Phytoparasitica (2011) 39:67–71
DOI 10.1007/s12600-010-0139-z

First record of the entomopathogenic fungus *Entomophaga aulicae* on the Bihar hairy caterpillar *Spilarctia obliqua* in Manipur, India

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Received: 4 August 2010 / Accepted: 9 December 2010 / Published online: 6 January 2011

Abstract *Entomophaga aulicae* (Hoffman in Bail) Batko (Zygomycotina: Entomophthorales) was found to cause epizootics in *Spilarctia obliqua* (Walker) (Arctiidae: Lepidoptera), a polyphagous pest in Manipur, India. Infected caterpillars climbed to elevated positions on plants where they hung upside down, attached by their abdominal prolegs. Infection levels reached up to 57%. The conidiophores are simple, conidia are ovoid to pyriform in shape, and the nuclei stained readily in aceto-orcein. Secondary, tertiary conidia and resting spores were also observed. No rhizoids and cystidia were found. This is the first report of *E. aulicae* infecting *S. obliqua* in Manipur.

Keywords Epizootics · Medicinal and aromatic plants · Zygomycotina

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Introduction

The Bihar hairy caterpillar, *Spilarctia obliqua* (Walker) (Arctiidae: Lepidoptera), is a sporadic pest, widely distributed in India, China, Bangladesh, Myanmar, Nepal and Pakistan (CPC 2004). In India it is a serious pest in Bihar, Madhya Pradesh, Uttar Pradesh, Punjab, Manipur, and other states. Due to its highly polyphagous nature, it attacks pulses, oilseeds, cereals, certain vegetables, mulberry, medicinal, aromatic and other economic plants, and causes severe economic damage (Gupta and Bhattacharya 2008). It is one of the major pests of mulberry, and can cause a total foliage loss up to 4.9% in mulberry plantations in Karnataka, India (Shree and Manjunatha 1998). An individual larva of *S. obliqua* can consume up to one kg of mulberry leaves during its larval period (Sharma and Tara 1988). Caterpillars feed gregariously during the early (first to third instar) larval stages (Plate 1–Fig. 1) and solitarily in the late (fourth to fifth instar) larval stages (Gupta and Bhattacharya 2008). Chemical control of this pest is difficult and very high doses of pesticides are required. At present, parasitoids like *Apanteles* sp. (Shetgar et al. 1990) and *Meteorus* sp. (Kumar and Yadav 1987), along with other natural enemies such as nucleopolyhedrovirus (NPV) (Varatharajan et al. 2006), and fungal pathogens like *Metarhizium anisopliae* (Metschnikoff) and *Beauveria bassiana* (Balsamo) Vuillemin (Purwar and Sachan 2006), are being evaluated to manage early instars.

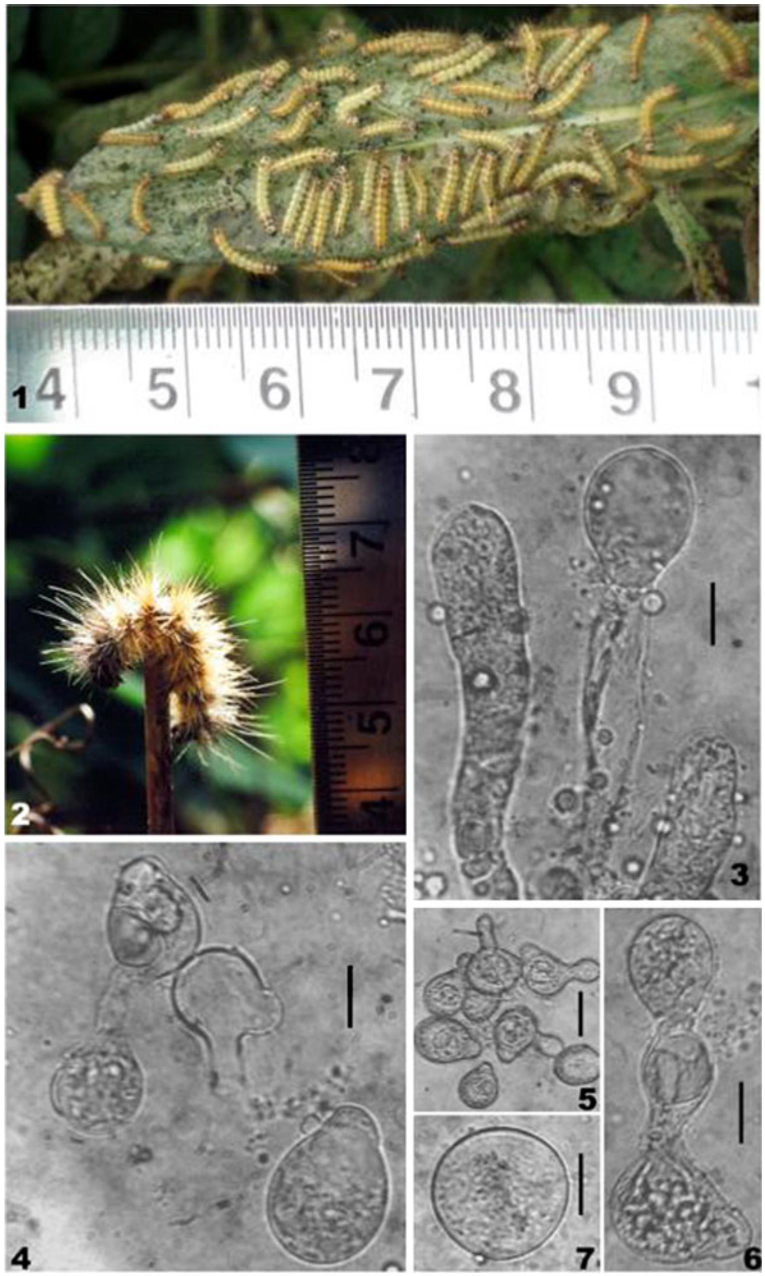

Plate 1–Fig. 1 Gregarious early instar larvae of *Spilarctia obliqua*.
Plate 1–Fig. 2 Cadaver of *S. obliqua* infected by *Entomophaga aulicae*.
Plate 1–Fig. 3 Hyphae and conidiophores.
Plate 1–Fig. 4 Primary conidia, remnants and development of replicative conidium.
Plate 1–Fig. 5 Development of secondary conidia and germ tube.
Plate 1–Fig. 6 Development of secondary and tertiary conidium.
Plate 1–Fig. 7 Resting spore. *Bars* in Plate 1–Figs. 3, 4, 6 & 7=10 µm, magnification x1250; Plate 1–Fig. 5=20 µm, magnification x500

Manipur (94°31′ to 94°78′ E and 23°83′ to 25°68′ N, 550–3600 m above MSL), one of the eastern-most border states of India, is within the “Indo-Burma” center of biodiversity hotspots of global significance. The area has prevailing monsoon rainfall with an average annual rainfall of 2100 mm and average air temperature range from −1°C to 38°C in a temporal cycle. The mean daily relative humidity is highest during the months of July–September, varying from 80% to 96% r.h. (Singh et al. 2003). Due to a favorable elevation and pattern of precipitation, it has resulted in a subtropical type of climate in most areas of the state. This communication describes the occurrence of a natural infection in *S. obliqua* by an entomopathogenic fungus, *Entomophaga aulicae* (Hoffman in Bail) Batko, in Manipur State and is reported for the first time from Manipur.

Materials and methods

To study the disease prevalence, 50 early 4th instar larvae collected from the field randomly during May and June 2008, were transferred to plastic containers (L × W × D=9″×4.5″×3.5″) at the rate of five larvae per container and were fed with weed plants (*Alternanthera philoxeroides* Griseb.). The containers were covered with perforated lids for aeration. A dry stick was also provided to enable insects to climb up before dying, similar to their natural habitat. Observations on mortality were recorded daily until the insects either died or pupated. Dead insects were examined under a microscope to confirm the cause of death. The whole setup was incubated under ambient room temperature conditions.

Another set of experiments was conducted in the field during June 2008, to record the death of insects in a known area under natural field conditions. For this, an area of 2 m^2 was marked randomly in the field with the help of four wooden sticks and they were tied with threads to demarcate the area. Observations were carried out daily in the morning to record the density of cadavers. Dead insects were immediately removed after counting to avoid duplication. The experiment was replicated ten times over an area of ~20 m^2.

For morphological studies, conidia and cadavers were mounted in lactophenol cotton blue, and nuclei were stained in aceto-orcein. Semi-permanent slide mounts were prepared, and identification was done based on earlier literature (Humber 1997; Keller 1987). Measurement of conidia was carried out using an ocular micrometer calibrated with a stage micrometer. All observations were carried out under a Leitz-Ortholux microscope equipped with a photomicrograph facility.

Results and discussion

Infected caterpillars were first observed in the fields of medicinal and aromatic plants located inside the North-East Institute of Science and Technology's substation campus during June 2006 (but not quantified), coinciding with the period of heavy rainfall, sunshine and high relative humidity which persisted until the end of July 2006. These favorable environmental conditions might have induced the infection. The medicinal and aromatic plants in which infected caterpillars were observed included *Alpinia galanga* Wild., *Curcuma amada* Roxb., *C. caesia* Roxb., *Cymbopogon winterianus* Vent., *Hedychium spicatum* Buch-Ham., *H. coronarium* Koenig., *Mentha piperita* Linn., *Meriandra bengalensis* Benth., *Ocimum gratissimum* Linn., and *Solanum torvum* Swartz. Apart from these plants, dead cadavers were also observed on cabbage, *Ipomoea carnea* Jacq. and several other weed species. The pest did not occur during 2007. In 2008, the pest incidence was noticed during May and June and *E. aulicae* infection reached epizootic levels during these months. Subsequently, the pest incidence was minimal, due to floods in 2009 and 2010.

The infected larvae were restless and climbed to elevated regions on the plants. Such larvae died within 24 h. The death of insects usually occurred after dusk. Diseased insects usually move about for some time before death, carrying the pathogens with them, in a few cases even dispensing conidia as they go (Sawyer et al. 1994). Cadavers were found to be clinging by their abdominal prolegs on the peak of the stems, on the margins of the top leaves or on the downward side of top leaves mostly hanging upside down (Plate 1–Fig. 2). Moribund caterpillars infected with *E. aulicae* crawl up the dead stems of both grasses and herbs to die at high parts of the plants (Yamazaki et al. 2004). Some fungi and viruses alter behavior of host insects, causing them to climb up vegetation and to die at a high site (Goulson 1997; Yamazaki et al. 2004). These behavioral alterations have been interpreted to be adaptive for the pathogens, aiding in the dispersion of spores and viruses (Goulson 1997; Sawyer et al. 1994). The infected larvae did not exhibit any overt symptoms of mycosis but a huge powdery mass of discharged conidia was found to be attached to the body, particularly on the hairs. These spores along with the hairs remained on the leaves even after some of the cadavers were carried away by ants. Rain splashes and water flow on plant surfaces may disperse conidia and resting spores (Sawyer et al. 1994).

Laboratory assays to quantify the disease prevalence resulted in 57% (n=50) mortality during May and June 2008. The prevalence of death in the field was in the range of 0.3–1.2 cadavers per 2 m^2 (see Fig. 8). The epizootics prevailed for a period of about 8 days. The ability of the fungus to cause more than 50% mortality under natural conditions can be well

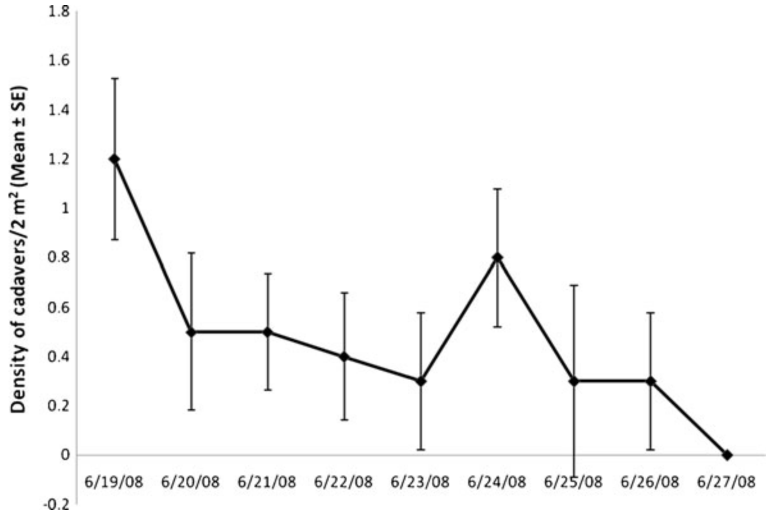

Fig. 8 Prevalence (mean ± SE) of *Entomophaga aulicae* on *Spilarctia obliqua* in the field

exploited along with other natural enemies to control the pest effectively.

The conidiophores are simple with pear-shaped conidia (Plate 1–Fig. 3). Secondary, tertiary conidia and resting spores (Plate 1–Figs. 4, 5, 6 and 7) were also observed; rhizoids and cystidia were not found. The average length of primary conidia was 31.52± 0.51 μm (±S.E.) (range: 20–46 μm; *n*=100) and the average width was about 22.71±0.39 μm (±S.E.) (range: 14–38 μm; *n*=100) at x1250 magnification with a length:width ratio of 1.39±0.01 (range: 1.18–1.63; *n*=100). Average length of secondary conidia was 26.42 μm±0.36 (±S.E.) (range: 22–31 μm; *n*=50) and the average width was ~19.38 μm±0.27 (±S.E.) (range: 15–23 μm; *n*=50) with a length:width ratio of 1.37±0.02 (range: 1.13–1.67; *n*=50). The diameter of the resting spores was in the range of 18–35 μm (average: 22.8 μm±0.39; *n*=50). Nuclei stained readily in aceto-orcein, and conidia were found to be multinucleate. The total number of nuclei ranged from 10 to 18 per conidium (average: 13.98± 0.28; *n*=50) and the number of nuclei in hyphae varied from 6 to 17 (average: 11.8±0.37; *n*=50). These morphological features fit well with the earlier descriptions of *E. aulicae* (Hajek et al. 1996; Humber 1997; Keller 1987; Yamazaki et al. 2004).

The *E. aulicae* species complex has a Holarctic distribution with records from Europe, Asia and North America. Around the Pacific Ocean, members have been reported from British Columbia and Japan (Hajek et al. 1996). This species complex includes several members that are well known for the epizootics they cause, e.g. in *Euproctis chrysorrhoea* (L.), *Estigmene acrea* (Drury), *Lambdina fiscellaria* Hulst, *Choristoneura fumiferana* Clemens, *Orgyia vetusta* Boisduval (see Hajek et al. 1996), *Chionarctia nivea* (Ménétriès) (Yamazaki et al. 2004), etc. In Canada, it is a naturally occurring fungal pathogen of eastern hemlock caterpillar and the eastern spruce budworm and is being used for biological control of these pests (Nolan 1993). This fungus is well adapted to serve as a pathogen under relatively cool and moist boreal and maritime coniferous forest conditions (McDonald and Nolan 1995). The environmental factors which are generally considered to affect a fungal pathogen of insects include relative humidity, temperature, light, air movement, nutrient availability, and host physiological status (Tanada and Kaya 1993). The prevailing climatic conditions of this region (moderate temperature, high humidity and rainfall) are highly suitable for the establishment of fungal pathogens. *S. obliqua* is a polyphagous pest, which attacks many crop plants and hence the discovery of this pathogen with high epizootic potential (Fig. 8) may prove to lead in the future to a promising biological control agent.

Acknowledgments CMSK gratefully acknowledges the assistance of Dr. A. B. Jensen, University of Copenhagen, for helpful comments on the manuscript. Thanks are due to Drs. R.A. Humber and S. Keller for sending us pertinent literature. We thank the anonymous reviewers for their valuable suggestions, and the Directors of NEIST & IBSD for providing facilities.

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