

PRELIMINARY STUDY ON EFFICACY OF *BEAUVERIA BRONGNIARTII* BIOINSECTICIDES AGAINST COMMON COCKCHAFFER UNDER FIELD CONDITIONS

Ana-Cristina Fatu¹, Mihaela-Monica Dinu¹, Constantin Ciornei², Ana-Maria Andrei^{1*}

¹ Research-Development Institute for Plant Protection, Bucharest

² Forestry Research Station, Bacău

*correspondence address:

Research - Development Institute for Plant Protection

Bd. Ion Ionescu de la Brad nr.8, CP 013813, S1

Bucharest, ROMANIA

Tel.: 004-021-269 32 34

Fax.: 004-021-269 32 39

e-mail: anamaria_111@yahoo.com

Abstract: Among the root pests, the beetles in the family Scarabeidae are now the major biotic stress in forest nurseries. In the last two decades common European cockchafer, *Melolontha melolontha* L., recorded mass propagation and produced significant damage in Romania. The stringent environmental, economic, and social standards for responsible forest management and FSC certification of many forests in Romania have imposed restriction in the chemical insecticides use. Considering these aspects, RDIPP was funded by the National Forest Administration RNP-ROMSILVA to develop obtaining and application technologies for *M. melolontha* biocontrol. This paper presents the preliminary results of *Beauveria brongniartii* bioinsecticides application against *M. melolontha* adults under field conditions.

Key words: *Beauveria brongniartii*, *Melolontha melolontha*, biocontrol

INTRODUCTION

In the last two decades common European cockchafer, *Melolontha melolontha* L., recorded mass propagation and produced significant damage in Romania thus becoming the major biotic stress in forest nurseries.

In Romania, the larval evolution lasts for 3-4 years, depending on region. Flight period lasts for 3-4 weeks. After 15-20 days of feeding (supplementary feeding) for maturation of sex organs, egg deposition starts, in soil at 10-12 cm depth, in groups of 20-40. A female lays 90-120 eggs. Incubation lasts 4-6 weeks. Throughout the feeding period that begins in spring and lasts until autumn, the larvae perform vertical and horizontal movements, in relation to environmental factors (Manolache et al. 1967).

Beauveria brongniartii (Sacc.) Petch is a naturally occurring fungus and has been extensively studied for control of the May beetle or European cockchafer, *Melolontha melolontha*, in Switzerland, Denmark, Italy, Austria and Germany.

The presence of the swarming fly of the common cockchafer in spring 2012 in some north eastern areas of Romania (Moldavia) give the possibility to experiment some methods for adults biocontrol, by giving them treated food (by ingestion) and by direct infection with *B. brongniartii* spores (by contact) and followed by their releasing in the field.

The aim of this study was to evaluate the efficacy of biological *B. brongniartii* treatments against *M. melolontha* adults, as well as the extent to which this method of treatment ensures the spread of pathogens in the insect-target population.

MATERIAL AND METHOD

The foliar application of *B. brongniartii* insecticide have been conducted in north-eastern Romania, in forest nurseries situated at 180-350 m altitude. Two forest nurseries belonging to Roman and Dorohoi forest districts and one in Adancata forest district were selected for biological treatments administraton, considering the earlier records of feeding damage caused by *M. melolontha*.

A Stihl equipment for fine spraying was used to treat the trees (Fig. 1). Two liters of conidial suspension (10^{12} cfu/l) was obtained by washing one kilogram of barley kernels colonized with *B. brongniartii*, produced by RDIPP's Useful Organisms Department.



Fig. 1. Aspects during treatments application of conidial suspension of *Beauveria brongniartii* with ground suspension preparation and spraying with Stihl equipment, in Basta Nursery - Roman

Silk filters were used in order to avoid the obstruction of spraying equipment.

In all three forest nurseries, a 150 meters long of hardwood band was treated with 1 litre of conidial suspension /10 meter long. A 50 meters long of hardwood band was used as control. The presence of adults was determined in each field site, before the first treatments. The treatments were done at the end of aprilie 2012 and repeated after 3 days. The effectiveness of biological treatment was not made directly, by quantifying treated adults mortality, but indirectly by determining the number of larvae in the soil, resulting in the next generation of adults treated.

When direct treatment of the *M. melolontha* adults was performed, the adults were collected using light traps, from 26 April to 3 May 2012 (Fig. 2).



Fig. 2. Light traps installed in the Izvor nursery - Gura Humorului (a,b) in order to capture and treat *Melolontha melolontha* adults through direct contact with the RDIPP bioinsecticid(c,d)

After each night of collecting they were put in direct contact with the fungus by spraying (dipping) with a conidial suspension (10^{12} cfu/l) or by let them walking on barley kernels colonized with *B. brongniartii*, for one hour and released. A total of 710 adults (609 males and 101 females) were released back in the field. One treatment was carried out in a cherry and oak nursery from Dorohoi forest district. The expectations for a very high intensity fly were justified by the soil infestation, 0.7-1 adults /sqm.

First instar larvae were present also (0.2/sqm). The biological treatments efficacy was established two and four months after the treatment.

The second treatment was done in Izvor nursery from Gura Humorului forest district. Before swarming fly, 1.7 adults/ sqm could be found, leading to a very high intensity fly in that area. A total of 818 adults were captured, treated and released. No chemical treatments were used in soil.

RESULTS AND DISCUSSIONS

After four months, the effect of the foliar treatments on the reproduction/survival rate was estimated according to the larval density in soil. Although no first instar larvae were found in soil four months after treatments, but only second instar ones, the difference of density between the control and the treatment sites was obviously (Fig. 3).

A reason for these could be the very high temperature and lack of precipitation registered during the summer. According to Huiting and others (2006), the young L1 larvae are affected by extreme high temperatures and drought. The older larvae are able to avoid this by moving to deeper soil. No mycosed larvae were found.

The results on the first instar larvae in soil, after the direct treatment of the *M. melolontha* adults at different observation moments are presented in figure 4. Only for Izvor nursery a decrease of first instar larvae in treated sites from 4 to 2,33 was registered.

At the end of the summer (four months after treatment), in Pustoaia nursery, no presence of larvae was identified in the field. No chemical treatments were applied on soil. No sign of natural infection was found in any experimental areas. The results obtained during the observation period, are due to the low larvae density that prevented the spread of the pathogen. Extreme weather conditions in the summer of 2012 had also significantly influenced the biological tests results.

Many trials on the treatment of *Melolontha* adults with the *B. brongniartii* were conducted by Keller who reported, in 1978 the application of spores to adult female cockchafer and subsequent mortality in their larvae, thus demonstrating spread of the disease progeny (Gillespie et al., 1988).

In 1976, blastospores were applied along the edge of a forest to swarming beetles. The fungus was successfully introduced into the population, but population collapse occurred only during the second generation after treatment. In the following four generations, the population density remained stable at low level and fungus-induced mortality was still relatively high. In 1982, two further sites were treated in the same manner with similar results (Keller 1986, Keller et al. 1997). The studies revealed that the treatment of swarming adults in a forest area comprising about 2% of the breeding area would contaminate a much larger area. Preliminary trials using this approach under controlled conditions demonstrated that the fungus can be transmitted successfully to the soil environment, where a certain proportion of the young larvae succumbed to the disease (Keller 1997, Keller 1978). Related to the efficacy of this control method of white grubs, the same author suggested that it is likely to be unsuitable in crops where economic threshold is lower.

CONCLUSIONS

The aim was to use adults as vectors to transmit the disease into the breeding area. Effect of food treatment on *Melolontha* adults with *B. brongniartii* conidia, in spring 2012 cannot be clarified at this time because, even with density differences recorded between treated and control areas, no diseases of the larvae were identified yet. This will be followed and analyzed throughout the entire generation of pest development in experimental surfaces.

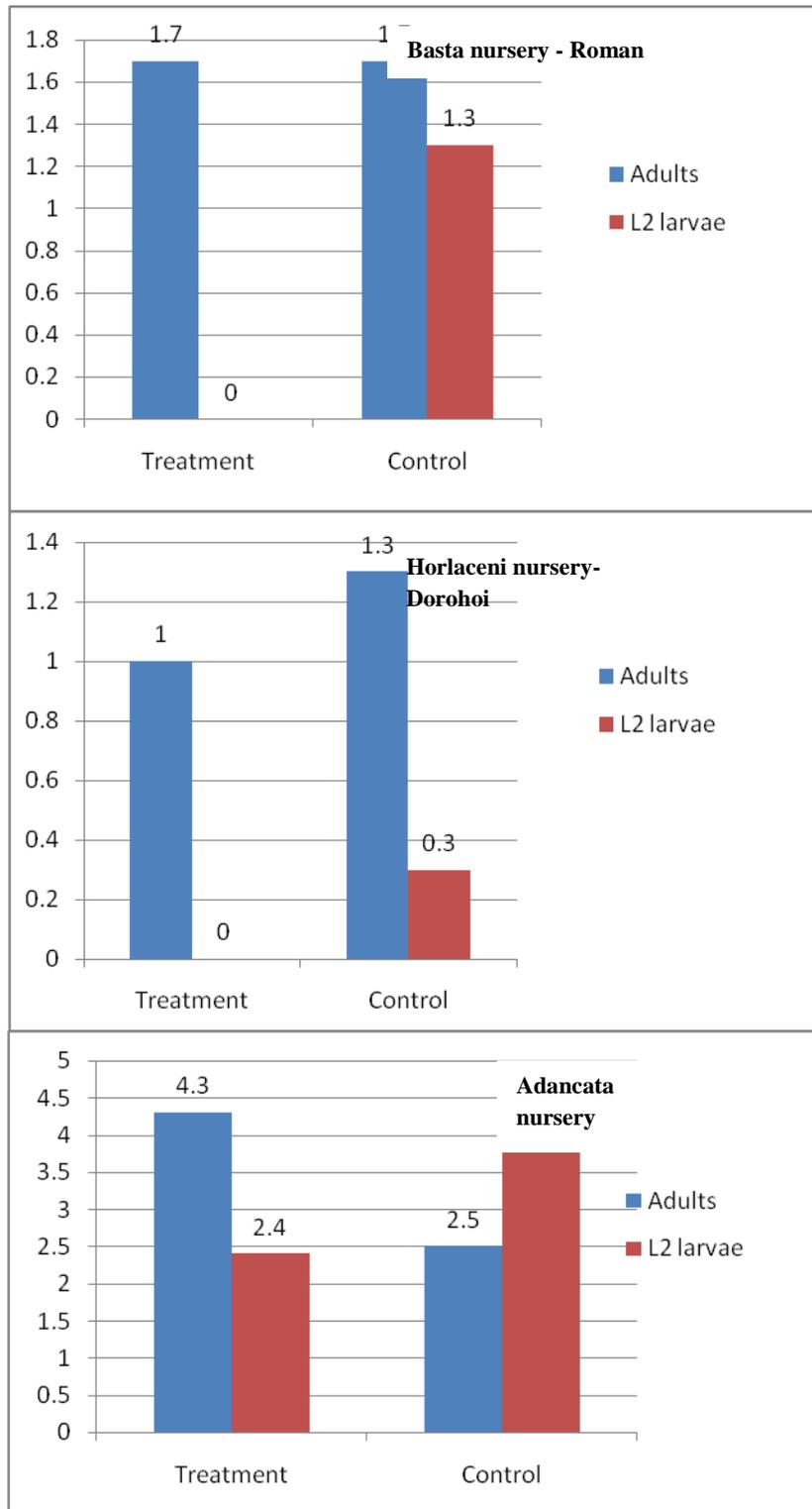


Fig. 3. The infestation level before and after the treatment of the trees, in the treatment sites

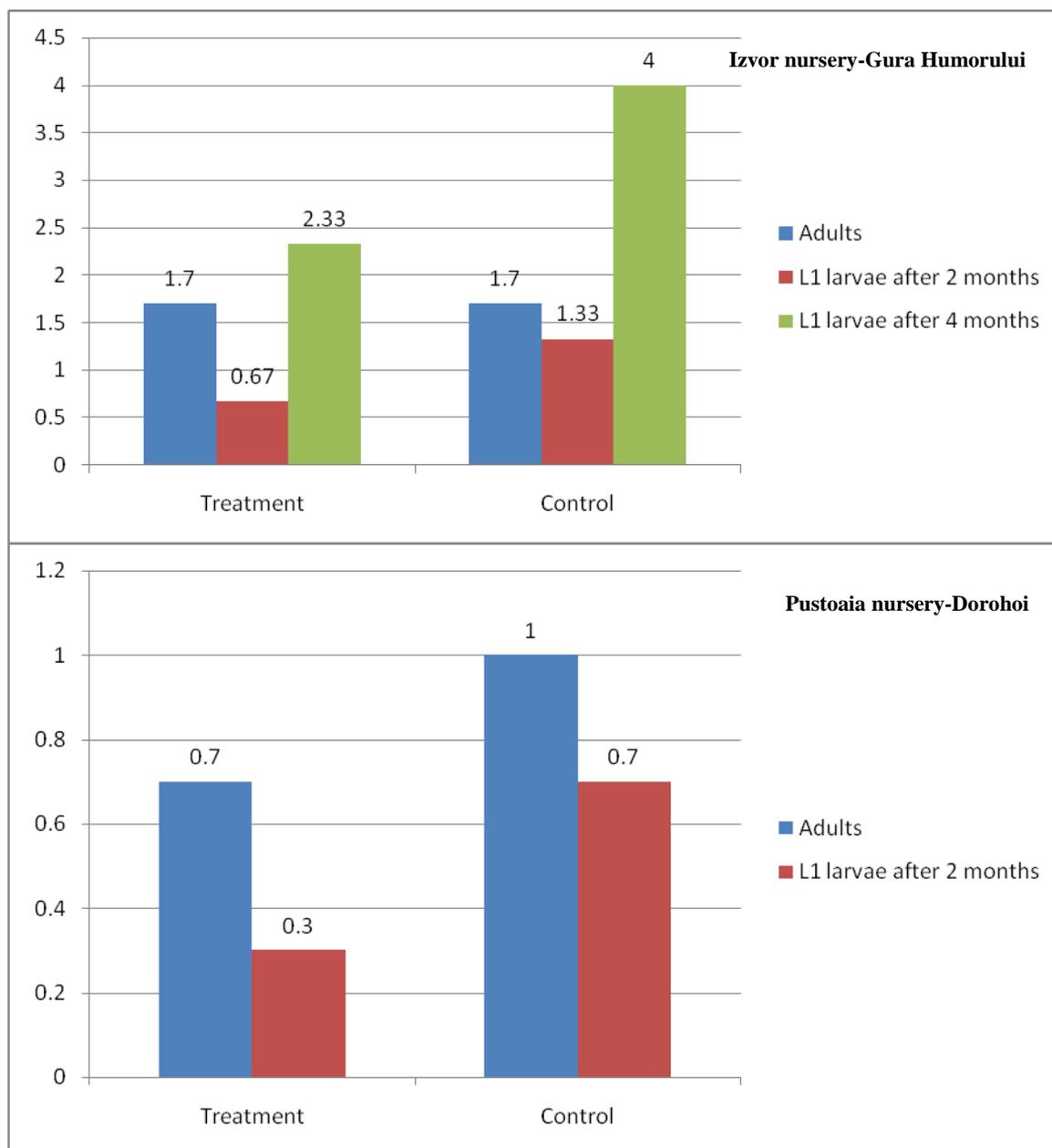


Fig. 4. The infestation level before and after direct treatment of the *Melolontha melolontha* adults

REFERENCES

- Gillespie, A.T. (1988). Use of fungi to control pests of agricultural importance. In *Fungi in Biological Control Systems* (Edt. M.N. Burge), p.44. Manchester University Press, UK. Available on: http://books.google.ro/books?id=qoK7AAAAIAAJ&pg=PA44&lpg=PA44&dq=larvae+lab+melolontha+beauveria&source=bl&ots=ZC1CrrxVNi&sig=9rt68qjWy3-0xt_Y2j07rWLSGdI&hl=ro&sa=X&ei=BKSQUZ6vBMHGtObQyYGgDA&ved=0CHIQ6AEwCTgK#v=onepage&q=larvae%20lab%20melolontha%20beauveria&f=false
- Huiting, H.F. Moraal, L.G. Griepink, F.C. & Ester, A. (2006). Literature report on biology, life cycle and pest incidence, current control possibilities and pheromones. In *Biology, control and luring of the cockchafer, Melolontha melolontha*, p. 13
- Keller, S. (1978). Infektionsversuche mit dem Pilz *Beauveria tenella* an adulten Maikafern (*Melolontha melolontha* L.) mitteilungen der Schweizerischen Entomologischen Gesellschaft 51, 13-19.
- Keller, S. Schweizer, C. Keller, E. and Brenner, H. (1997). Control of White Grubs (*Melolontha melolontha* L.) by Treating Adults with the Fungus *Beauveria brongniartii*, *Biocontrol Science and Technology*, 7:1, 105-116.
- Manolache, C. Boguleanu, GH. (1967). Daunatorii pomilor fructiferi. In *Entomologie agricola*. Ed. Didactica si Pedagogica-Bucuresti, p. 336