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BIOLOGICAL CONTROL OF *Melolontha melolontha* L. LARVAE WITH ENTOMOPATHOGENIC BIOINSECTICIDE BASED ON *Beauveria brongniartii*

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Abstract

From 2010 to 2013 tests were conducted with entomopathogenic bioproduct based on Beauveria brongniartii, in nurseries of the Botosani, Neamt and Suceava Forest Departments. At RDPP Bucharest a technology for multiplication on nutrtive solid substrate of entomopathogenic fungus B. brongniartii was developed, on the basis of which the experimental bioinsecticide BioMelCon (G) was obtained. Soil treatments were performed with the BioMelCon (G) in doses ranging from 100 to200 kg/ha. The tested dosages were achieved by applying two treatments per year. Application of biological product held generally early spring on surfaces free from crops, to ensure a uniform distribution and to create the possibility of periodic inspection of the treatment effect by soil surveys. Checking the effectiveness of treatment was carried out in several stages, in different site conditions. The paper presents results concerning the effect of applying several biological treatments on Melolontha melolontha larvae (L_2-L_3). The cumulative effect of the biological product and the agricultural practices of experimental fields have led to a decreased density of larval populations under economic threshold level.

Key words: *Melolontha melolontha, Beauveria brongniartii, microbial control.*

INTRODUCTION

The European cockchafer or May bug, *Melolontha melolontha* (L.) (Coleoptera: Scarabeidae), is considered a serious pest in agriculture, horticulture and forest nurseries.

M. melolontha grubs are feeding underground on the roots of a wide range of plants and adult cockchafers feed on forest tree leaves - in particular oaks (*Quercus* sp.), beech (*Fagus* sp.), maple (*Acer* sp.) - as well as on fruit trees, producing damages to trees and crops. The unavailability of a selective pesticide and the restrictive measures laid down by EC Directives 91/414 requirements lead to development of alternative control measures including biological control.

Natural epizootics of entomopathogenic fungi often prevent European cockchafer outbreaks and contribute to decreasing of pest populations. *Beauveria brongniartii* (Sacc.) Petch is a naturally occurring fungus which was repeatedly observed to cause epizootics among *M. melolontha* populations (Keller et al., 2007). In Romania, Ciornei et al. (2006) discovered natural infections of *M. melolontha* with *Beauveria* sp. on 3rd instar larvae (L_3), pupae and adults, during a screening of 116 nurseries from 38 eastern Romanian forest districts. Numerous studies were conducted regarding the efficacy of treatments with different formulations of *B. brongniartii* based products against European cockchafer

(Vestegaard et al., 2002; Kessler et al., 2004; Keller et al., 2007; Keller & Schweizer, 2007; Fătu et al., 2013). Several biological products based on the entomopathogenic fungus *B. brongniartii*, have been marketed in Europe: Melocont®–Pilzgerste, *Beauveria brongniartii* Myzel, *Beauveria* Schweizer, Engerlingspilz (Faria and Wraight, 2007). Unfortunately, these strains has not been approved anymore and bioproducts based on entomopathogenic fungi are no longer commercially available for biological control of *M. melolontha* in Europe.

This paper presents the results obtained during four years of investigations on the effects of consecutive applications of *B. brongniartii* barley kernels on *M. melolontha* population density, in some Romanian forest nurseries.

MATERIALS AND METHODS

Experimental areas

The experiments were conducted in three forest nurseries situated in north and north-east of Romania: Basta-Roman (NFA Branch Neamt), Zavoi-Siret (NFA Branch Botosani) and Izvor (NFA Branch Suceava). The experiments started in 2010 and were the first trials carried out in Romania with this entomopathogenic fungus species. The experimental areas were selected based on the feeding damage caused by *Melolontha melolontha* larvae, in forest nurseries, the presence of more than one *M. melolontha* larva per square meter (1 L_3/m^2) means a high threshold, a single L_3 larva can completely destroy a one year old forest culture (Georgescu, 1957). The forest nurseries to be treated were situated in different stationary conditions (altitude of 180, 274 and 468 m respectively) and placed on medium texture soils (clays), on the riverside. Each trial site has been located after the Latin rectangle method including the control. The experimental surfaces ranged between 20 and 30 ares and was equally divided for each treated plot (variant). Surfaces between 1 and 3 ares were used for control. No chemical treatments were applied before and during the tests on the experimental areas, except Izvor nursery where 50 kg/ha Nemathorin 10 g was applied in 2012.

Experimental bioproduct

A patented Romanian *B. brongniartii* strain, BbgMm1a/09, was used in the tests. This strain was obtained from natural diseased *Melolontha* grubs, identified in forests located in the northeast of Romania. In previous studies, *B. brongniartii* was detected two years before application in only one experimental area, Basta Roman nursery, NFA Branch Neamt (Ciornei et al., 2006). The experimental bioproduct ($1x10^{13}$ conidia/kg) was obtained using an original fermentation method on barley grains (Fătu et al., 2014). New series of bioproduct were used for each annual application. The viability of conidia was cheeked before treatments.

Application method

For providing better conditions for surviving and activity of entomopathogenic conidia, the experimental areas preparation, as well as the granulated bioproduct incorporation into the soil was executed with a motorised cultivator between trees rows. A rotary hoe in non-cultivated areas was used. Treatments were applied two years consecutively so that the treated areas received 2.0-5.0x10 ^{15}spores ha^{-1}. The product was applied in three variants resulted in three annual doses: 100 kg/ha (V_1), 150 kg/ha (V_2) and 200 kg/ha (V_3). In 2010, one half of the annual dose of bioproduct was applied in two treatments, one month after the first one. Next year a single treatment was done and the biological product has been applied simple or in mixture (Izvor nursery) with beech humus in the same annual doses. The long term effects of treatments on density of *M. melolontha* larvae were evaluated by classical soil survey (1mx1mx1m) in each treated nursery, usually in spring and autumn. Biological treatment effect was annually evaluated during four years (2010-2013).

The infection rate was calculated with formula: 100M/Σ (M+L), where M - mean mycosed larvae and L- mean living larve.

RESULTS AND DISCUSSIONS

Infection rate in Başta-Roman nursery

The first trial carried out in Başta-Roman nursery, was in *Pseudotsuga taxifolia* Britt. cultivated field. In 2010 (19.09.) the field observations show the following rates of L_3 infection: 100% in plots treated with 100 kg/ha (V1) and 50% in plots treated with 200 kg/ha (V3). No infection was recorded in control and

V2 (150 kg/ha). These results suggest that after the first year of biological treatment, no correlation between the dosage of *B. brongniartii* bioproduct and the *M. melolontha* larvae infection rate could be registered.

In 2011, 56 days after biological treatment application, the mean infection rate was 100% only in V3, densities of living larvae being null in the other. No natural mortalities were registered in control areas. *B. brongniartii* mycelium identified in the soil samples taken from the experimental plots has induced larva mortality in 2013, resulting in a mean infection rate of 12.5% only in V2.

Infestation rate

In the autumn 2010 (01.09.) the relative density of grubs/m^2 decreased compared to spring, only in V1 and V2 (Figure 1). However, a direct proportion between the bioproduct dosage and the relative larvae density was observed one year later in the spring, when the infestation was totally reduced compared with the untreated area, where 100% grubs infestation was recorded. In 2012 when no treatment was applied, *M. melolontha* density was higher than the initial infestation recorded in 2010. At 2.5 years after the last treatment, the insect density from areas who received doses of 150 and 200 kg/ha has maintained the same level as that from the beginning of observations. The high infestation rates in control compared with treated variants at the end of the investigations suggested that the high spore treatments are responsible for the reduction of cockchafer population.

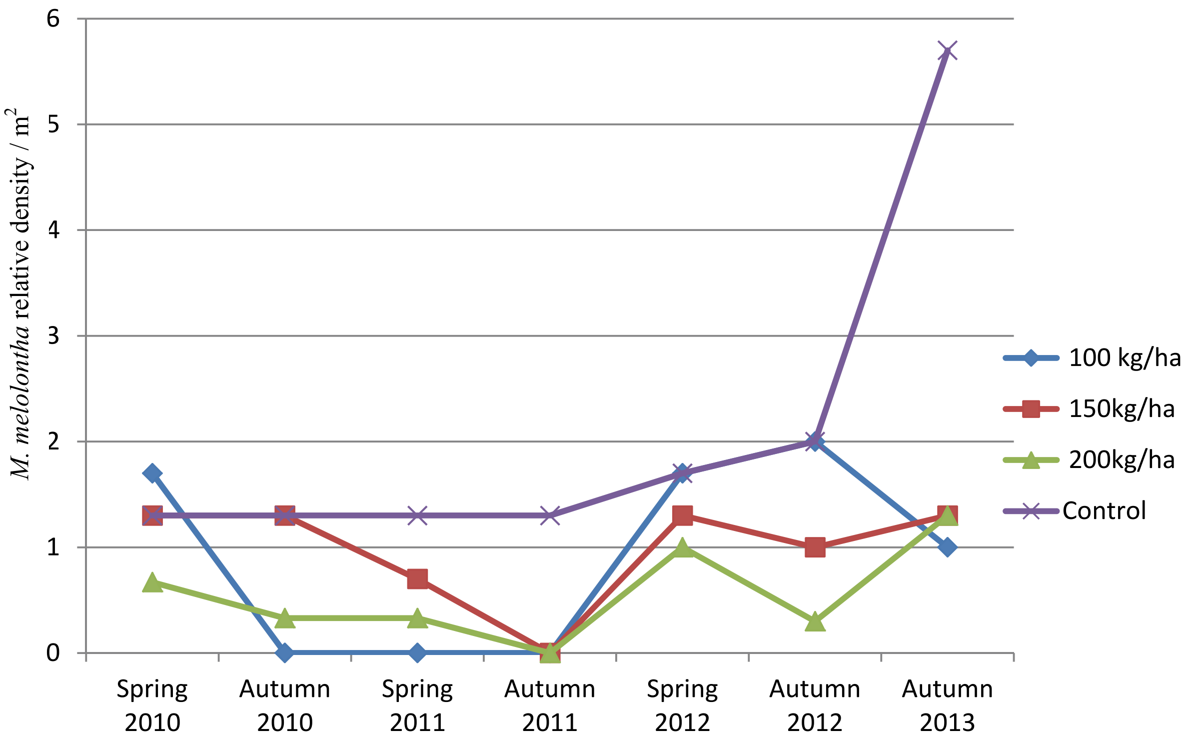

Figure 1. Level of mean infestation of *M. melolontha* at Basta-Roman nursery

Infection rate in non-cultivated nursery, Zavoi-Siret

There was a low mean L_3 infection rate, which ranged between 8% and 30% in all three variants in the first year of application. One third of the infections have been found at second instar larvae (L_2) in 100 kg/ha treated areas. Also 9% of *B. brongniartii* infected larvae was identified in control. These results could be influenced by the water flooding from July after which the Siret River had stalled over two weeks at 1 meter level. Also 75% death larvae by asphyxia were founded in one repetition in V1 where the water has stalled about a month. Next year, the mean infection rate increased with 80% in V1, 72% in V2 and with 50% in V3. In the flight year 2012 the *B. brongniartii* mycelium was present in the soil. In 2013 a mean infection rate of 20% and 23% was identified in V1 and control areas respectively.

Infestation rate

At Zavoi-Siret nursery, in the treated areas, the larval density gradually decreased from spring 2010 to autumn 2011, proportionally with the applied dosage (Figure 2). A slowly decline trend was registered also in control maybe because of the presence of *B. brongniartii* after the 2010 summer flood of which influences were evident even after one year since the last application (05.04.2011). Thereafter a fast increasing of infestation level was noted only in control and 100 kg/ha treated areas, the level corresponding with the maximum dose treated areas remaining constantly up to spring 2013 followed by a decline in all variants including the control, at the last period of experimental observations. These results could correspond to the infection with *B. brongniartii* in control and V1 as mentioned before or suggest that other factors then the treatment application could influence the *M. melolontha* density.

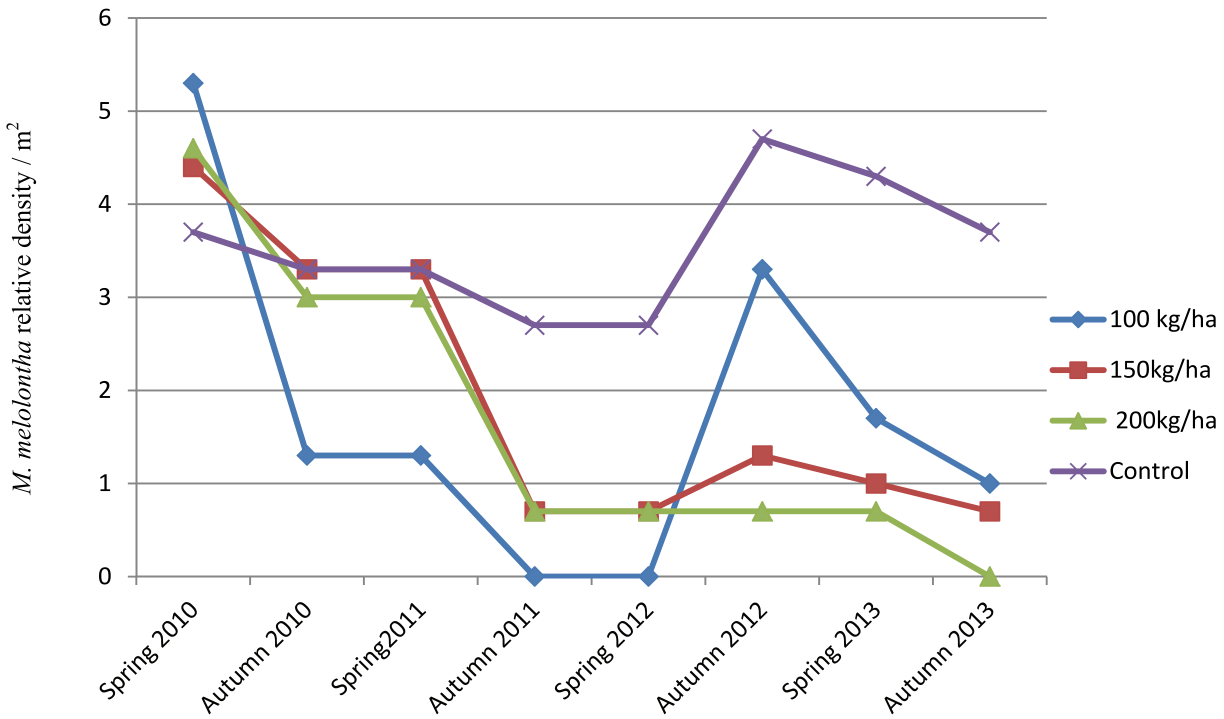

Figure 2. Level of mean infestation of *M. melolontha* at Zavoi-Siret nursery

Infection rate in Izvor nursery

In this nursery, the best conditions for the application of treatments has been provided by the very homogeneous incorporation of the biologically product into the soil. The total mortality produced after two treatment applications were at: 25% V1, 37% in V2 and V3, 50% with a positive dose-effect correlation in the first year. In the following year, the mean infection rate was at maximum level of 100% in V1 and V2, no larvae being detected in V3. No typically fungus infection mortalities were recorded in non-treated areas. Also in 2012 and 2013 no mycosed insect was identified.

Infestation rate

The overall picture shows that the maximum decreasing level of insect density was reached after 1.3 years since the beginning of the treatments which is corresponding to the high infection level due to the fungus (Figure 3). Thereafter the trends of the infestation level followed the same pattern for all variants. In Izvor nursery treatments carried out with the same annual doses in the years 2010 and 2011, on non-cultivated, followed by spruce cultures in 2013 have led to a decrease in the density of larvae beetles in fall 2013 (12.09), compared with fall 2012, as a percentage of 76.9% in V1 and 100% in V2 and V3. Natural mortality was 15% in the control area.

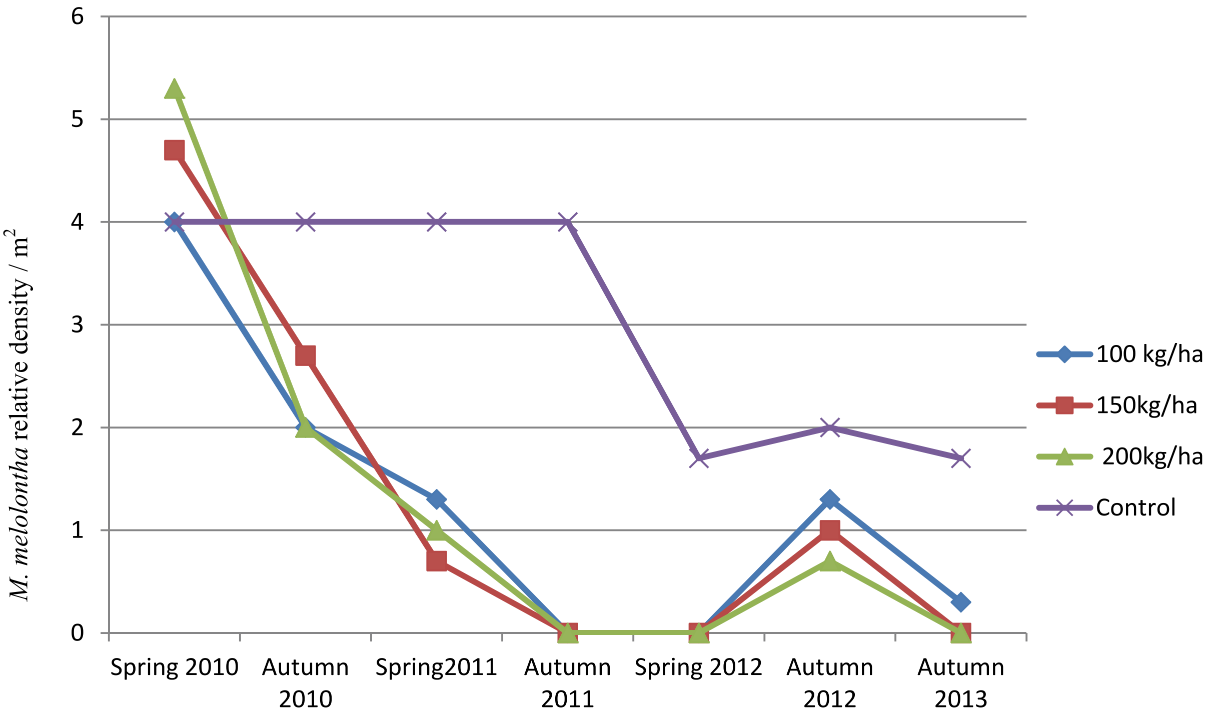

Figure 3. Level of mean infestation of *M. melolontha* at Izvor nursery

The results on the effect of treating the soil two years consecutively (2010-2011) with Romanian bioproduct demonstrates a positive correlation between dosage and mortality due to fungus infection only at one forest nursery (Zavoi-Siret). The long-term action analyzes of the soil colonization by *B. brongniartii* reveals that obvious mortality rates have been produced at the end of larval development especially (pupation year). However the positive effect of treatments on insect density was obvious at 1.5 years (on second generation) at nurseries who received the higher cumulative dose even if not in all cases clear fungus infections were detected. An increased infection rates in the generation following the treatment was reported by Keller (1997) who experimented a method to contaminate the breeding sites of the cockchafer by applying on adults, doses of 2.0-3.7 x 10^{14} blastospores of five *B. brongniartii* strains. Keller (2002) recommended also that the repeated applications to be done with reduced doses to increase inoculation spots in the soil instead increasing the dosage.

Dolci et al. (2006) reported a decrease of pest density in orchards treated with 1×10^9 conidia/m^2 rye kernels in northwest of Italy, the damage caused by *M. melolonta* being limitated. Benker and Leuprecht (2005) obtained an 80% decrease of insect density due to fungal control and 89% decrease using mechanical treatment. Also they found that chemical treatment didn't improve the effect of the rotary hoe.

CONCLUSIONS

The experiences made in three forest nurseries from north east of Romania during 4 years clearly show that cockchafer density decreased under economical threshold level which is more than 1 larvae/m^2 due to cumulated action of biological and mechanical treatments.

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