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CROP PROTECTION

No evidence of coconut stem bleeding disease transmission by bark beetles in Brazil

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ABSTRACT: *Stem bleeding disease, caused by the fungus Thielaviopsis paradoxa is one of the most important diseases of coconut palm Cocos nucifera worldwide and some insect species have been found in association with T. paradoxa infected coconut palms. The present research investigated whether coconut palm-infesting bark beetles (Coleoptera: Curculionidae: Scolytinae) could disseminate stem bleeding disease. Adult bark beetles collected from coconut palms presenting stem bleeding symptoms were inoculated in PDA culture medium. Results showed no germination of the fungus T. paradoxa from either cuticle or digestive tract of bark beetles. Thus, we found no evidence that bark beetles carry viable spores of T. paradoxa that could infect healthy coconut palms and disseminate stem bleeding disease.*
Key words: *Cocos nucifera, Thielaviopsis paradoxa, Ceratocystis paradoxa, Chalara paradoxa, Scolytinae.*

Não evidência de transmissão da doença resinose do coqueiro por escolitíneos no Brasil

RESUMO: *A resinose, causada pelo fungo Thielaviopsis paradoxa, é uma das doenças mais importantes do coqueiro Cocos nucifera em todo o mundo, e algumas espécies de insetos têm sido encontradas em associação com coqueiros infectados por T. paradoxa. O presente trabalho investigou se os besouros escolitíneos (Coleoptera: Curculionidae: Scolytinae) poderiam disseminar a resinose a partir de coqueiros infectados. Escolitíneos adultos coletados de coqueiros com sintomas da resinose foram inoculados em meio de cultura BDA. Os resultados mostraram que não houve germinação do fungo T. paradoxa da cutícula ou do trato digestivo dos insetos. Assim, não encontramos evidências de que besouros escolitíneos carreguem esporos viáveis de T. paradoxa que pudessem infectar coqueiros e disseminar a doença resinose.*
Palavras-chave: *Cocos nucifera, Thielaviopsis paradoxa, Ceratocystis paradoxa, Chalara paradoxa, Scolytinae.*

Coconut palm (*Cocos nucifera* L.) originated in the Pacific and Indian Ocean basins (GUNN et al., 2011) and has been cultivated and disseminated by humans since remote times throughout the tropical lowlands of the world. The species has nowadays hundreds of uses as a source of food, drink, fiber, construction material, charcoal, and oil worldwide, being referred as one of the 20 most important crops upon which human life depends (HOWARD, 2001; GUNN et al., 2011). Coconut plantations are attacked by several diseases, including the lethal stem bleeding disease, caused by the fungus *Thielaviopsis paradoxa* (De Seyn) Hölh (syn. *Chalara paradoxa*, *Ceratocystis paradoxa*). Stem bleeding symptoms include conspicuous reddish-brown stains coming from a hole or wound that seeps down the coconut stem.

Stem bleeding infections most commonly initiate from *T. paradoxa* spores or mycelium that survive on decaying plant litter and are spread from soils to coconut stems by splashing rain or irrigation water (NELSON, 2005; WARWICK & TALAMINI, 2009). However, in Venezuela, PARRA et al. (2003) recovered *T. paradoxa* colonies from the alimentary canal of *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae). WARWICK et al. (2009) also obtained *T. paradoxa* cultures from *R. palmarum*, *Metamasius hemipterus* (L.) (internally and externally) and *Homalinotus coriaceus* (Gyllenhal) (Coleoptera: Curculionidae) (externally) in Brazil, demonstrating the possible role of these important species of coconut borers in disseminating stem bleeding. Furthermore, MARAMOROSCH et al. (1972) reported *Platypus*

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rugulosus (Chapuis) (Coleoptera: Curculionidae: Platypodinae) and *Xyleborus ferrugineus* (Fabr.) (Coleoptera: Curculionidae: Scolytinae) infesting stems of coconut palms affected by stem bleeding in Puerto Rico, but a relationship between these insects and the disease could not be established.

In Brazil, coconut stem bleeding was first recorded in 2004 in the northeastern state of Sergipe (WARWICK & PASSOS, 2009). Since then, this disease has spread to several other coconut producing states. As stem bleeding in Brazil is considered to be recent, several epidemiological aspects of this disease are still to be clarified in order to establish management programs. Recently, some unidentified species of bark beetles (Coleoptera: Curculionidae: Scolytinae) have been observed inhabiting stem bleeding-infected coconut palms in Sergipe (unpublished data). Since symbiotic interactions are prevalent in all bark beetle communities (HOFSTETTER et al., 2015) and adult insects belonging to this group present a wealth of fungi-carrying structures (HULCR et al., 2015), we investigated whether coconut palm-infesting bark beetles could disseminate stem bleeding disease.

Sections of bark and cortex were cut from ten coconut palms presenting stem bleeding symptoms (Figure 1A) in a commercial plantation located in Neópolis, Sergipe State (10º20'39.72"S 36º42'30.66"W, 125 meters above sea level), Brazil. Adult bark beetles (Figure 1B, C) found in these sections were collected and killed in freezer (0ºC) for two hours. In order to verify whether *T. paradoxa* could be carried either on the insect cuticle or in its digestive tract, beetles were: 1) straightly inoculated in Petri dishes containing PDA (potato-dextrose-agar) culture medium + chloramphenicol (500mg L^{-1}), a broad spectrum bactericide; or 2) externally disinfected with 70% alcohol solution (2 seconds) and sodium hypochlorite (60 seconds), washed with distilled water, macerated, and subsequently inoculated in Petri dishes with PDA medium + chloramphenicol (500mg L^{-1}). Dishes received three insects each and were kept in an incubation chamber at 25±1ºC and daily evaluated, during seven days. The experiment consisted of a completely randomized design with 60 replications (insects) per treatment.

Although this methodology has been successfully used for isolating *T. paradoxa* from plant tissue or insect body (WARWICK & PASSOS, 2009; COSTA E CARVALHO et al., 2011a, 2011b), there was no *T. paradoxa* germination from either cuticle or digestive tract of bark beetles in our experiment. The fungus *Aspergillus* sp. grew in PDA medium but it was considered a contaminant. Bark beetles are known for attacking diseased, weakened or recently dead trees to which they are attracted, and for making galleries in the tree trunk inside which they develop, breed, and oviposit (RAFFA et al., 2015). Owing to their habit, bark beetles were expected to acquire and transmit the fungus *T. paradoxa* while tunneling in stem bleeding-infected trees. However, it is possible

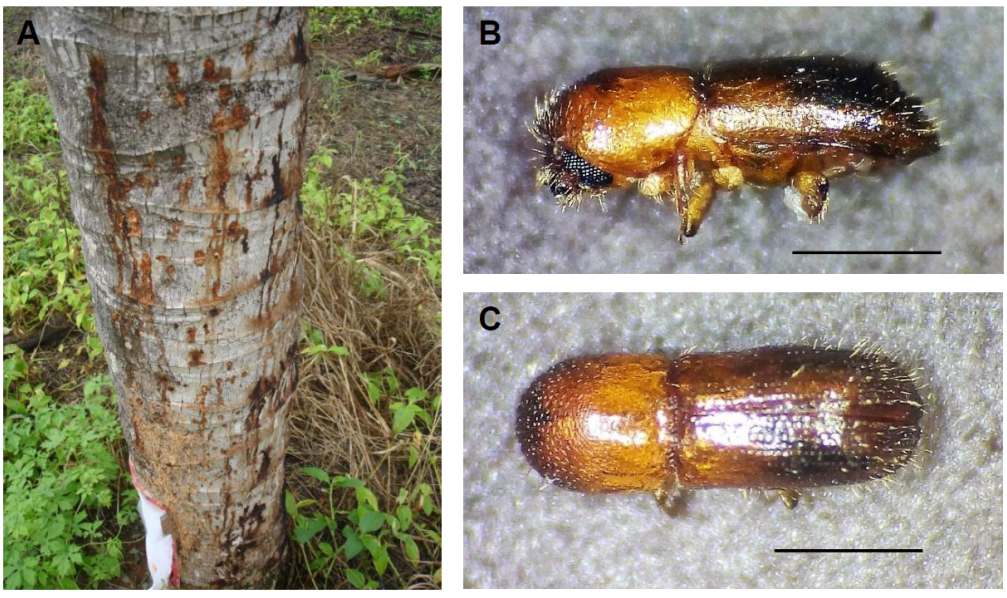

Figure 1 - Stem bleeding symptoms in coconut palm (A) and adult bark beetle in lateral (B) and dorsal (C) views. Scale bars: 1mm.

that the insects we assayed had been attracted to the stem bleeding-infected coconut palms but they had not enough time to be contaminated by *T. paradoxa* spores and/or mycelia. Nevertheless, we assumed that *T. paradoxa* could not have colonized the entire stem and its galleries remained uninfected. Furthermore, most of bark beetles symbiotically-associated fungi may inhibit other fungi (HOFSTETTER et al., 2015), possibly explaining the reason why we did not obtain *T. paradoxa* from their bodies. Our study is not yet conclusive and further investigations should be carried out in order to elucidate the possible role of bark beetles in transmitting and disseminating *T. paradoxa*. In the light of current knowledge, we still have no evidence that bark beetles could carry viable spores of *T. paradoxa* that could infect healthy coconut palms and disseminate stem bleeding disease.

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