Metarhizium cylindrospora CHEN et GUO (Deuteromycotina:
Hyphomycetes), the Causative Agent of an Epizootic on
Graptopsaltria nigrofuscata MOTCHULSKI
(Homoptera: Cicadidae)

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(Received April 20, 1989)

An epizootic of a *Metarhizium* species occurred in a population of the large brown cicada, *Graptopsaltria nigrofuscata*, in forests of Japanese alder, *Alnus japonica*, in Ibaraki, Japan. The average density of the cadavers of *G. nigrofuscata* per trunk base (defined as the bottom 6 m of trunk) was 9.3 in the center of the epizootic. Living adults were seldom observed. The isolates produced 2 types of conidia, macro- and microconidia, on a common phialide. Macroconidia were cylindrical to banana-shaped, $16.9 \times 3.8 \mu\text{m}$, and microconidia were ellipsoid, $5.4 \times 2.7 \mu\text{m}$. The conidial chain was united in a column. Cultures derived from either micro- or macroconidia produced 2 types of conidia. This fungus was identified as *Metarhizium cylindrospora*, although only macroconidia had been described in its original description.

INTRODUCTION

In September of 1988, an epizootic occurred in an adult population of the large brown cicada, *Graptopsaltria nigrofuscata* MOTSCHULSKI, in forests of Japanese alder, *Alnus japonica* STEUD. This cicada is one of the most common species in Japan. Adults oviposit on trunks of various trees and sometimes damage fruit orchards, and nymphs injure roots. The causative agent of this epizootic was identified to be a rare species of genus *Metarhizium*. Moreover, epizootics of *Metarhizium* in cicada population have not been common. Characterization and identification of the pathogen were therefore carried out in addition to field observation.

MATERIALS AND METHODS

Two study plots, A and B, were set up in forests of *A. japonica* along Kokai River, Shimotsuma, Ibaraki, Japan, to investigate the prevalence of the disease. Plot A was about 100 m from plot B, separated by a marshy area and a vegetable field. Ten trees in each plot were chosen randomly and number of cicada cadavers on the bottom 6 m of the trunks were counted on September 20, 1988.

The conidia from the cadavers were streaked on plates of SABOURAUD's dextrose

agar containing 1% yeast extract (SDY) to obtain fungous isolates. The isolates were cultured on either SDY, silkworm pupal extract agar containing saccharose and soy sauce (PES), or autoclaved silkworm pupae. Conidiogenous structures of the fungus on slide cultures, host cadavers and autoclaved silkworm pupae were investigated with a microscope. Size of the conidia on the host was measured with vernier calipers on the screen of a microscopy television set.

Monospore cultures were isolated from solitary conidia by a hanging drop culture of the conidial suspension in SDY on a sterilized cover slip. Types of conidia from the monospore isolates were checked.

Three isolates from the microconidia were planted on SDY plate in a 9 cm petri dish, and placed at 25°C in total darkness. Diameters of the colonies were measured every 2 or 3 days for 18 days. Four replicates were measured for each isolate.

RESULTS

Adult cicadas killed by the fungus were seen on the trunks of almost all of the trees in the center of the epizootic (plot A). Some cadavers were also found on the leaves of the understories and on the ground. The number of cadavers ranged from 3 to 17 per tree with a mean of 9.3 ± 1.4 (SE). On plot B, cadaver count ranged from 0 to 5 with a mean of 1.3 ± 0.5 (SE). Living adults were rarely found.

The cadavers on the trunk had stiffened and were fastened to trunks by their legs (Fig. 1). Mycelia extruded from the intersegmental membranes and had sporulated in most of them. On other cadavers, outer mycelial growth was not present until placement into a humid chamber at 25°C. All the cadavers examined were infected with this fungus.

The fresh conidial masses were pale grayish green, and turned to olive green as they grew older, but never became as dark as those of *M. anisopliae*. The conidia were united in dense heaped masses, contrasting with those of *M. anisopliae* (which are flat and layer-like and easily fall off the cadavers).

Isolates from the cadavers developed white cottony hyphae on SDY and PES, and some produced dark brown pigment in the media. This fungus sporulated fairly well on autoclaved silkworm pupae, but poorly on slants of SDY and PES.

The conidia were borne basipetally on phialides. Phialides were clavate, branched from a conidiophore. Seven phialides were usually borne from a conidiophore.

There were 2 types of conidia, long (macroconidia) and short (microconidia), which formed on common phialides. Macroconidia were cylindrical to banana shaped, both ends narrower, $12.9\text{--}19.7 \times 2.3\text{--}3.4$, mean 16.9 ± 0.4 (SE) $\times 3.8 \pm 0.1$ (SE) μm . Microconidia were ellipsoid, $4.2\text{--}7.2 \times 2.3\text{--}3.4$, mean $5.4 \pm 0.1 \times 2.7 \pm 0.03$ μm (Fig. 2). Both types of conidia were slightly green under the microscope.

Usually, phialides bore microconidia prior to the formation of macroconidia (Fig. 3). Microconidia and macroconidia were therefore the top and base in a conidial chain (Fig. 4), respectively. The conidial chains united in a column structure. As 7 phialides were branched from a conidiophore, a conidial column usually consisted of 7 conidial chains. Each conidial column was very coherent, because conidial production phases on phialides were synchronized very well with the other phialides branched from a common conidiophore. The tendency to form conidial columns was more prevalent in this fungus than in *M. anisopliae*. Macroconidia in particular tended to stick

Figs. 1–4. *Metarhizium cylindrospora*. 1: On adults of *Graptopsaltria nigrofuscata*. 2: Micro- and macroconidia. Note macroconidia forming bundles. 3: Young phialides bearing microconidia. 4: Old phialides bearing micro- and macroconidia.

together laterally rather than longitudinally in order to form macroconidia bundles (Fig. 2).

Single spore isolates from the microconidia produced both macro- and microconidia. Macroconidia formed bundles, making it difficult to obtain a solitary macroconidium. However, an isolate obtained from the macroconidia bundle produced both types of conidia, indicating the production ability of this fungus.

The growth rate of the isolates is shown in Fig. 5. It varied among the isolates

Fig. 5. Mycelial growth of 3 isolates of *Metarhizium cylindrospora* on SDY plate. Each line represents the average of diameters of colonies of 4 replicates, vertical bars show ranges.

even though their parent culture was the same. Average diameter of a colony was 25.4 to 36.9 mm at 11 days and 44.3 to 60.3 mm at 18 days.

DISCUSSION

This fungus was considered to belong to genus *Metarhizium*, based on its mode of the conidial arrangements and the formation of conidial column. Genus *Metarhizium* is divided into 6 species (8 varieties) today, which are *Metarhizium album* PETCH (ROMBACH et al., 1987), *Metarhizium anisopliae* (METSCH.) SOROK. var. *anisopliae* (TULLOCH, 1976), *Metarhizium anisopliae* (METSCH.) SOROK. var. *majus* (JOHNSTON) TULLOCH (TULLOCH, 1976), *Metarhizium cylindrospora* CHEN et GUO (GUO et al., 1986), *Metarhizium flavoviride* GAMS et ROZSYPAL var. *flavoviride* (GAMS and ROZSYPAL, 1973), *Metarhizium flavoviride* GAMS et ROZSYPAL var. *minus* ROMBACH, HUMBER et ROBERTS (ROMBACH et al., 1986), *Metarhizium guizhouense* CHEN et GUO (GUO et al., 1986), and *Metarhizium ping-shaense* CHEN et GUO (GUO et al., 1986). The present species is characterized by the formation of 2 types of conidia on a common phialide. This does not coincide with the description of any species of *Metarhizium* already known. Except for this characteristic, this fungus resembles *M. cylindrospora*. GUO et al. (1986) described only 1 type of conidia of *M. cylindrospora*, with shape and size almost identical to those of the macroconidia of the present fungus. Their illustration and SEM photograph, however, showed that *M. cylindrospora* formed both long and short conidia on the same phialide, and that the latter were chained beyond the former. Their isolate does not appear to produce short conidia as abundantly as the fungus in this study. They consider short conidia to be immature ones (GUO, personal communication). However, the short conidia on the top part must be older than the long ones on the bottom of the conidial chain, which are basipetal. I have also found immature conidia of the present fungus which were sometimes similar to microconidia, but were distinguishable by being chained between phialides and macroconidia. Both types of conidia of the present fungus germinated during culturing and hence were proved to be matured. I conclude that my isolates and GUO's isolate belong to the identical species, *M. cylindrospora*, and the original description by GUO et al. (1986) lacks an explanation of microconidia.

The formation of 2 types of spores should be added to the characteristics of *M. cylindrospora*.

Species of *Metarhizium* sometimes cause epizootics in insect populations. However, those in cicada populations have seldom been recorded. AOKI (1957) investigated fungal epizootics on various wild insects in Japan, and the numbers of diseased insects found in the field during 1 season were recorded. Although there are several examples of cicadas infected with *M. anisopliae* in his records, the individual numbers are so small that they were not thought to be prevalent. There are also a few records of *M. anisopliae* var. *majus* (=var. *major*) on the periodical cicada (TULLOCH, 1976) and on *Cyphotympana mandarina* (LIU, 1988). It is uncertain, however, whether epizootics occurred in these cases or not. Epizootics on leaf- and planthoppers, which are members of auchenorrhyncha as well as cicadidae, have been reported; *M. flavoviride* var. *minus* on *Nilaparvata lugens* and *Recilia dorsalis*, and *M. album* on *Nephotettix virescens* and *Cofana spectra* (ROMBACH et al., 1987).

According to GUO et al. (1986), *M. cylindrospora* was originally isolated in China from an unidentified soilborne insect, but it is not clear from his paper whether it was a species close to *G. nigrofuscata*, and whether the fungus caused an epizootic or not. This species has not been reported from other countries. Even in China, this species is very rare (Guo, personal communication). Therefore, the present epizootic in *G. nigrofuscata* population caused by *M. cylindrospora* can be regarded as an unusual phenomenon. The weather in Japan in 1988 was very unusual; temperature was low and rainfall was extraordinarily heavy in August and September in that year. Such weather conditions in 1988 might promote the prevalence of this fungal disease.

ACKNOWLEDGEMENTS

I wish to thank Dr. Takeshi MATSUMURA of the National Institute of Agro-Environmental Sciences for informing me of the occurrence of the epizootic and informing me of the sites, and Dr. Masakazu GUSHIKEN of the Okinawa Forestry Experiment Station for his kind cooperation in the field investigation.

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