NEW *BEAUVERIA BASSIANA* (Bals.) Vuill. ISOLATE FROM *IPS DUPLICATUS* (Sahlberg)

Mihaela Monica Dinu[1], Daniela Lupăştean[2], Gabriel Cardaş[3], Ana-Maria Andrei[1]*

[1]Research-Development Institute for Plant Protection, Bucharest
[2] "Stefan cel Mare" University – Faculty of Forestry, Suceava
[3] Forest National Administration RNP-ROMSILVA, Botoşani Forestry District

[*]correspondence address: frommaca@gmail.com

Abstract: Adults of Northern bark beetle *Ips duplicatus* (Sahlberg) showing symptoms of white muscardine were found under a Norway spruce bark - *Picea abies* (L.) H. Karst. Isolation and fungi identification procedures were performed. Fungus was identified as *Beauveria bassiana* (Bals.) Vuill. Koch's postulates were used to prove a causal relationship between *B. bassiana* and the diseased *I. duplicatus*. Inoculated insects showed symptoms similar to the previously observed. The mortality caused by *B. bassiana* to *I. duplicatus* reached 100% within 4 days after the artificial inoculation.
The study is the first report of an occurrence in natural outbreaks of this association between the entomopathogenic fungus *B. bassiana* and the bark beetle *I. duplicatus*.

Key words: *Beauveria bassiana, Ips duplicatus*

INTRODUCTION

In Romania, *I. duplicatus* was first detected in 1948 in Rarău Mountains (the North of Eastern Carpathians) and until 1970 the species was sporadically observed along the Southern Carpathians, too. At that time, all records about *I. duplicatus* had a faunistic appoach (Negru et al.,1957; Vasiliu et al., 1978). In 2011, a study demonstrated the presence of *I. duplicatus* in almost the entire spreading area of the Norway spruce in the Carpathian Mountain, but also in some spruce stands installed outside the natural area. The epidemic level of the *I. duplicatus* population was recorded in the northeastern Romania, in Norway spruce stands outside the natural area and growing below the altitude of 1,000 m (Duduman et al., 2011). Bark beetles (Coleoptera: Scolytidae) are widely recognized as some of the most damaging forest insects. They prefer weakened tree but they can infest healthy trees as well, especially when the climate conditions favor an outbreak in the pest population density: extended drought, stagnosols (pseudogleic soils), high density of spruce trees (over 2,000 per hectare), etc. (Holuša, 2001; Olenici et al., 2011).

B. bassiana (Bals.) Vuill. is an enthomopathogenic fungus that grows naturally in a wide range of habitats (Steinhaus, 1956). It is a facultative pathogen infecting species from most insect orders, being an important agent for controlling pests (Andrei, 1999).

In order to avoid application of toxic pesticides in forests, efforts are now directed towards searching for natural enemies of pests - predators and parasitoids, nematodes and entomopathogens and combination of some of them with pheromone traps (Vaupel and Zimmermann, 1996; Kreutz et al., 2001).

MATERIAL AND METHOD

The *P. abies* logs were brought from a Norway spruce forest from northeastern Romania (40 year-old trees). The samples (logs with girth of 43 cm and length of 11 cm) were taken from a plot in the hilly area of Botoşani County (Direcţia Silvică Botoşani, Ocolul Silvic Mihai Eminescu, UP II Vorona). Logs were skinned with a knife and many live larvae and young adults of *I. duplicatus* were found under bark (Fig. 1).

Fig. 1. Samples of *Picea abies* logs infested with bark beetles

Two adults of *I. duplicatus* were found dead under the bark of one of the *P. abies* logs.The bark beetles had signs of infection and extrusion of fungal structures on the body (Fig. 2-3).

Fig. 2. *I. duplicatus* adult found dead under bark

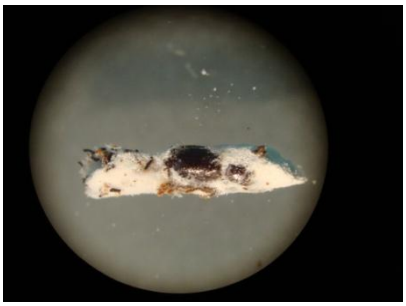

Fig. 3. Detailed view with stereo binocular microscope

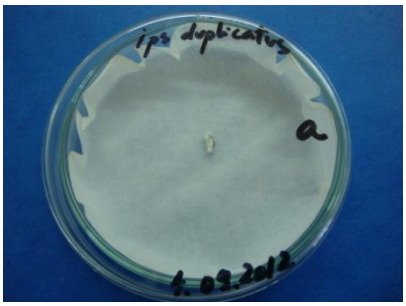

Fig. 4. *I .duplicatus* in a Petri dish moist chamber

One of the two adults was collected and transferred to a Petri dish moist chamber containing sterile and moistened filter paper, and incubated at 26 ± 2°C, 14 h photoperiod, for 7 days, then submitted to the process of fungi isolation (Fig. 4).

Samples from the second specimen (Fig. 5) were taken and submitted to the process of fungi isolation in Petri dishes containing PDA culture medium (Fig. 6). The plates were incubated for 10 days and samples of pure colonies were observed under the microscope (Fig. 7). Noting the presence of conidia, the fungus was inoculated on sterilized potato dextrose agar slants and incubated for 7 days (27 ± 1° C, 4 h photoperiod). The morphologic characteristics defined by Barnett (1962) were used for identification. The fungus was identified as *Beauveria bassiana* (Bals.) Vuill.

Fig. 5. *I. duplicatus* adult found dead under bark

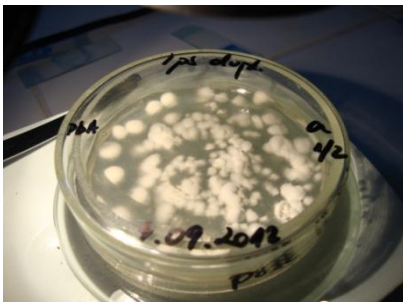

Fig. 6. Samples from. *I. duplicatus* on PDA culture medium

Fig. 7. Microscopic view of conidia

Afterwards, a bioassay was conducted to verify the pathogenicity of the isolate as postulated by Koch. The isolate fungus was multiplied on a PDA culture medium in Petri dishes and a suspension was obtained (1×10^9 conidia/ml). The artificial inoculation was made by spraying a number of 30 *I. duplicatus* adults with *B. bassiana* conidial suspension. The inoculated adults were transferred to an insect growth chamber and evaluated every day over a period of 4 days (Fig. 8).

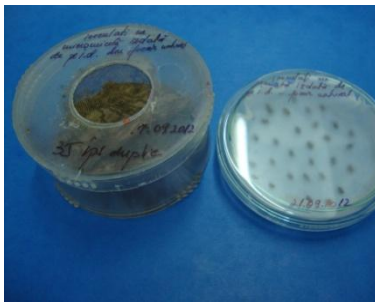

Fig. 8. Insect growth chamber and Petri dish moist chamber

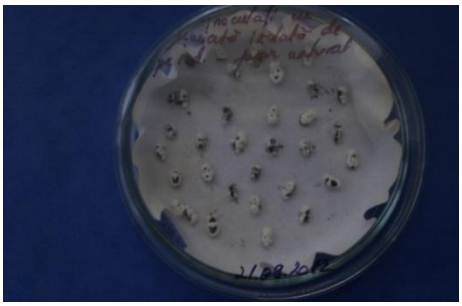

Fig. 9. Adults with white muscardine

Fig. 10. Detailed view by stereo binocular microscope

Five insects were not inoculated with conidial suspension but were transferred along with the 30 inoculated ones to the same insect growth chamber.

RESULTS AND DISCUSSION

Four days after inoculation, all 35 beetles were found dead. Dead insects were collected and then transferred individually to a Petri dish moist chamber as described before. One week later, all the dead adults of Northern bark beetle were showing symptoms of white muscardine (white aerial mycelia and conidia emerging from the dead insects) (Fig. 9-10). The new fungal strain had the ability to adhere and penetrate the insect cuticle, proving the pathogenicity character; *I. duplicatus* showed a high degree of susceptibility to *B. bassiana* infection.

Based on the morphological characteristics, the recovered fungus was the same as the innoculated one and was identified as *B. bassiana*: mycelium white with a white powdery appearance; conidiophores single, irregularly grouped or in verticillate clusters, inflated at the base, tapering to a slender fertile portion that appears zigzag after several conidia are produced; conidia (sympodulospores) hyaline, rounded to ovoid, l-celled (Fig. 11).

Many records can be found dealing with entomopathogenic fungi in bark beetles. "The possible role of vectors (e.g. predators, parasitoids) in spread of infection through transmission of fungal spores within bark beetle galleries was mentioned in early investigations (Karpinski,1935; Doane, 1959). Many fungi were found in galleries of bark beetles (31different species) from different tree species, and even if beetles were not present int heir galleries, several of them were known as entomopathogenic fungi (*Beauveria bassiana*, *Beauveria caledonica*, *Conidiobolus coronatus*, *Paecilomyces farinosus*, *Tolypocladium cylindrosporum* and *Verticillium lecanii*), *B. bassiana*, *B. caledonica* and *P. farinosus* were the most abundant species (Kirschner, 1998, 2001; Landa et al., 2001)" (Wegensteiner, 2007). Doberski and Tribe (1980) reported *B. bassiana* isolation originated from infected insects on or under the bark of elm trees and from soil at the base at Culford, Suffolk and near Cambridge during 1976 and 1977.

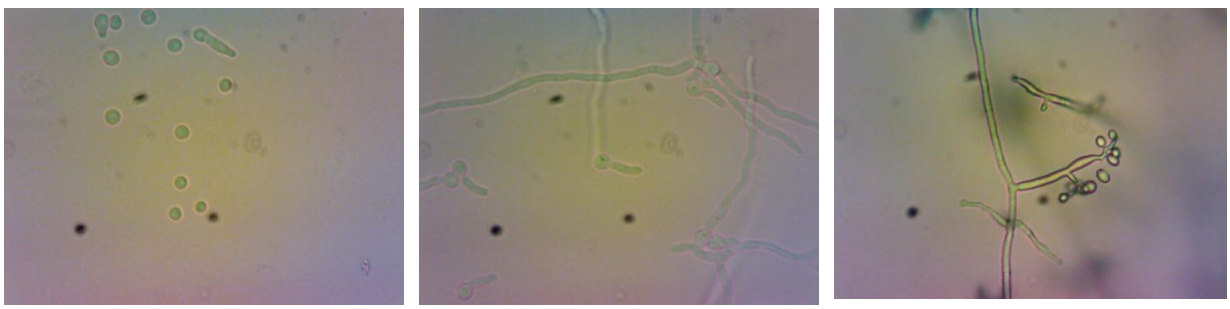

Fig.11. Microscopic view of early development stages of *B. bassiana* isolated from *Ips duplicatus*

CONCLUSIONS

A new *B. bassiana* strain was isolated from the bark beetle *I. duplicatus*. This is the first record of a naturally occurring fungal infection and disease caused by *B. bassiana* to bark beetle *I.*

duplicatus. The bioassay carried out on host insects showed the high potential of *B. bassiana* to control this pest. Further studies should be conducted to evaluated this new *B. bassiana* strain in order to use it in the biological control of the Northern bark beetle *I. duplicatus*

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