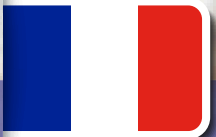




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An entomopathogenic fungus recently discovered to attack *Amrasca biguttula biguttula* (Hem.: Cicadellidae) in Benin: provisional identification and research to be undertaken

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Abstract:

In June 2023, high populations of *Amrasca biguttula* were observed in Cotonou, Benin, infected by an entomopathogenic fungus on leaves of *Abelmoschus esculentus* (okra). This first reporting in Africa could be due to an Entomophthorale. Recommendations are given to better identify the fungus.

Keywords: okra, leahopper, Entomophthorale, Sub-Saharan Africa.

Introduction

The leafhopper *Amrasca biguttula biguttula* (Ishida) (= *Amrasca devastans* (Distant)) (Hemiptera- Cicadellidae), known as the Green or Indian cotton jassid, has recently been observed as an invasive exotic species on cotton plants in many West African countries as well as in Madagascar. During a mission carried out in southern Benin in June 2023, high populations of this insect were observed on the leaves of okra plants (*Abelmoschus esculentus*, Malvaceae)

grown at the headquarters of the Institut National de Recherche Agricole du Bénin (INRAB), and close to the International Institute of Tropical Agriculture (IITA), in Calavi (outskirts of Cotonou). Dead adults, golden yellow in color, attached under the leaves of all varieties, with their wings spread apart (Figures 1 and 2) were suspected to be contaminated by an entomopathogenic fungus.



Figure 1. Dead adults of *A. biguttula* on okra leaves (lower parts). © P. Silvie, CIRAD



Figure 2. Dead adults of *A. biguttula* on okra leaves (details). See the spread wings, an indication of probable fungal infection. © P. Silvie, CIRAD

Provisional Pathogen Identity

The examination under a binocular microscope of the samples brought back at IITA's laboratory allowed us to observe the presence of granular particles of the conidia type on the wings of some individuals (Figure 3).

These particles suggested that the fungus must belong to the *Entomophthorales* group. Insects who had already sporulated were placed on water-soaked cellulosic

paper placed in the lid of a Petri dish to obtain conidia by projection (according to the methods described by Silvie and Papierok (1991)). This operation yielded conclusive results on Wednesday evening, with conidia having been projected onto the slides. With a rotund shape, they may belong to a species of the genera *Conidiobolus*, *Entomophaga*, or *Batkoa*. The confirmation of the leafhopper's identity was done molecularly on healthy individuals collected in parallel.



Figure 3. Adult of *A. biguttula* with spread wings viewed with a binocular. Note the rounded conidia of the fungus projected onto the wings, a characteristic feature of the *Entomophthorales* group. © G. Goergen, IITA

A comprehensive literature review gave rise to a well-founded hypothesis regarding the identification of the pathogenic species. Thus, the fungus *Batkoa amrascae* Keller & Villacarlos, described by Villacarlos and Keller in 1997, was observed to infest *A. b. biguttula* in the Philippines thirty years ago (in 1993). More recently, in India, the same

species was reported on *Cofana spectra* (Hem.: Cicadellidae), an insect present in the “rice system” (Baiswar and Firake, 2021) which Keller and Yubak Dhoi had suspected as early as 2007, but on another Hemipteran insect, *Pyrilla perpusilla* (Fulgoridae), occurring on sugarcane and collected in September 2000. Fungi from

the Entomophthorales group, especially the species *Zoophthora radicans* (McGuire et al., 1987; Butt et al., 1988; Galaini-Wraight et al., 1991; Wraight et al., 2003), are also known as pathogens of species of the Cicadellidae family (Batista Filho et al. 1997; Souza et al., 2021).

Research needs

To confirm the hypothesized species, it is imperative to measure both primary and secondary conidia, which should be spherical in shape. The presence of rhizoids—filaments that attach the cadavers to leaves—must also be verified, along with the number of nuclei in the conidia, using staining methods such as lactophenol-aceto-orcein (0.5%) or cotton blue and orcein. Additional morphological attributes that need to be verified include the conidiophores, which should be simple and unbranched, as well as the form of the hyphae.

The occurrence of this pathogen should be further investigated in populations found on cotton plants. If present, its role should be considered in any study on the population dynamics of the leafhopper, as epizootics are frequent when specific humidity conditions are met - specifically, several hours of 100% relative air humidity per night. Rainfalls recorded in Cotonou in June 2023, combined with the high leafhopper density on okra plants, undoubtedly facilitated the observed epizootic event.

The species *B. amrascae* can be cultured in artificial media. If indeed present in Benin, the species should be isolated for two main purposes: firstly, to further characterize the species, and secondly, to explore the

possibility of infecting *A. b. biguttula* populations in other humid regions of the country. For instance, trials could be conducted in lowland areas cultivated with okra during the dry season, provided the insect is abundantly present. Another strategy could involve the collection of okra leaves infested with mycosis-affected dead individuals for direct dissemination into living leafhopper populations. However, it should be noted that manipulating this group of pathogens is challenging, and large-scale fermentation culture is probably not economically viable at time.

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