

BATKOA AMRASCAE KELLER & VILLACARLOS, A NEW SPECIES OF ENTOMOPHTHORALES (ZYGOMYCETES) INFECTING THE COTTON LEAFHOPPER, *AMRASCA BIGUTTULA* (ISHIDA) (HOMOPTERA: CICADELLIDAE) IN THE PHILIPPINES

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ABSTRACT

Batkoa amrascae a new species of Entomophthorales infecting *Amrasca biguttula* is described. the fungus was first noted on cotton leafhopper attacking okra and eggplant in VISCA, Baybay, Leyte in 1993. Is is a potential biological control agent of the cotton leafhopper.

Key Words: *Batkoa amrascae*, Entomophthorales, *Amrasca biguttula*, insect pathogen.

INTRODUCTION

In early 1993 epizootics due to an Entomophthorales were observed on populations of cotton leafhopper *Amrasca biguttula* (Ishida) on okra (*Abelmoschus esculentus* (L.) Moench). These occurred in almost regular pattern on heavily infested okra fields in the Visayas States College of Agriculture (VISCA), Baybay, Leyte, Philippines (Villacarlos, 1995). Infected leafhoppers were also observed on eggplant. This was not the first time a fungus infecting cotton leafhopper was observed in the country. Cendana and Baltazar (1947) reported an epizootic in a population of *Empoasca flavescens* (now *A. biguttula*) caused by a fungus which was identified as *Cephalosporium* sp. They indicated that the fungus was an important factor in the control of the pest when the environmental conditions were favorable for fungal growth. The present authors suspect that the infection may have been due to a species of Entomophthorales that was overgrown by *Cephalosporium*, a common saprophyte on Entomophthorales-infected insect cadavers. In Illinois, a closely related species of potato leafhopper, *Empoasca fabae*, has been often observed to be infected with *Zoophthora radicans*, an Entomophthorales with wide host range (McGuire *et al.*, 1987).

Villacarlos (1995) identified the fungus infecting *A. biguttula* under the genus *Entomophaga*; however, closer examination of its characteristics, by the junior author proved it to be under *Batkoa*, a genus closely related to *Entomophaga*. This paper

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describes *Batkoa amrascae*, a new species of Entomophthorales infecting *A. biguttula* in the Philippines.

MATERIALS AND METHODS

Collection of specimens

Infected insects were collected during an on-going epizootic from okra fields with heavy leafhopper infestation. Dead insects showing the characteristic growth of the fungus were collected and placed in vials containing 70% alcohol. Leafhoppers still attached to the leaf were collected and some placed in a plastic plate lined with tissue paper for air drying.

Processing of specimens for identification

Freshly dead insects were individually placed in moist chamber consisting of a petri plate lined with moist tissue paper. Conidial deposits were collected on glass slide placed 3-5 mm above the cadaver. These were stained in lactophenol acetoorcein (LPAO) or lactophenol cotton blue (LBPC) (Keller, 1987). An ocular graticule calibrated to μm was used to measure various structures. The mean dimensions were based on sample size which varied from 1-5 series, each series consisting of 50 measurements if not otherwise stated.

Fungus culture

Some of the freshly dead insects were individually glued with molten agar in the inner side of a petri dish cover whose bottom contained either Sabouraud destrose agar (SDA) or SDA enriched with egg yolk (1 egg yolk per 200 ml SDA) (SDAEY). Small pieces of agar from the edge of the fungal growth were transferred to SDAEY and SDA slants to get a pure culture. The fungus was also grown in a liquid medium used for culturing *Zoophthora radicans* (Pell et al., 1993). It consisted of 20 g D-glucose, 5 g yeast extract, 50 ml fresh milk and 450 ml distilled water mixed in a breaker and dispensed in 250 ml flasks at 50 ml each. These together with test tubes containing 2-ml medium were sterilized at 10 psi for 20 minutes. Small pieces of fungal mat from 2-week old SDA culture were aseptically triturated with the use of sterile glass rod in the test tube containing 2-ml medium. The resulting suspension was carefully added into the flask with 50 ml medium and placed in an orbital shaker at 250 rpm for 3-5 days. The inoculum was harvested by filtration using a Buchner funnel lined with Whatman No. 2 paper. The mycelial mat collected was placed in a sterile plate and incubated in the dark for 16-20 hours. This was further incubated in moist chamber for 3-6 hours for optimum sporulation.

RESULTS AND DISCUSSION

Symptoms

There was no indication of the disease in infected leafhoppers. They died on the surface of the leaf where they were attached mainly by 7-9 strands of rhizoids. Infected adults were usually observed during early morning with their wings spread out and sometimes containing conidia (Figure 1). Creamy mycelial mat was concentrated on the intersegmental regions of the thorax and the abdomen.

Descriptions of the pathogen

Bathkoa amrascae KELLER & VILLACARLOS, sp. nov.

Figs. 1-11

Conidia primaria (20-) 22-26 (-31) x (17-) 18-23 (-28) μm , subsphaerica vel ovoids, distincts papilla rotunda vel conica, 6-13 nucleis continentia. *Conidia secundaria* habitu primariis similia. *Conidiophora simplicia* apicaliter inflata. *Corpora hyphalia* rotunda vel elongata, 5-16 nucleis diametro 3.5-5.5 μm impleta. *Rhizoidea mononemata* diametro 7-23 μm , discoideis vel formae specialis terminata. *Cystidia et sporae perdurantes* absunt.

In *Amrasca biguttula* Ishida (Homoptera, Cicadellidae) (hospite typico).

Holotypus: ZT, Baybay, Leyte, Philippines, in *Amrasca biguttula*, IX 1993 et V 1996, coll. L. T. Villacarlos, leg S. Keller no AB5. *Paratypi* ZT: Ab6, Ab14, Ab23; K: Ab20; BPI: Ab22.

Hypal bodies irregularly rounded to elongate, simple, rarely spherical to ellipsoidal, sometimes arranged in chains (Figure 2). They contain 5-16 nuclei with an average of 10-11 nuclei (2 series) with a diameter of 4.5-4.6 μm (3.5-5.5 μm) (series plus 1 series with n=30). Conidiophores unbranched with 5-12 nuclei (2 series, n=38 and 48) terminally enlarged (Figures 3, 4 & 5). Primary conidia 22.2-26.4 x 18.4-23.1 μm (20-31 x 17-28 μm), L/D=1.16-1.23 (5 series, n=38-50) Figure 6); unitunicate, subspherical to ovoid, apex rounded, papilla rounded or conical, sometimes pointed, 6-13 nuclei (1 series, n=19) (Figure 7). Secondary conidia like primary, 21.9-23.0 x 18.5-19.0 μm (17-28 x 25-28 μm), L.D=1.18-1.21 (3 series, n=50), formed on short conidiophore laterally of the primary conidia (Figure 8). Rhizoids monohyphal with a diameter of 12.6 (7-23) μm , (Figure 9) emerging ventrally from the thoracic region, sometimes branched before the endings; endings disc-like, sometimes finger-like (Figure 10). Cystidia and resting spores not observed.

In *Amrasca biguttula* (type species) (Homoptera: Cicadellidae).

Culture

Grows easily on all media used. On SDA and SDAEY it initially formed separate distinct white colonies which later coalesced after two weeks forming an elevated mass with rugose surface. When about to sporulate glistening droplets appeared on the fungal surface. Primary conidia 27.5-28.4 x 22.2-23.1 μm (25-35 x 20-30 μm), L/D=1.24-1.25 (3 series, n=50), containing 4-6 nuclei (1 series, n=29) (Figure 11). In liquid media it took 3-5 days to get matured mycelia. Mycelial mat kept in the dark overnight and transferred to moist chamber produced conidia within 4-6 hours. The fungal mat appeared creamy white in all the media. Both the inocula grown on SDA and liquid media successfully infected healthy *A. biguttula* (Villacarlos, 1995).

Distribution and incidence in the field

The species was first noted infecting *A. biguttula* on okra (*Abelmoschus esculentus* (L.) Moench) and eggplant (*Solanum melongena* L.) from August to November, 1993 causing epizootics in the Visayas State College of Agriculture, Baybay, Leyte, Philippines (type locality). Subere (1996) showed that epizootics were greatly influenced by insect density, inoculum source and weather conditions. She further observed that the incidence of *B. amrascae* was highly localized. It ranged from 0-43% in June to November in on area and 0 in another field sampled from Oct.-Dec. 1994.

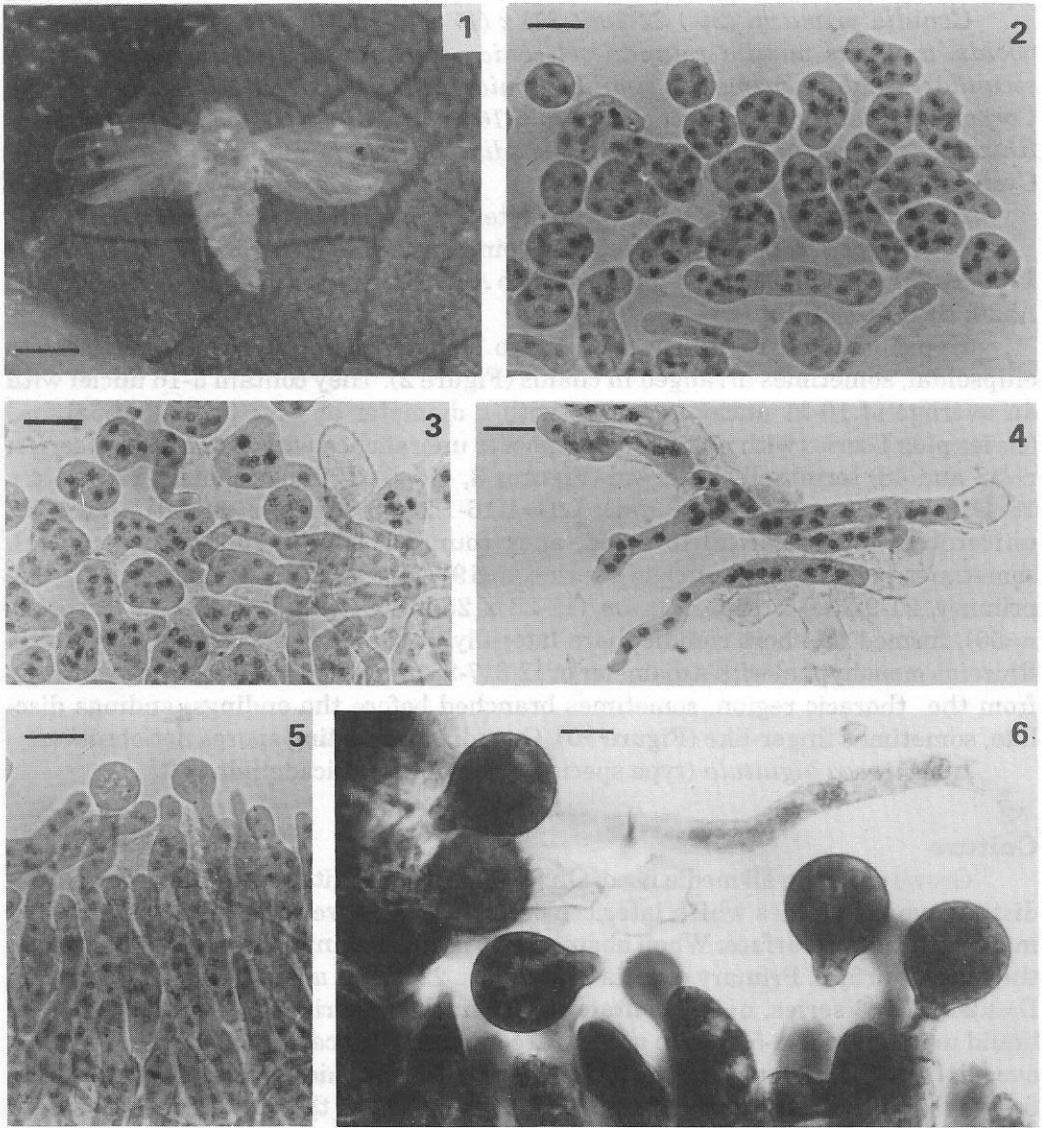
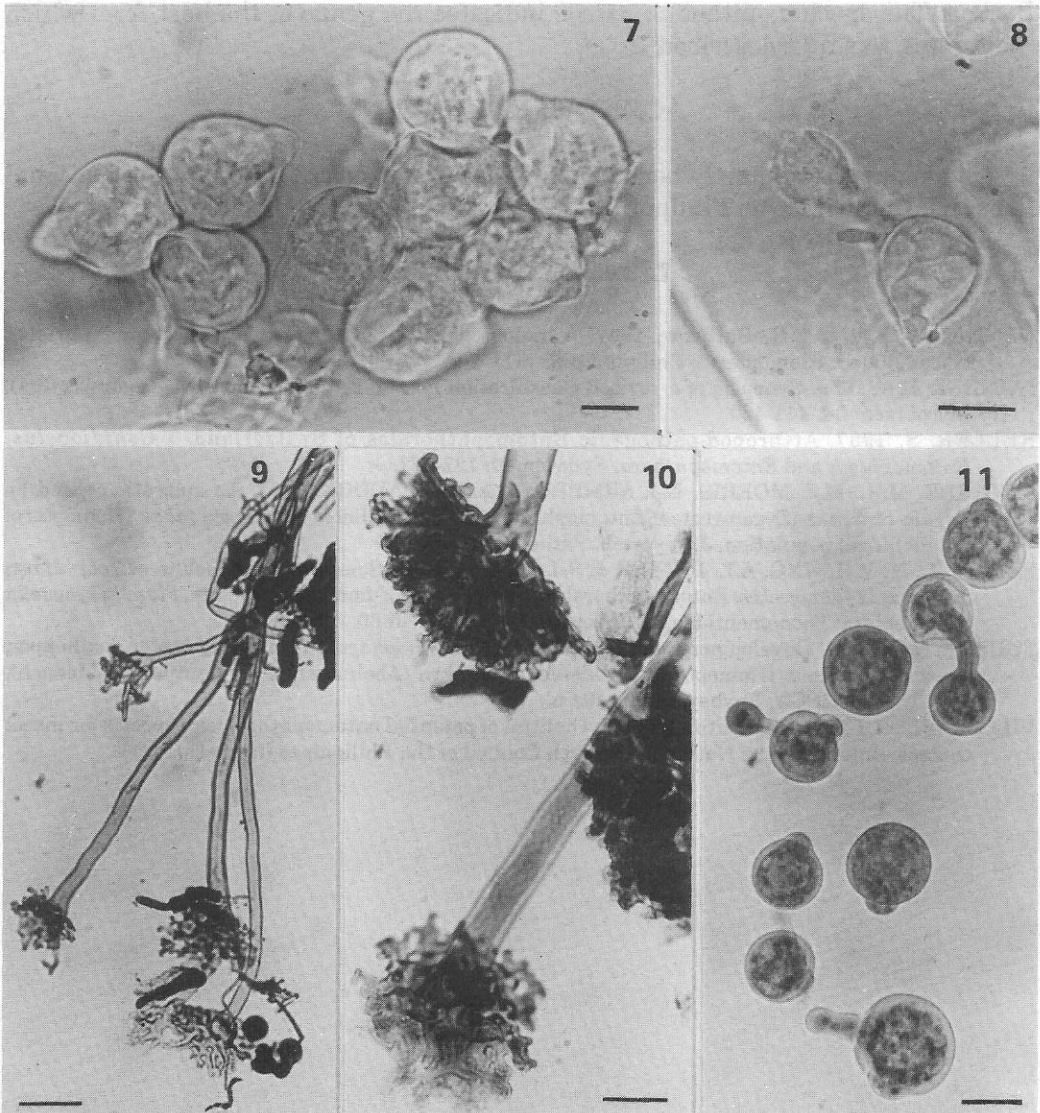


Figure 1-6. Structures of *Batkoa amrascae* Keller & Villacarlos, n. sp.: 1. photomicrograph of infected *Amrasca biguttula* showing fungal mat along intersegmental regions of the abdomen (bar= 1 mm); 2. hyphal bodies with nuclei (LPAO); 3. hyphal bodies starting to germinate (LPAO); 4. young conidiophores with shells of hyphal bodies (LPAO); 5. conidio phores and formation of conidia (LPAO); 6. fully developed primary conidia on conidiophore and projected conidium (LPCB) (bar=10 μ m). Bar in Figs. 2-5: 5 μ m.



Figures 7-11. Structures of *Bathoa amrascae* Keller & Villacarlos, n. sp.: 7. primary conidia (LPCB) (bar= 10 μ m); 8. formation of secondary conidium (LPCB); 9. rhizoids (LPCB) (bar= 50 μ m); 10. endings of rhizoids (LPCB); 11. primary conidia from culture, some forming secondary conidia (LPAO). Bar in Figs. 8, 10 and 11: 20 μ m.

Remarks

The species has characteristics of both the genera *Batkoa* Humber (1989) and *Entomophaga* Batko emend. Humber (1989). The simple rounded hyphal bodies are typical for the genus *Entomophaga*. Also the low number of nuclei corresponds better with this genus. However, due to the presence of rhizoids, the shape of the conidia and the good growth on artificial media is to be attributed to the genus *Batkoa*. The specific epithet *amrascae* indicates the genus of the host from which the fungus was first described.

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LITERATURE CITED

- CENDANA, S.M. & C.R. Baltazar, 1947. A biological study of *Empoasca flavescens* Fabricius (Cicadellidae), Homoptera). Philipp. Agric. 3(1): 1-22.
- HUMBER, R.A. 1989. Synopsis of a revised classification for the Entomophthorales (Zygomycotina). Mycotaxon. 34: 441-460.
- KELLER, S. 1987. Arthropod-pathogenic Entomophthorales of Switzerland. I *Conidiobolus*, *Entomophaga* and *Entomophthora*. Sydowia 40: 122-167.
- MCGUIRE, M.R., M.J. MORRIS, E.J. ARMBRUST & J.V. MADDOX, 1987. An epizootic caused by *Erynia radicans* (Zygomycetes: Entomophthorales) in an Illinois *Empoasca fabae* (Homoptera: Cicadellidae) population. J. Inverteb. Pathol. 50: 78-80.
- PELL, J.K., N. WILDING, A.L. PLAYER & S.J. CLARK. 1993. Selection of an isolate of *Zoophthora radicans*. (Zygomycetes: Entomophthorales) for biocontrol of diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). J. Inverteb. Pathol. 61: 75-80.
- SUBERE, V.Q. 1996. Development and host range of *Entomophaga* sp. infecting cotton leafhopper, *Amrasca radicans* (Homoptera: Cicadellidae) on okra (*Abelmoschus esculentus* (L.) Moench). M.S. thesis, VISCA, Baybay, Leyte. 101 p.
- VILLACARLOS, L.T. 1995. Identification and culture of potential entomophthoralean species for insect control. Bulletin of the National Research Council of the Philippines (in press).