed on PDA medium, but also on those structures scraped from infected tissues, by using appropriated fungi descriptions (Morgan-Jones, 1967).

According to Elliott (2015) while there are no reliable symptoms to predict which palms are infected with *C. paradoxa* (= *T. paradoxa*) and which ones are not, two symptoms that might be observed include the lowest leaves dying prematurely and hanging down from the canopy and stem bleeding. In the disease of the acai palm reported here, besides these two symptoms, a very important and diagnostic one has to be considered: the ringing (constrictions) of the stem associated to small cracks, frequently, observed in naturally infected plants (Figures 1, 2).

The predominantly isolated fungus showed white colonies on PDA medium, becoming black two to three days later (Figure 3a), and matched very well with morphological descriptions of *C. paradoxa* (Morgan-

Jones, 1967). The fungus produce perithecia, partly or completely immersed, dark brown to black, globose, with long neck, black, pale brown towards the tip, tapering, ostiolar hyaline hyphae, erect or moderately divergent (Figures 3b, c). Ellipsoid, often with unequally side curved, hyaline, non-septate, smooth, 6-10 x 2-3 µm ascospores (Figures 3c, d, e). Slender conidiophores arising laterally from the hyphae, septated, phialidic, hyaline to very pale brown, tapering towards the tip and producing a succession of conidia through the open end. Conidia cylindrical to somewhat oval when mature, hyaline to mid-brown, smooth-walled, 6.5-10 x 3-5 µm (Figures 3c, d, e). Aleuroconidia terminal, in chains, obovate to oval, thick-walled, brown, 9-24 x 6-9 µm (Figure 3f).

The method used to inoculate the 12-years-old acai palm plants with isolates of the both phases of the pathogen was efficient in reproduce of all the disease symptoms (Figures 2 g, h). No difference in terms of symptomatology was observed when the plants were inoculated with isolates of the anamorph and teleomorph. Both forms were also re-isolated in PDA medium from tissues taken from the advancing margins of lesions, and also from those showing symptoms of discoloration. Although, *C. paradoxa* (= *T. paradoxa*) have already been reported on several other palm species around the world (Garofalo & McMillian, 2004), this, apparently, is the first report of this fungus causing disease on acai palm, not only in Brazil, but also, worldwide.

*Ceratocystis paradoxa* appears to only be able to infect a palm if a fresh wound is present. Likewise, diseases caused by this fungus may progress more rapidly if the palm is under stress. On other palm species, most infections occur in non-lignified or lightly lignified tissue. Since most lignified fibers are in the lower trunk, the disease is most frequently observed in the upper third of the stem (Elliott, 2015).

*Ceratocystis paradoxa* is a soil-borne fungus that occurs, naturally, in Southern Bahia, not only on acai palm, but also, on some other palm species, such as coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Lacq.) (Oliveira, M.L. data not published). The fungus can be spread by wind, insects, by man and probably in storm water. It enters the host through wounds, causing disintegration of the stem and/or bud, and root rot. The fungus can also enter through the spear-leaf, young leaf bases, inflorescence, mechanical damage, growth cracks, and leaf pruning cuts (Garofalo & McMillian, 2004).

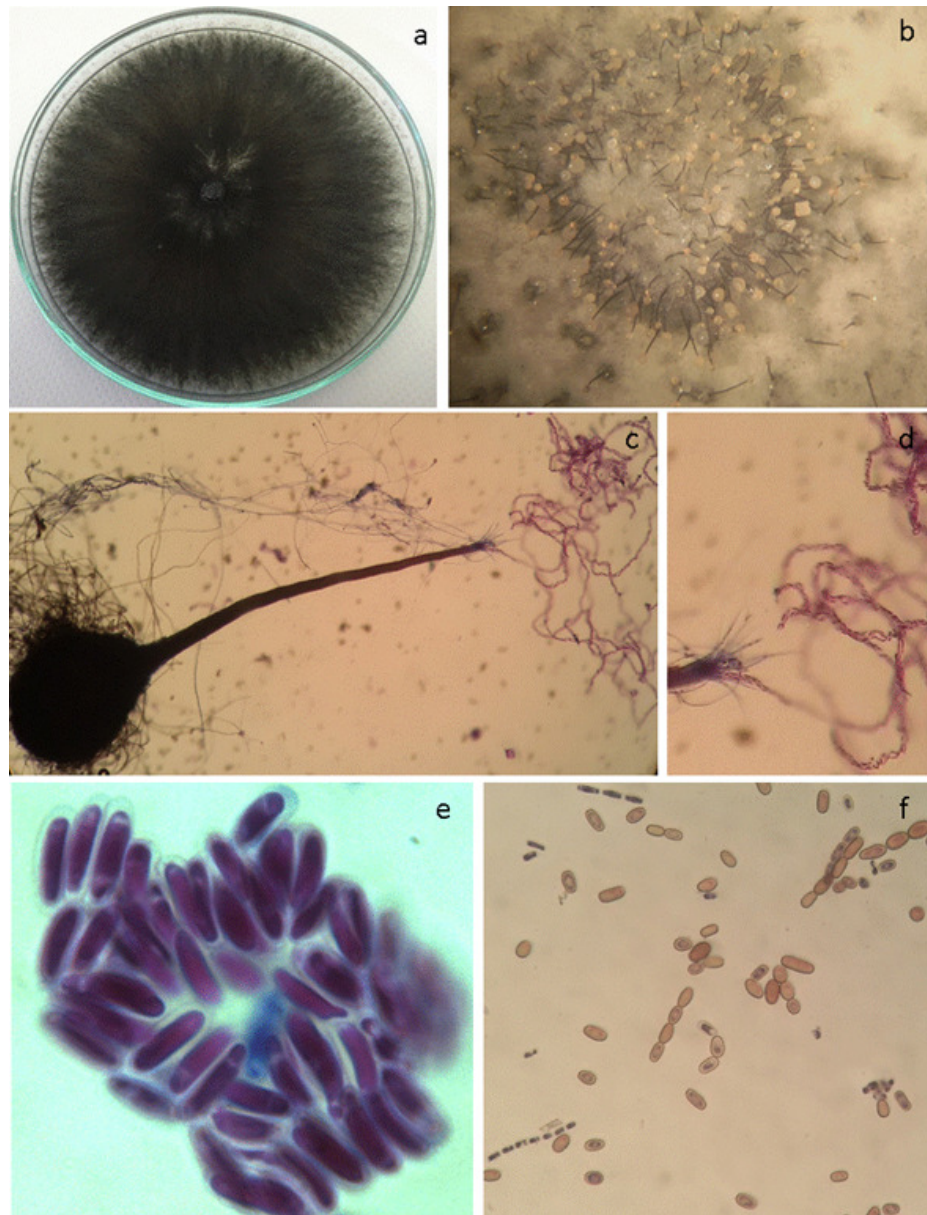


Figure 3. Aspects of a black colony of *Ceratocystis paradoxa* (= *Thielaviopsis paradoxa*) (a) and massive production of perithecia in PDA medium (b). Light micrographs of a black and globose perithecium showing ascospores emerging from its long neck with ostiolar hyphae (c, d); details of the ellipsoid, hyaline, non-septate, often with side unequally curved ascospores (e). Two types of conidia produced by *T. paradoxa* in PDA medium: thin-walled hyaline conidia and darkly pigmented aleuroconidia both produced in chains (f).

While *C. paradoxa* can be found throughout the world, its host range is primarily restricted to monocot plants grown in warm climates. Although, it was not registered on every palm species, all of them are considered potential hosts (Elliott, 2015). The fungus was already reported causing stem bleeding (Nambiar et al., 1986; Warwick & Passos, 2009; Freire &

Martins, 2010) and fruit basal rot on coconut under field conditions (Rossetti, 1955; Camargo & Gimenes-Fernandes, 1997; Tzeng & Sun, 2009), and also external (Tzeng & Sun, 2009) and internal post-harvest rot diseases on coconut (Pinho et al., 2013). The pathogen can also causes post-harvest diseases in banana (*Musa* spp.), carambola (*Averrhoa*

*carambola* L.), guava (*Psidium guajava* L.), pineapple (*Ananas comosus* (L.) Merr.) (Reyes et al. 1998; Junqueira et al., 2001; Ploetz, 2003), stem rot in *Dracena marginata* (Santos et al., 2012), besides infecting some hardwoods, and several herbaceous ornamentals (Garofalo & McMillan, 2004).

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