***Beauveria bassiana* on overwintering adults of *Stereonychus fraxini* in Croatia**

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**Abstract**: The ash weevil, *Stereonychus fraxini*, occurred in high infestation levels in 2009 causing serious damage on different ash species in lowland forests in Lipovljani, Croatia. Overwintering adults in moos on the bottom of trees showed high mortality rates(over 80%). Most of these beetles were infected with the fungus *Beauveria bassiana*, which reduced the population density of adults by 53.7%. The naturally occurring strain was isolated and tested on overwintering individuals under laboratory conditions. Laboratory experiments demonstrated that this strain was very virulent on adult weevils. Due to restrictions in use of insecticides in Croatian forests, biological control using *B. bassiana*, is a promising option.

**Key words**: *Beauveria bassiana*, *Stereonychus fraxini*, *Fraxinus* spp.

**Introduction**

The ash weevil, *Stereonychus fraxini* Deg. (Coleoptera, Curculionidae), is known as a major defoliator on different ash species (*Faxinus* spp.) in lowland forests in Croatia (Tomiczek et all. 2008) and has also been reported as a pest in some South European countries (Schwenke 1994, Miklos 1977, Markova 1992). The adults overwinter on the ground, either in the soil, litter or in the moos on the bottom of the trees. In the spring adults move to tree crown where they damage buds by feeding or boring holes for egg deposition. Damage can cause late sprouting. The young larvae feed on buds and leaves causing, in severe attacks, defoliations. To overcome this problem forest managers used chemical control successfully until few years ago when chemical control measures were banned according to the guidelines of national standard for forest certification (FSC - Forest Stewardship Council). Since then, defoliations caused by the ash weevil are very common (Fig. 1.) and alternative measures are discussed.

Because little is known about the natural enemies of *S. fraxini* (Markova 1998), the aim of this preliminary study was to find the infection rate of the disease caused by *B. bassiana* and to test the virulence of a newly out of the infected beetles developed strain.

**Material and methods**

The study was carried out in winter 2009/2010 in a mixed oak-ash stand in Lipovljani in Croatia. In October 2009 moos from 20 randomly chosen trees (oaks and ashes), has been collected, brought and incubated in the laboratory. In total 3.2 m2 of moos were observed. Beetles were counted as healthy and dead. Healthy beetles were transferred to Petri dishes and stored at 4°C. Dead beetles covered by the white mycelium of *Beauveria bassiana* (Bals.) Vuill. were separated and stored at 4°C. A small amount of the fungus from infected beetles has been transferred onto a medium containing Potato dextrose agar. On December 15th, 30 healthy beetles were left to move for 5 minutes in cultures of *B. bassiana*. Another 30 healthy individuals were left to move for 5 minutes in empty Petri dishes serving as control beetles. Afterwards they were transferred into separated Petri dishes and stored in clime chambers at 22°C (±2°C) and under a 16L: 8D photoperiod.

**Results and discussion**

Out of 3.2 m2 moos brought from 20 trees in the field, 1.640 beetles were counted which amounts 512 beetles per 1 m2 moos. Only 19.6% of beetles (Table 1.) were alive. Accordingly, this is at least the percentage of beetles capable to attack buds in the spring. On the other hand 1.318 or more than 80% of beetles were dead, from which 881 (53.7%) had a *B. bassiana* infection (Table 1.).

Table 1. *Stereonychus fraxini* beetles examined from 3.2 m2 moos and their infection by the entomopatogenic fungi *Beauveria bassiana* (Bb)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Total number of beetles | Alive beetles | | Death beetles | | Bb infected beetles | |
| N | n | % | n | % | n | % |
| 1640 | 322 | 19.6 | 1318 | 80.4 | 881 | 53.7 |

This result shows that *B. bassiana* was the main mortality factor on overwintering *S. fraxini* adults. Although the density of overwintering beetles in the moos shows a high population, the recorded high mortality decreased the population density in 2010 (Fig.1). This is also confirmed by the high decrease in number of overwintering beetles in the winter 2010/2011 when only 15 beetles per 1m2 of moos could be counted.

Figure 1. Percentage of defoliated trees in the forest district Lipovljani, Croatia

Laboratory tests with treated beetles show a clear susceptibility of infections of adult weevils by the isolated strain of *B. bassiana* (Fig. 2). 80% of treated beetles had a positive *B. bassiana* infection, compared to only 13% of untreated beetles.

Figure 2. Mortality (%) of *Sterenychus fraxini* beetle treated with *Beauveria bassiana* (Bb) strain

Because chemical control could not be used in Croatian forests, new gradations of ash weevil population are expected in subsequent years. Considering the fact that *B. bassiana* is acting as very important natural enemy of *S. fraxini* and the strain isolated and tested under laboratory conditions showed high virulence for weevil adults, further studies especially in the field are necessary.

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