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Record of thrips (Insecta: Thysanoptera) and fungi affecting soursop trees in the state of Acre, Brazil

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ABSTRACT

The drying and death of soursop seedlings (*Annona muricata* L.) were reported on Viveiro da Floresta in Rio Branco, state of Acre, Brazil, in March 2017. The seedlings were obtained from municipalities of Dona Euzébia, Minas Gerais state, and the seeds from Capixaba, Acre state, Brazil. Seedlings with dieback symptoms were taken to the Laboratories of Entomology and Phytopathology of Embrapa Acre and analyzed with a stereomicroscope. The thrips found were preserved in ethanol (70%), mounted on slides, and identified in the specialized literature. Moreover, the isolation of fungi from leaves and stems was performed, which were inserted on Petri dishes with a PDA medium. The insect was identified as *Pseudophilothrips* sp., and the fungi pertained to the genera *Colletotrichum* and *Lasiodiplodia*. The high infestation of thrips in soursop seedlings, related to the nutrition of insects, is probably associated with the entrance of opportunistic fungi. Thus, the fungi colonized already fragile plants by the initial attack of insects, causing the drying and death of seedlings.

Keywords: Annonaceae, Botryosphaeriaceae, Glomerellaceae, Phlaeothripidae, Tubulifera.

Registro de tripes (Insecta: Thysanoptera) e fungos associados à gravioleira no estado do Acre

RESUMO

Foi observado secamento e morte de mudas de gravioleira (*Annona muricata* L.) depositadas no Viveiro da Floresta em Rio Branco, estado do Acre, Brasil, em março de 2017. As mudas eram provenientes dos municípios de Dona Euzébia, MG e de sementes provenientes de Capixaba, AC, Brasil. Mudas com sintomas de secamento de ponteiros foram trazidas aos Laboratórios de Entomologia e Fitopatologia da Embrapa Acre, onde foram triadas sob microscópio estereoscópio. Os tripes encontrados foram preservados em etanol (70%), montados em lâminas de microscopia e identificados com auxílio de literatura especializada. Ademais, foi realizado isolamento de fungos de folhas e hastes, os quais foram inseridos em Placas de Petri, contendo meio PDA. O inseto foi identificado como *Pseudophilothrips* sp. e os fungos como pertencentes aos gêneros *Colletotrichum* e *Lasiodiplodia*. A alta infestação de tripes observada nas mudas de gravioleira, associada às injúrias causadas por sua alimentação, provavelmente estão associadas à entrada de fungos oportunistas. Assim, os fungos colonizaram as plantas, já debilitadas pelo ataque inicial desses insetos, causando o secamento e a morte de mudas.

Palavras-chave: Annonaceae, Botryosphaeriaceae, Glomerellaceae, Phlaeothripidae, Tubulifera.

The soursop tree (*Annona muricata* L.,) is a species from Tropical America, which reaches up to six meters in height, and has a small crown with few ramifications. The plant produces yellow flowers from which large fruits emerge, weighing between three and five kilograms. (Steyemark et al., 1995; Sacramento et al., 2009; Cavalcante, 2010). The food industry uses these fruits to make creams, desserts, ice creams, and juices (Cavalcante, 2010). Other parts of plants (flowers, leaves, and seeds) are also used in folk medicine to treat several diseases (Mors et al., 2000; Lorenzi and Matos, 2002).

This tree is cultivated from south Florida to Brazil, including some Caribbean islands, Hawaii, Australia, and some African and Asian countries (Silva et al., 2016). In Brazil, the North and Northeast regions are the leading soursop producers (Silva and Garcia, 1999; IBGE, 2017). According to the Brazilian agriculture and husbandry census of 2017, soursop tree's planted area in Brazil was 2,760 ha. The state of Bahia (Northeast region) was the leading producer with 1,531 ha destinated to the crop. In the Amazon region, the states of Amazonas and Pará stand out with 104 ha and 81 ha of crop area in 2017 (IBGE, 2017). In other states of the Amazon region, the soursop tree is commonly cultivated in forest gardens and small rural properties (Silva et al., 2016).

Phytosanitary aspects (pests and diseases) are the main limiting factors for crop expansion in the Amazon (Silva et al., 2016). The main pest insects associated with soursop crops in Brazil are Annona fruit borer [*Cerconota anonella* (Sepp) (Lepidoptera: Elachistidae], *Bephratelloides pomorum* (Fabricius) and *Bephratelloides maculicolis* (Bondar) (Hymenoptera: Eurytomidae), *Cratosomus bombina* (Fabricius) and *Heilipus catagraphus* Germar (Coleoptera: Curculionidae), caterpillars [*Gonodonta* sp. (Lepidoptera: Noctuidae), *Cocytius antaeus* (Drury), and *Cocytius duponchel* (Poey) (Lepidoptera: Sphingidae)], *Thecla ortygnus* Cramer (Lepidoptera: Lycaenidae), *Horiola picta* (Coquebert) and *Membracis suctifructus* Boulard and Couturier (Hemiptera: Membracidae), *Empoasca* sp. (Hemiptera: Cicadellidae), lesser snow scale [*Pinnaspis aspidistrae* (Signoret) (Hemiptera: Diaspididae), *Saissetia coffeae* Walker and *Ceroplastes floridensis* Comstock (Hemiptera: Coccidae), and aphids [*Aphis gossypii* Glover, and *Aphis spiraecola* Patch (Hemiptera: Aphididae)] (Ledo, 1992a; 1992b; Hamada et al., 1998; Gallo et al., 2002; Sacramento et al., 2009; Moura and Coelho Júnior, 2010; Silva et al., 2016).

In Acre state, the insects *C. anonella*, *C. bombina*, *B. pomorum*, and *T. ortygnus* are the main pests reported, limiting commercial soursop production in the state and reducing fructification (Ledo, 1992a; 1992b; Silva et al., 2016). Regarding pathogens, the primary diseases related to soursop trees in Brazil are anthracnose [*Colletotrichum gloeosporioides* (Penz) Sacc.], black bread mold [*Rhizopus stolonifer* (Ehrenberg) Vuillemin], black rot of soursop [*Lasiodiplodia theobromae* (Patouillard) Griffon and Maublanc], *Sclerotium coffeicola* Bull., *Sclerotium rolfsii* Sacc., brown eye spot [*Cercospora annonae* A.S. Muller et Chupp], *Phytophthora* spp., *Rhizoctonia solani Khun* and *Fusarium* spp., thread blight [*Corticium koleroga* (Cooke)], *Phomopsis* sp., root rot [*Cylindrocladium clavatum* Hodges and L.C. May, and *Pythium* sp.], and *Cytorhabdovirus* sp. (Sacramento et al., 2009; Junqueira and Junqueira, 2014).

In this sense, this study aims to report thrips and fungi infestation and additional phytosanitary threats to the crop, which cause mortality in soursop seedlings in Acre. In March 2017, we observed damages in leaves, drying, and death of soursop seedlings stored at Viveiro da Floresta, Rio Branco, AC, originated in the municipalities of Dona Euzébia, Minas Gerais state, and seeds from Capixaba, Acre state (Figures 1A and 1B).

Figure 1. A. Soursop seedlings stored at the greenhouse of Viveiro da Floresta, municipality of Rio Branco, Acre state, Brazil. B. Aspect of the soursop seedlings attacked by thrips and fungi. (Pictures: Juscélia Aparecida Batista de Almeida).

Infected seedlings were taken to the Laboratories of Entomology and Phytopathology of Embrapa Acre, where they were analyzed with a stereomicroscope. Thrips were observed in leaves collected with a thin brush and deposited into a flask with ethanol (70%). Afterward, the insects were sent to the taxonomist Dr. Élison Fabrício Bezerra Lima (Universidade Federal do Piauí – UFPI, municipality of Floriano, Piauí state, Brazil), mounted on permanent slides (Mound and Marullo, 1996). Then, they were identified regarding genus (the lowest taxonomical level) based on the study of Mound et al. (2010). Witness specimens were deposited in the *Coleção de História Natural da Universidade Federal do Piauí* (CHNUFPI - Natural History Collection of the Universidade Federal do Piauí).

Furthermore, seedlings with visual symptoms of fungal infection were taken to the Phytopathology Laboratory of Embrapa Acre, where leaves, stems (with necrotic and dead tissue) were prepared to isolate fungi probably present on them. For such a procedure, the samples were cleaned in running water with neutral detergent, sliced in 0.5 x 0.5 cm pieces in the interface between healthy and diseased tissue. In a vertical laminar flow hood, the fragments were immersed in ethanol (70%) for 30 seconds and then transferred to a sodium hypochlorite solution (1.25%) for three minutes.

Afterward, they were cleaned in sterile distilled water, dried in a sterile paper filter, and conditioned in Petri dishes with a PDA medium + 100 ppm of chloramphenicol. The dishes were maintained in a BOD at 25 ºC with a photoperiod of 12 hours. After 12 days, the phytopathologists identified the fungi observing the colonies with a magnifying glass. They also mounted the slides and observed fungi reproductive structure with an optic microscope (Alfenas et al., 2016).

The insects identified were *Pseudophilothrips* sp. (Thysanoptera: Phlaeothripidae) (Figures 2A and 2B). Nowadays, 13 valid *Pseudophilothrips* are recorded in the Americas (Mound et al., 2010), and seven are present in Brazil (all phytophagous). There are records of the genus in the states of Amazonas, Goiás, Espírito Santo, Minas Gerais, São Paulo, Rio de Janeiro, and Paraná (Silva et al., 2019; Monteiro and Lima, 2021).

Nevertheless, a taxonomical review of the genus *Pseudophilothrips* is highly recommended as some species, previously described as *Liothrips*, were transferred to *Pseudophilothrips* (Johansen, 1979; Mound et al., 2010). Therefore, it is probable that other species should also be transferred after a detailed analysis of species of these genera. Thus, it was not possible to identify the thrips species at a specific level.

Female *Pseudophilothrips* (suborder Tubulifera) do not have terebra (serrated ovipositor) to perform the endophytic egg-laying, as occurs with species of the suborder Terebrantia. Consequently, they do not damage plants during egg-laying. Nevertheless, due to the gregarious habit of *Pseudophilothrips* nymphs, they stay longer in the same place to feed, causing injuries at the site (Cuda et al., 2009).

Insects can interfere in the pathogen-host interaction regarding survival, dissemination, and infections of phytopathogenic agents (Bergamin Filho et al., 2018). Thrips can transport fungal propagules in different body parts and open scars during their nutrition or egg-laying. Picanço et al. (2003) verified an increase in the number of fungal injuries caused by *C. gloeosporioides* and *Pestalotia* sp. in guava fruits (*Psidium guajava* L., Myrtaceae), due to higher densities of *Pseudophilothrips* sp.

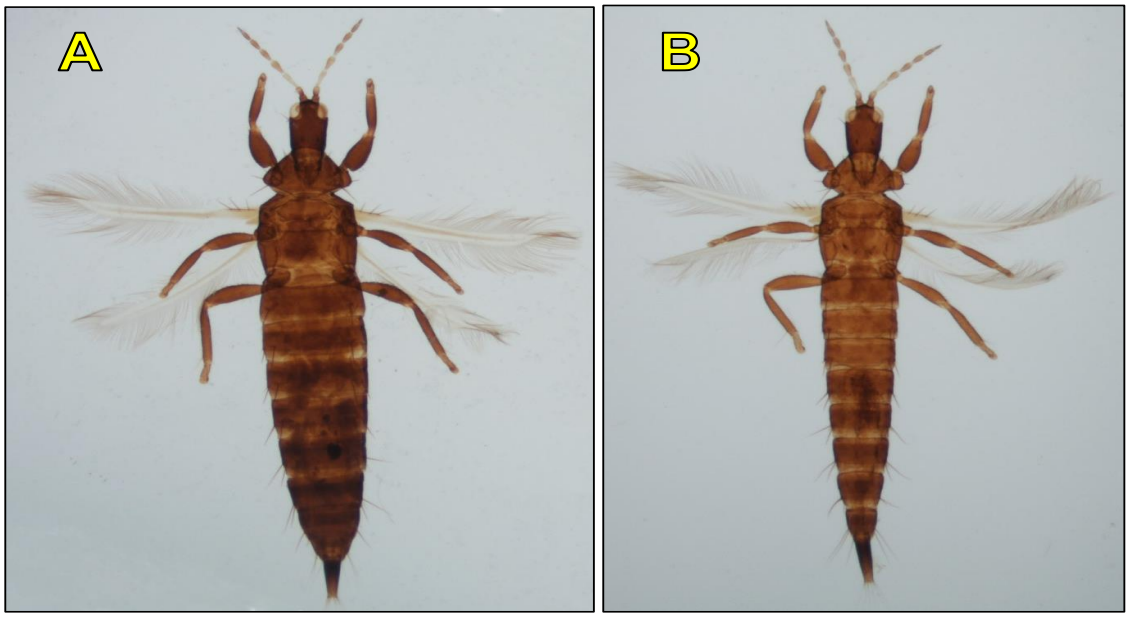

Figure 2. *Pseudophilothrips* sp. (Thysanoptera: Phlaeothripidae): **A**. Adult female. **B**. Adult male. (Pictures: Élison Fabrício Bezerra Lima).

Regarding indirect damages, thrips transmit different types of viruses related to pollens (*Ilarvirus*, *Sobemovirus*, and *Carmovirus*), besides Orthotospovirus, which have an intimate biological relation with the species of thrips, involving the transmission leaf-to-leaf (Ullman et al., 2002; Whitfield et al., 2005; Riley et al., 2011). *Pseudophilothrips* species are not reported as transmitters of viral diseases. Nevertheless, some are considered pests for certain crops (Picanço et al., 2003; Monteiro and Lima, 2021). No symptoms of viral infection were found in the soursop seedlings analyzed. We found fungi of the genera *Colletotrichum* (Diagnosis: formation of sporodochia, mucilage of salmon coloration containing cylindric conidia rounded at the extremities) and *Lasiodiplodia* (Diagnosis: the presence of pycnidia with mature uniseptate conidia of brown-yellow coloration, with longitudinal stria).

Filamentous *Colletotrichum* fungi are considered important phytopathogens widely disseminated, mainly in tropical and sub-tropical regions. Saprophyte and pathogenic forms are found among *Colletotrichum*. The latter form is responsible for diseases of severe economic damage, such as anthracnose, which occur widely in a wide range of hosts (Menezes, 2006). Anthracnose may penetrate plant tissues (branches, leaves, fruits, and inflorescences) directly or indirectly by injuries caused by insects (Manica et al., 2001). The leaves affected presented brown spots of oval or irregular shape and varying sizes. The injuries appear on the apex, margin, or the center of the leaf, which can rupture with high incidence (Tavares et al., 2005).

The occurrence of anthracnose in soursop trees was already studied in Acre by Ledo (1992a), which reports that this is the leading disease in growing soursop trees. The fungus preferentially attacks young tissues of leaves and fruits, causing the death of branches, tips, grafts, as well as the loss of fruits and flowers (Junqueira and Junqueira, 2014; Spósito et al., 2016).

Fungi of the genus *Lasiodiplodia* are cosmopolite, polyphagous, and opportunistic, related to several diseases of fructiferous trees (Cardoso et al., 1998; Oliveira et al., 2012). They are associated with pathogenic processes in stressed plants submitted to natural or inflicted damages. On the other hand, they are considered fungi with low pathogenic specialization (Olunloyo and Esuruoso, 1975). Oliveira et al. (2013) verified *L. theobromae* in cashew trees (*Anacardium occidentale* L., Anacardiaceae) attacked by a non-identified coleopterous species. Thus, it is plausible that the high thrips infestation in soursop seedlings is the main cause of injuries in the leaf tissue of seedlings, which favors opportunistic fungi entrance.

Lasiodiplodia species can survive in the environment, in dead or alive vegetal tissues, and then be disseminated by pruning, wind, or insects (Oliveira et al., 2013). Moreover, Cardoso et al. (2006) verified that *L. theobromae* also endophytically survives in *A. muricata* seeds. This pathogen can cause different symptoms in infected plants, including dieback; galls in branches, stems, and roots; injuries in cuttings, leaves, fruits, and seeds; besides provoking the death of seedlings and grafts (Oliveira et al., 2013).

Two or three days after the seedlings were deposited in the nursery, they started presenting visible injuries in leaves, and drying and death of tips. Therefore, it is not dismissed or proved that the seedlings acquired were already infested by *Pseudophilothrips* and fungi, even before reaching the state of Acre. Furthermore, storage conditions (seedlings on the floor of nurseries and the presence of invading plants), temperature, and humidity can also favor fungi proliferation.

It is worth mentioning that nurseries, accredited in the *Registro Nacional de Sementes e Mudas* (RENASEM - National Register of Seeds and Seedlings) should be monitored by the responsible agency to produce seedlings, aiming to verify sanitary conditions, purity of varieties, and other constant factors of patterns of seedlings (Castro, 2011). Inspection of seedlings, associated with the phytosanitary analysis report conceded by an official laboratory, is fundamental to control the propagation of pests between the country's states.

This study is the first report about *Pseudophilothrips* sp. infection of soursop seedlings in the state of Acre. From the high population verified, *Pseudophilothrips* sp. damages seedlings during their nutrition, propitiating the entrance of fungi. It is also the first record of *Lasiodiplodia* sp. colonizing soursop seedlings in Acre state. It is confirmed that anthracnose, caused by *Colletotrichum* is the most frequent fungal disease in soursop trees on Acre state.

Authors' Contribution

Dr. Rodrigo Souza Santos collected the thrips from the soursop seedlings in the laboratory and participated in the writing and reviewing of the manuscript. Dra. Sônia Regina Nogueira isolated and identified the fungi of the soursop seedlings, besides inspecting the manuscript. Dr. Rivadalve Coelho Gonçalves participated in the writing and review of the manuscript.

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