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Local infection associated with vascular bundles: the colonization of coconut palm leaflets by two *Camarotella* species

Kelly Lana Araújo · Silvaldo Felipe da Silveira · Vicente Mussi-Dias · Juan Manuel Anda Rocabado · Flavio Costa Miguens

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Abstract Two *Camarotella* (Phyllachoraceae) species, *C. torrendiella* and *C. acrocomiae* are the causative agents of small (SV) and large verrucosis (LV), respectively, which are important diseases affecting Brazilian coconut palms. The small verrucosis produces necrotic lesions in coconut palm leaflets, whereas LV just produces chlorosis. Semi-thin sections of asymptomatic leaflets and of leaves presenting stromata in different development stages were compared through light microscopy in order to characterize the colonization process of these two coconut palm verrucosis agents. *Camarotella torrendiella* initially colonized the adaxial epidermal cells and the cells underlying the epidermis close to the vascular bundles. In latter colonization stages, the hyphae of *C. torrendiella* remained limited to the underlying necrotic tissue adjacent to the mature stromata, mostly in the intracellular spaces and in the collapsing cells of necrotized tissues around the vascular bundles. This species does not colonize intracellular intact fiber cells, xylem vessels or phloem sieve tube elements. In contrast, *C. acrocomiae* presented a typical biotrophic parasitism model such as that of some gramineous *Phyllachora* spp. High densities of *C. acrocomiae* hyphae were found inside intact sieve tube elements; however, with no evidence of cellular death. The extensive hyphal colonization by *C. acrocomiae* within sieve tube elements was also observed in tertiary and quaternary bundles, as well as in anastomosing vascular bundles. The dependence of both species on the colonization sites associated with vascular bundles indicates the need for additional studies about these intricate host-pathogen relationships. These studies could be important to define new strategies to control coconut palm verrucosis diseases.

Keywords *Cocos nucifera* · Small verrucosis · Large verrucosis · *Camarotella torrendiella* · *Camarotella acrocomiae* · Histopathology

Introduction

The Phyllachoraceae (Phyllachorales, Ascomycota) encompasses a large diversity of fungi, including important airborne plant pathogens. The genus *Phyllachora* and some related species are biotrophic foliar fungal pathogens that feed on the hosts' living cells. These fungi cause "tar spot", which is a disease of little economic importance in most of its natural hosts, in dicotyledonous and monocotyledonous plants (Silva-Hanlim and Hanlim 1998). However, two tropical species of *Camarotella*, a Phyllachora-related genus, are found in almost all regions where coconut palms are cultivated in

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K. L. Araújo · S. F. da Silveira (✉) · V. Mussi-Dias · J. M. A. Rocabado
Laboratório de Entomologia e Fitopatologia, Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, Av. Alberto Lamego, 2000, Parque Califórnia, CEP 28013-602 Campos dos Goytacazes, Rio de Janeiro State, Brazil
e-mail: silvaldo@uenf.br

F. C. Miguens
Laboratório de Biologia Celular e Tecidual, Centro de Biologia e Biociências, UENF, Rio de Janeiro, Brazil

Brazil. These species cause small [*Camarotella torrendiella* (Bat.) Bezerra & Vitória] and large verrucosis [*Camarotella acrocomiae* (Mont.) Hyde & Cannon], which are in certain ways similar to the "tar spot" disease (Subileau et al. 1993; Hyde and Cannon 1999; Vitória et al. 2008; Warwick and Leal 2003). The LV and SV diseases are easily distinguished from each other through their symptoms, since they differ in the location, appearance of the lesions and in the reproductive structures (disease signals) of the pathogen, which present distinct fruiting body and ascospore morphologies (Vitória et al. 2008). The SV is more harmful than the LV, since it induces extensive necrosis in coconut palm leaflets, whereas LV induces occasional leaf chlorosis in the lateral limbs of the plant. The 5 to 7 cm long diamond-shaped SV lesions typically develop in the midrib of leaves, which are initially chlorotic and later become necrotic. The damage caused by SV can be observed in the large number of coalescing necrotic lesions in the leaflets; it culminates in the collapse of the entire leaf (Warwick and Abakerli 2001). Severely attacked coconut palms present premature defoliation, and their normal fruit development is compromised, they also present the physical and physiological weakening of their fruit bunches. The small verrucosis is considered the most damaging foliar disease in coconut palm crops in the Southeastern coastal region of Brazil, mainly in the South of Bahia State (Bezerra 1989), in the Northeast of Rio de Janeiro and Espírito Santo States (Monteiro et al. 2013). Verrucosis in coconut palms has also been reported in Cuba, Chile, French Guiana and Paraguay (Hyde and Cannon 1999).

The name "verrucosis" derives from the superficial and abundant salient black stromata produced by the fungi in the infection spots. *Camarotella* spp. are characterized by the formation of black, pulvinate, carbonaceous, erumpent or salient and rough stromata. A central cavity containing curved filiform cells emerges at the beginning of the stromata development. As the biological function of the curved filiform cells produced inside the initial stromata (=pycnidial) cavity was not scientifically proved yet, it is impossible saying whether they are "pycniospores" or "spermatia". The second hypothesis (spermatia) seems to be the most accepted one, because these cells do not germinate or infect coconut palm leaflets (Vitória et al. 2008). The sexual stage is characterized by the upper formation of perithecia submerged in salient stromatal tissue. These perithecia have a uniform basal layer of unitunicate asci intermingled with hyaline filiform paraphyses. *Camarotella torrendiella* stroma is smaller (less than 1 mm in diameter), firmly attached to the leaf surface and black. The ascospores are unicellular, hyaline, monostic to distic, subfusoid to ellipsoid; they have sub-acute poles and are involved in a mucilaginous matrix. The *C. acrocomiae* stroma is larger (approximately 2 mm diameter) than other species and loosely attached to the surface of the leaf. It is greenish-gray to black and mostly produced at the edge of the leaflet's limbs. The unicellular ascospores of *C. acrocomiae* are also involved by a mucilaginous matrix, but they differ from *C. torrendiella* ascospores due to their darker color (from light to dark-brown), distic-ellipsoid shape, and obtuse extremities (Vitória et al. 2008; Souza et al. 2008; Hyde and Cannon 1999; Subileau et al. 1993).

Most of the pathological studies about Phyllachoraceae have focused on *Phyllachora* spp., which show distinct host colonization models between species (Gabel 1989; Luttrell 1981). According to Luttrell (1981), the 'tar spot' is a "tissue-replacement disease", because the phytopathogenic fungi replace the host organ by their somatic or reproductive structures. Orton (1924) (cited by Luttrell 1981) states that such tissue replacement is the "most striking physiological characteristic of this parasite, since the tissue change does not cause any external evidence of necrosis in the host". The LV and SV symptoms in coconut palms resemble that of 'tar spot', but there are no detailed pathological studies about these pathosystems, so far. The signal and symptom differences between LV and SV and their relation with "tar spot" diseases caused by *Phyllachora* deserve attention due to the colonization and parasitism processes. Additionally, the restricted regions where verrucosis diseases occur in *C. nucifera*, which is a non-native species in South America, suggest that the pathogens have recently adapted to this "new" host. The aim of the current study is to characterize and compare the colonization processes, as well as the structural changes induced by the two *Camarotella* spp. in coconut palm leaflet tissues.

Materials and methods

Leaflets presenting symptoms and signals of small (SV) and large verrucosis (LV) were collected from fourteen-year-old coconut palms (variety Brazilian Green Dwarf of Jiqui) cultivated in the experimental area of Antônio Sarlo Agricultural School (21°42′59″ S and 41°20′33″ W), in the Campos dos Goytacazes County, Rio de Janeiro State, Brazil. The current study used leaf samples from field plants exposed to natural inoculation, since there are no methods of cultivation and reproduction in vitro for the pathogens. Since it was not possible to precisely determine the age of the analyzed lesions and structures based on the inoculation data, the sampling was focused on the leaflets containing symptoms and signs of diseases at different stages. The samples were collected from 5 randomly chosen plants. Six (two opposite) leaflets from each plant were removed from the mid third of the leaves number 10, 15, and 18 or 20 (counted from the first apical leaf). The leaves were accessed with ladder aid in the same canopy sector (axial direction) and represented vertically the upper, middle and lower portion of the palm canopy, respectively. None of the youngest leaves (leaf number 9 or previous) showed symptoms of SV and LV. As some plants had green leaves less than twenty, these samples were taken from the leaf number 18 (which is the closest to leaf 20 in the same palm tree canopy sector). Asymptomatic coconut palm leaflets were also collected and analyzed for comparison purposes and anatomical characterization (Araújo et al. 2013). Leaflet fragments presenting SV and LV symptoms and signals were selected and classified according to the size and appearance of the stromata, as well as to the developmental stages of the lesions: (1) Stromata in initial formation showing dark spots smaller than 0.5 mm diameter; (2) small protruding stromata, 0.5 mm diameter, showing signs of initial lesions without ascospores (= pycnidial or spermogonal phase), (3) salient and mature stromata (with asci and ascospores) in young lesions without chlorotic rings or necrosis around them; (4) protruding and mature stromata containing mature ascospores in advanced lesions surrounded by chlorosis (LV) or necrosis (SV). Samples showing advanced lesions and dry or empty stromata (without ascospores) were excluded from the histopathological analyses.

Digital photos from all enumerated samples were taken to associate the observed symptoms and the developmental stages of external fungal structures (stromata) with the colonization process and the histological changes occurring inside the host's tissue. In addition, images from advanced LV and SV stage symptoms were also included in the study to illustrate the final and relative damage resulting from the colonization of these pathogens in coconut leaflets. We characterized the leaflet anatomy of green dwarf coconut leaves using sample fragments presenting no initial symptoms. These results were separately described in another article in order to help further understanding the colonization process of these pathogens (Araújo et al. 2013).

The leaflet fragments were fixed using microcentrifuge tubes (200 μL) containing 2.5% glutaraldehyde fixing aqueous solution (*v*/v) and 4.0% formaldehyde (*v*/v) diluted in Pipes buffer at 1% (*v*/v) for microscopy studies. These containers were stored in refrigerator at 4 °C up to the time the samples were prepared for light microscopy. The fixed material was washed three times in Pipes buffer at 1% (*v*/v), for 1 h, for histological preparation, and dehydrated in ascending aqueous ethanol series: 30%, 50%, 70%, 90% and 100% (1 h at each concentration). The fragments were progressively subjected to infiltration in ascending series of Unicryl® resin (25%, 50%, 75% and 100%) diluted in 100% ethanol, after dehydration. The fragments were kept in each stage for 24 h and placed in oven at 60 °C for 12 h, after their inclusion in pure resin, in order to polymerize the resin. Semi-thin sections (0.5 μm width) were obtained using an ultra-microtome (Reichet Ultracut S™) equipped with a diamond knife. These sections were mounted on a microscope slide, stained in 1% aqueous Toluidine Blue solution +0.1% boron sulfate and observed under light microscope. The images were obtained using an optical Axioplan Zeiss microscope, equipped with ZVS – Zeiss camera, connected to the AnalySIS™/Soft Imaging System.

Results

Leaflets showing symptoms of disease in the early stages of development (lesion stages 1 and 2) were hardly find even in the leaf 10. Leaflets presenting intermediated development stages 2 and 3 were mostly obtained from leaves number 15. Developed injuries and mature ascomata (with mature ascospores) were found mostly in the leaf 18 (lesion development stage 4). In the leaf 20 many ascomata were already dry or empty at this collection date. So, for SV and LV histopathology only a few (5 to 10) leaf fragments containing each of the representative lesion development stages were analyzed by light microscopy.

The invading hyphae remained filamentous in the mesophyll of the coconut leaflets during all the studied colonization phases of both *Camarotella* species. Masses of prosenquimatous to pseudo-parenchymatous cells have gathered in the upper stromata tissues, from the epidermal level to the periphery (Figs. 1a–c, 2a–b, d, and 3a–c).

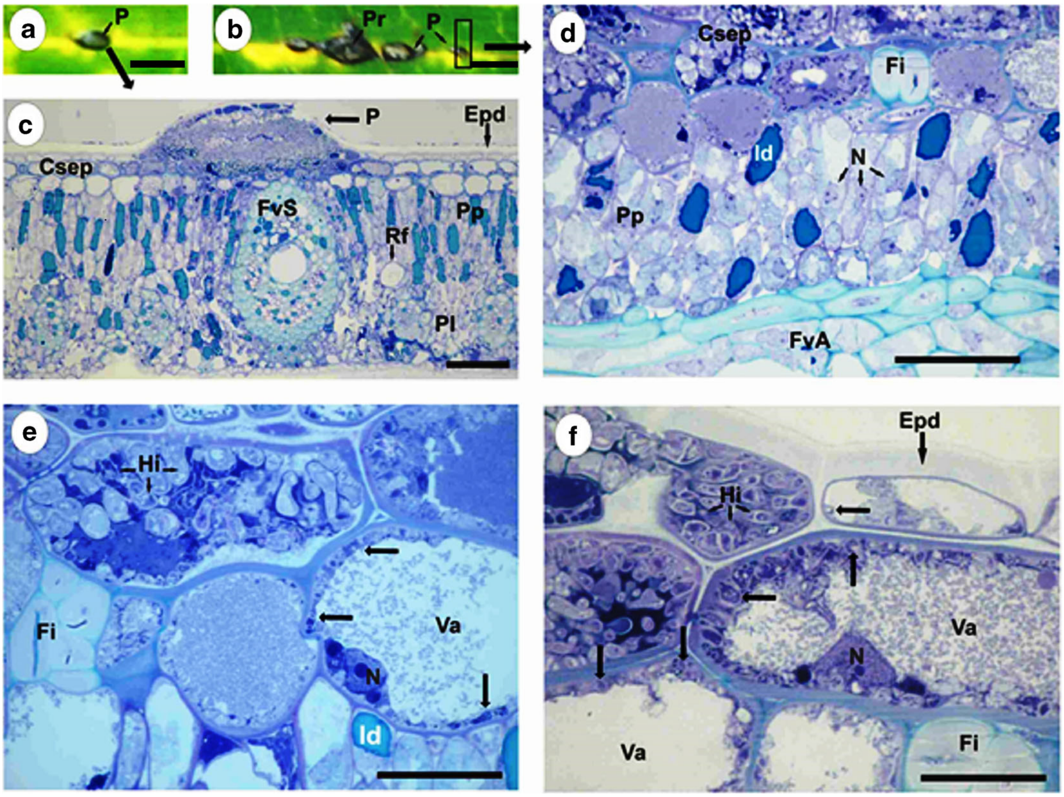
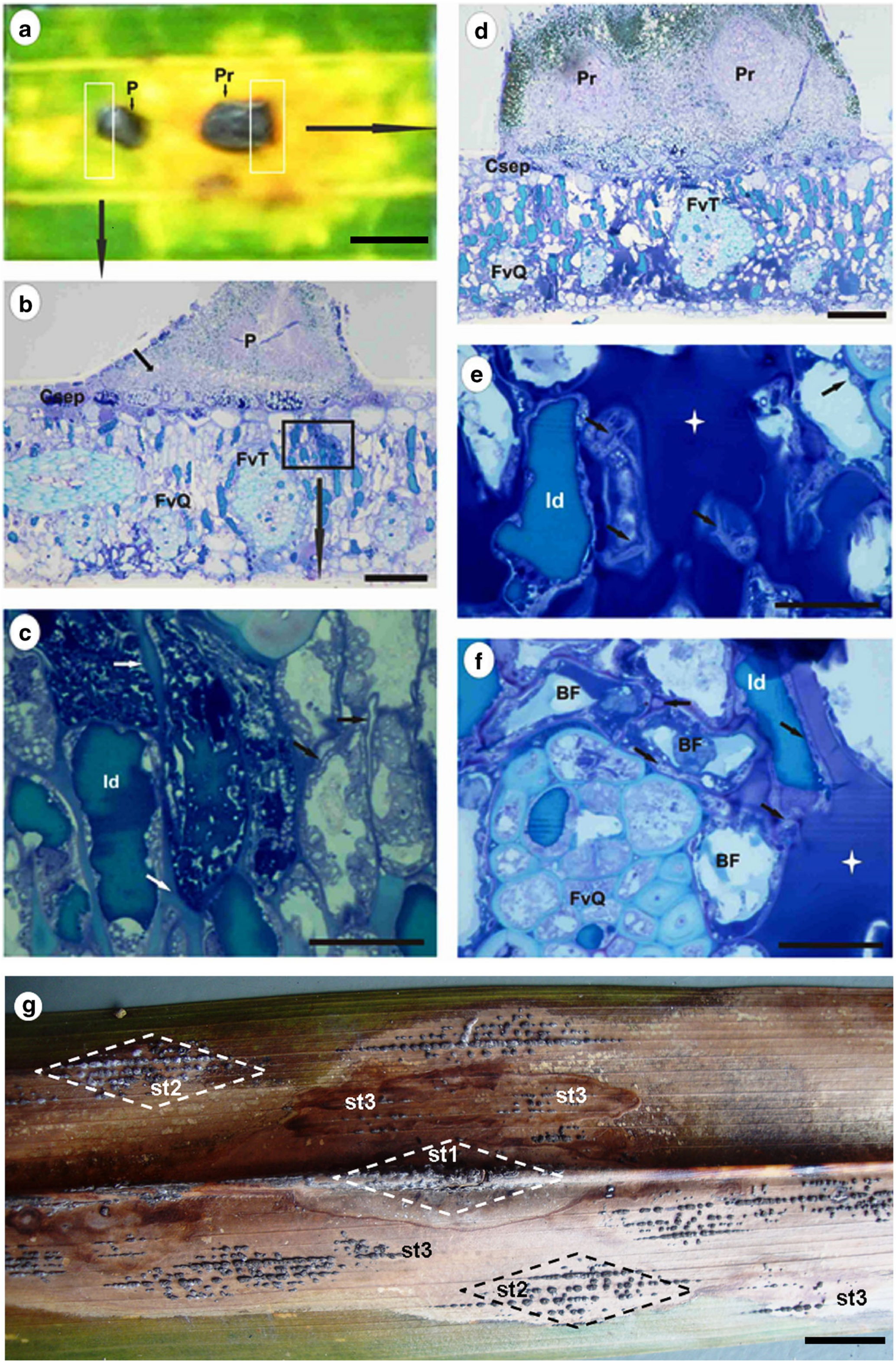


Fig. 1 (**a**, **b**) Coconut palm leaflet fragments presenting initial symptoms of small verrucosis (SV) and young stromata (bar =1 mm) and (**c** – **f**) transversal sections of these fragments in OM. (**c**) Transversal section showing the presence of pycnidia and mycelial growth in epidermal cells and in cells subjacent to the adaxial epidermis (bar =100 µm). (**d**) Detail of mesophyll cells subjacent to the pycnidia (bar =50 µm). (**e**, **f**) Epidermal and subepidermal cells showing initial colonization by hyphae (*arrows*) (bar =20 µm). Abbreviations: P – pycnidia; Pr – perithecia; Epd – adaxial epidermis; Csep – cells subjacent to the adaxial epidermis; FvS – secondary vascular bundle; PP – palisade parenchyma; Pl – lacunary parenchyma; Rf – raphide; Hy – Hyphae; Fi – Fibers; Va – vacuole; Id – tannic idioblast; N – core; FvA – anastomosing vascular bundle

Pycnidia and perithecia formation was often associated with the colonization of subjacent vascular bundles and of their adjacent tissues by both *Camarotella* species (Figs. 1c, 2b–c, and 3d). The vascular bundle colonization and its association with the development of upper and exterior stromata is easily seen in advanced symptomatic leaflets. Generally, the SV and LV stromata formation is often aligned to and above the primary and secondary leaf veins, respectively (Figs. 2g and 3c).

The first sign of SV in coconut leaflets (development stage 1) is characterized by pycnidia formation on the adaxial surface of leaflets (Fig. 1a, b). At this point, *C. torrendiella* colonizing hyphae were evident in the epidermal cells and in cells subjacent to the adaxial epidermis (Fig. 1c, d). Hyphae were not observed in palisade and lacunary parenchyma cells subjacent and adjacent to the pycnidia; these tissues did not show cellular disarray, their cells presented regular vesicles and core, as well as intact chloroplasts (Fig. 1d). The colonized cells presented unorganized formation

Fig. 2 (**a**) Coconut tree leaflet fragments presenting symptoms of ▶ small verrucosis (SV) and stromata (bars =1 mm) and (**b** – **f**) transversal sections of this fragment in OM. (**b**) Transversal section of leaflets showing the presence of pycnidia and the beginning of perithecia formation (*arrow*) (bar =100 µm). (**c**) Hyphae (*black arrows*) in cells of palisade parenchyma. Deposition of materials in the intercellular spaces (*white arrows*) (bar =20 µm). (**d**) Overview of a colonized mesophyll, demonstrating totally destroyed cells during perithecia development (bar =100 µm). (**e, f**) Hyphae (*arrows*) found in (**e**) lacunar parenchyma cells and in cells of (**f**) bundle sheathes. Material strongly stained in toluidine blue in the extracellular spaces (stars) (bar =20 µm). (**g**) Portion of a leaflet showing SV symptoms: extensive necrosis caused by primary (st1), secondary (st2) or tertiary (st3) SV lesions, containing typical diamond shape stromata groups (st) of *C. torrendiella*. Abbreviations: P – pycnidia; Pr – perithecia; Csep – cells subjacent to the adaxial epidermis; Id – tannic idioblast; FvT – tertiary vascular bundle; FvQ – quaternary vascular bundle; BF – cells of the bundle sheathes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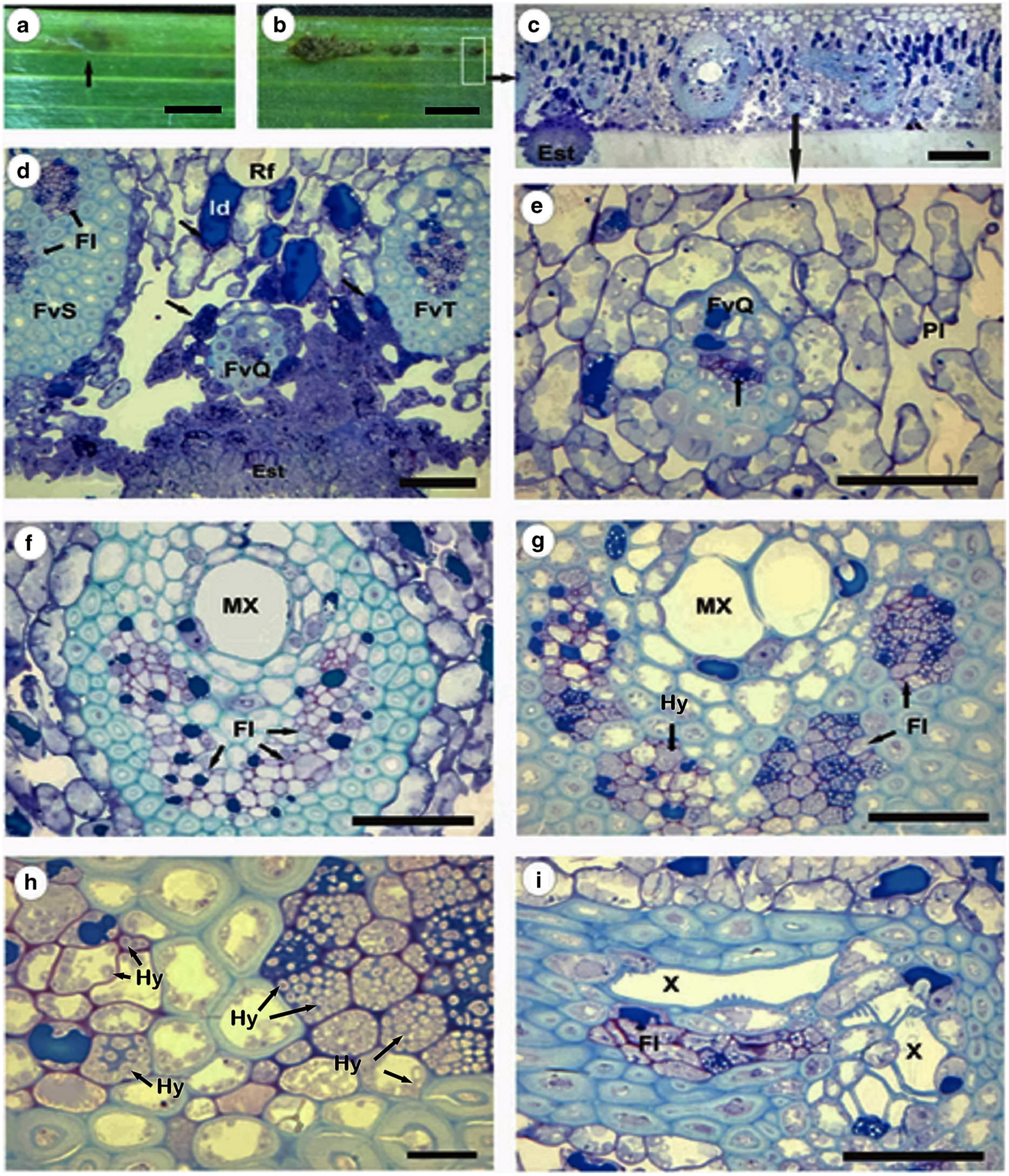

subjacent to the adaxial epidermis, without cellular content and with intracellular spaces totally filled by fungal hyphae (Fig. 1e, f).

The initial perithecial development (stage 2) occurred in the pycnidia basal tissues (Fig. 2b). Epidermis disruption was followed by the colonization of the subjacent palisade parenchyma tissue around the vascular bundles at this stage (Fig. 2b–f). Drastic changes were initially detected in palisade parenchyma cells subjacent to the stromata

◀ **Fig. 3** (**a, b**) Fragments of coconut leaflets with large verrucosis (LV) symptoms and young stromata (bars =1 mm). (**a**) Adaxial region showing no necrotic symptoms or yellowish color around the stromata (*arrow*). (**b**) Abaxial region of the same fragment. (**c** – **i**) Tranversal sections of the same fragment in OM. (**c**) Overview of the mesophyll showing no deposition of materials strongly stained in toluidine blue. (**d**) Hyphae (*arrows*) of *C. acrocomiae* in mesophyll cells and in vascular bundles (bar =50 μm). (**e**) Hyphae (*arrow*) of *C. acrocomiae* in a vascular bundle adjacent to the large verrucose stromata (bar =50 μm). (**f**) Detail of a secondary vascular bundle of an asymptomatic leaflet (bar =20 μm). (**g**) Overview (bar =10 μm) and (**h**) detail of colonized phloem cells with hyphae inside the sieve tube elements (bar =50 μm). (**i**) Details of an anastomosing vascular bundle showing the presence of hyphae (*arrows*) in the phloem (bar =50 μm). Abbreviations: Est – stromata; FVS – secondary vascular bundle; FvT – tertiary vascular bundle; Pl – lacunary parenchyma; Rf – raphide Hy – hyphae; Fi – fibers; Co – chollenchyma; Id – tannic idioblast; X – xylem; Fl – phloem; MX – metaxylem; FvQ – quaternary vascular bundle; Rf – raphide; Est – stromata

(Fig. 2b–f), they were characterized by cellular disarray and by the accumulation of material stained with toluidine blue in the extra-cellular spaces (Fig. 2c–f). Hyphae were found in the palisade parenchyma in the collapsing region, mainly in intracellular spaces (Fig. 2c). All the intercellular spaces subjacent and adjacent to the stromata around the vascular bundle were densely stained (Fig. 2e) in more advanced SV stromata development stages (3 and 4); it happened alongside to perithecia formation (Fig. 2d). High hyphal concentrations in the collapsing region, mainly in the intracellular spaces (Fig. 2e, f) were observed. Many of the mesophyll cells in the collapsing regions showed completely destroyed cellular wall. This cellular disarray model matched the initial macroscopic necrosis symptoms in leaflet tissues around the stromata, and it characterizes SV symptoms. Pathogen hyphae were also seen inside the tannic idioblasts (Fig. 2c, e, and f).

The initial large verrucosis (LV) in coconut palm leaflets (stage 1) was also characterized by stromatal pycnidia formation (Fig. 3a–b), and the LV stromatal pycnidia started forming in subjacent epidermic cells. The pycnidia were also found in many perithecia development stages (stage 2 and 3); however, both structures were observed on the leaflets' adaxial and abaxial surfaces in LV lesions. Occasionally, there were stromata on both sides of the same leaflet spot-point.

The analyses of different leaflet transversal sections infected with *C. acrocomiae* showed that this species colonizes the host tissues, as it was done by *C. torrendiella*, which is associated to vascular bundle sites. However, there were some differences in the cell colonization model (parasitic strategy) (Figs. 3 and 4). *Camarotella acrocomiae* intracellular hyphae were seen in epidermal and subjacent cells, as well as in palisade and lacunary parenchyma cells, even at the initial pycnidial phase - stage 1 (Fig. 3c–d). Colonization was inter and intracellular, outside the vascular bundle, but it was restricted to a few cells subjacent to the stromata on symptom stage 2 (Fig. 3d). No hyphae were identified in mesophyll cells surrounding the vascular bundles located away from the stromata (Fig. 3e). The hyphae of the fungus were found in areas subjacent (Fig. 3d) and adjacent to the stromata in vascular bundles (Fig. 3e) on symptom development stages 3 and 4. *Camarotella acrocomiae* hyphae in vascular bundles were mainly intracellular in sieve tube elements of phloem and companion cells (Fig. 3e-i). As it was previously described, the secondary vascular bundles of coconut palm leaflets were characterized by the presence of phloem divided into four strata (Fig. 3f) (Araújo et al. 2013). The presence of *C. acrocomiae* hyphae in all of the phloem strata was verified in sieve tube elements and in companion cells, without any cellular death reaction or tissue disruption/disorganization (Fig. 3g-i), even on final symptoms in development stage 4. Hyphae were also found in phloem-parenchyma and collenchyma cells, and in the sclerenchymatic sheath, although in a discrete manner. A completely colonized phloem strata, eventually in the same vascular bundle was observed, besides other strata presenting initial and final colonization stages (Fig. 3h). Intense mycelial growth was also observed in tertiary and quaternary bundles as well as in the anastomosing vascular bundles (Fig. 3i). No hyphae were observed in the xylem of any of the analyzed LV fragments.

The analyses of leaflet longitudinal sections around the LV stromata corroborated the assumption that phloem cells had an unorganized formation, without cellular content and with all intracellular spaces filled with *C. acrocomiae* hyphae (Fig. 4a, b). The deposition of materials in the intercellular spaces and, consequently, tissue necrosis, were not seen in any of the analyzed LV fragments. So, there were no tissue reactions or material accumulation in the advanced LV lesions, even in the blighted and dry leaflets; thus it differed from the LP lesions (Fig. 4c).

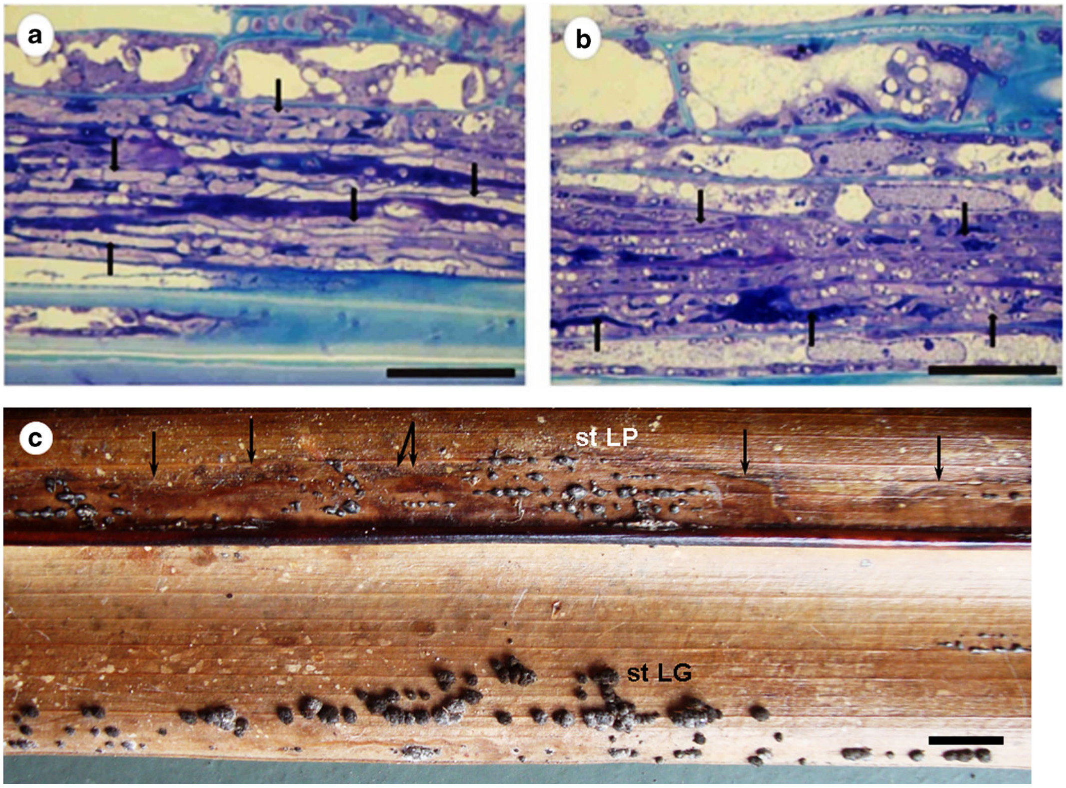

Fig. 4 (**a**, **b**) Longitudinal sections of a vascular bundle showing the *C. acrocomiae* hyphae (*arrows*) in phloem cells (bar =20 μm). (**c**) Portion of a blighted (senescent) leaflet of coconut palm showing above the leaflet midrib extensive necrosis of small verrucosis with stromata groups (st SV) of *C. torrendiella* (bar =10 mm). The limits of the primary necrotic caused by SV lesions, the blighted (dried and not colonized) tissues are highlighted (*arrows*). Below the leaflet midrib of the same leaflet there are large verrucosis stromata groups (st LV) of *C. acrocomiae* over blighted (dried) areas and no evidence of primary necrosis, or any reaction or discoloration of host tissues

Discussion

The symptoms and signs of coconut palm verrucosis and the similarity of the morphological reproductive structure between the two herein studied *Camarotella* species reinforce their close genetic relatedness (Vitória 2007; Vitória et al. 2008). However, according to the present study, the different symptoms found through the comparison between SV and LV can be explained by the divergent colonization strategies adopted by each of these pathogens.

Species belonging to genus *Phyllachora* are considered biotrophic fungi (Cannon 1991; 1996); however, many characteristics related with necrotrophism were found during the colonization process by *C. torrendiella*. Mendgen and Hahn (2002) have cited cell wall degradation, intense inter and intracellular mycelial growth and tissue necrosis in the host, among these characteristics. Hyphae were seen only in collapsing regions of leaflet fragments with SV. The first symptom of tissue necrosis in the host matches this cellular disarray. Additionally, the SV pathogen causes severe necrosis and shows extensive stromata colonization along the blighted areas of the leaflets. Necrotic symptoms have also been described for *phyllachoraceus* fungi and *Phyllachora maydis* Maubl. infection in corn (Hock et al. 1995), and for *Ophiodotella annonae* Bezerra, Freire & Andrade infecting *Annona squamosa* L. (Bezerra et al. 2006). According to Chalkley (2014) the apparent necrotrophic activity of *P. maydis* is unusual in fungi that require living plant cells to survive and it suggests that these fungi are not fully adapted to their host. The same concept can be applied to explain the *Camarotella torrendiella* pathogenesis in coconut palms, which is an exotic palm tree species in the South American continent.

The analyses of many coconut palm leaflet sections with SV stromata in different development stages have shown that the *C. torrendiella* colonization process was divided in two stages. In the first stage, the fungal mycelial growth is concentrated in epidermal cells and in cells subjacent to the adaxial epidermis. The second

C. torrendiella colonization stage was characterized by high cellular disarray caused by densely colored material accumulation in intercellular spaces (strongly stained with toluidine blue) and by the increased hyphal concentration in the inter- and intracellular spaces. The densely stained material accumulating in the intercellular spaces during the second *C. torrendiella* colonization stage was a remarkable characteristic of the collapsing tissues. However, it was difficult to conclude whether this accumulation was the reason for (before) or the consequence (after) of cellular collapse. Nevertheless, the second possibility is the most likely explanation. The accumulation of substances in many plant/pathogen interactions, mainly of phenolic compounds, is a plant reaction to limit or delay the colonization process in infected tissues (Rodrigues et al. 2003; Hong et al. 2001). Idioblasts containing phenolic compounds were frequently observed in palisade and lacunary parenchyma cells, as well as in vascular bundles of coconut palm leaflets (Araújo et al. 2013). The phenolic and tannic compounds are associated with defense mechanisms against herbivores and against the pathogenic infection in vascular plants. However, the presence of stained material in the analyzed coconut palm leaflets was not an effective barrier against *C. torrendiella* colonization in palisade and lacunary parenchyma cells, or even in idioblasts.

Different from *C. torrendiella*, *C. acrocomiae* colonization was mainly seen in phloem cells of vascular bundles subjacent and adjacent to the stromata. Probably, *C. acrocomiae* reaches vascular bundles located away from the LV stromata through the anastomosing vascular bundles. Anastomosing bundles link parallel vascular bundles in monocots (Esau 1977) and in coconut palms (Araújo et al. 2013). Fungal hyphae were observed also in epidermal and parenchyma cells subjacent to the LV stromata outside the phloem. The pattern of compatible intracellular colonization inside vascular bundle cells, including intact sieve tube elements of phloem, has already been described for *Phyllachora* species in grasses (Gabel 1989; Parbery 1963). According to Gabel (1989), the entire mesophyll is quickly invaded and colonized by perithecia and stromata development in warm-season grasses infected by *Phyllachora*. Such colonization causes tissue disruption and compression, as well as vascular bundle relocation. Additionally, Gabel (1989) concluded that these fungi are more destructive to warm-season grasses because of the complete vascular bundle colonization. It is of common sense in plant pathological studies that the hyphae growth inside the hosts' living cells causes cell death (necrosis), as previously described for many pathogens, such as *Colletotrichum* spp. (Mendgen and Hahn 2002). However, *C. acrocomiae* can colonize vascular bundles invading the phloem sieve tubes in coconut leaflets without causing tissue disruption or vascular relocation. The stromata of both herein studied *Camarotella* pathogens in coconut leaflets develop outside the mesophyll, from the epidermal level to the outside. No vascular bundle compression or leaflet inner tissue disruption was noticeable during *C. acrocomiae* colonization in coconut leaflet mesophyll, even in advanced stages. Coconut leaflet colonization by *C. acrocomiae* seems to have an unexpected and intimate dependence on vascular colonization, particularly inside the sieve tube elements of the phloem.

Significant cellular structural changes and evidences of the cell wall enzyme degrading activity were not observed in coconut leaflets infected with *C. acrocomiae* or with other *Phyllachora* species. Luttrell (1981) considers such parasitism (the host tissue replacement by colonizing hyphae does not lead to cell death) a type of biotrophism (obligate parasitism). According to Luttrell (1981), "*such fact may be worthy of further investigation, studies to elucidate the pathogenesis and mechanisms of these interactions at the biochemical or molecular level are still lacking*". An intimate and specific interaction between a pathogen native to South America and an exotic plant such as the coconut palm, which was introduced to this continent over 500 years ago, was also not expected. There is lack of studies to explain the rapid adaptation of obligate parasites to exotic host plants in the tropics. Moreover, the different colonization processes and parasitism strategies of the two studied *Camarotella* spp., recently classified in the same genus due to their morphology (Vitória et al. 2008; Vitória 2007), may indicate diversity in their genetic origins or even that the two species could migrate to coconut palms from different native host species (Warwick and Leal 2000). Further studies are necessary to better elucidate the evolutionary relatedness, and to determine the native hosts of the two *Camarotella* species parasitizing coconut palms in South America (Vitória 2007; Warwick and Leal 2000; Hyde and Cannon 1999).

The current study is the first to assess *C. torrendiella* and *C. acrocomiae* colonization processes in coconut palm leaflets and the results open new perspectives for additional studies. As *Phyllachora* spp. colonization in warm-season grasses is associated with vascular bundles (Gabel 1989), both *Camarotella* species in the present study limit the development of colonizing hyphae around and inside vascular bundle tissues in coconut palm leaflets. The intracellular phloem invasion suggests high dependency on vascular activities related to sap and water transportation in *C. acrocomiae* and, consequently, on the quality and quantity of nutrients to support the pathogen's growth. Based on the results of the current study we indicate new approaches for SV and LV control in coconut palm crops such as the selection of new highly vascular mobile fungicide molecules in Arecaceae (Monteiro et al. 2013); the selection of genetic resistance mechanisms or chemically induced resistance during post-infection stages in the associated vascular tissues of the host.

Further studies about *Camarotella* spp. initial events and post-penetration pathogenesis in coconut leaflets are needed in order to clarify the infection process (infectious structures) during the first stage. Vitória et al. (2008) have shown that *P. torrendiella* hyphae penetrate the leaflet through the host stomata. However, the way the hyphae invade and colonize the epidermal cells and underlie tissues without triggering the host's defense mechanisms is unknown. Additionally, it is important to understand the nutritional requirements and the trophic relation of the herein studied *Camarotella* species in coconut palms.

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