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EFFICACY OF BIOLOGICAL PRODUCT BIOMELCON (*BEAUVERIA BRONGNIARTII*) DURING THE SECOND YEAR OF APPLICATION IN FOREST NURSERIES OF MOLDOVA

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Abstract: In 2016, in some nurseries from Bacău county, very strong infestations with white grubs were reported and significant decreases of larval density were recorded following the two repeated applications of bioinsecticide based on *Beauveria brongniartii* (BioMelCon). However, in the second year, infestations with *Melolontha melolontha* were maintained at a medium level and those with other species of white worms, *Anomala* sp. and *Rhyzotrogus aestivus*, were high. The treatments were repeated in 2017 by applying in the spring a single dose of 150 kg / ha of BioMelCon by manually spreading and soil incorporation using a disc harrow. After second year of application the initial number of white grubs was reduced until the autumn evaluation in 2017 and greatest losses due to both the effect of the biological product as well as the soil plowing of larvae have been recorded at *M. melolontha* and *R. aestivus*.

INTRODUCTION

The most damaging insect pest in forest nurseries are larvae of carabid species, also called white grubs. The forbidden of chemical grub control imposed within FSC certification determines the use of prevention method like agrotechnical, physico-mechanical and biocontrol.

In 2016 high densities of white gubs were reported in FSC certificated forest nurseries in the north-eastern part of Romania (Fătu et al., 2016).

In attempt to minimize and control the infestations of nurseries with white grubs, an experimental biological product (BioMelCon) based on entomopathogenic fungus *Beauveria brongniartii* (an autochthonous strain) was applied in 2016, between 18 Aprilie and 10 May. The application was repeted one month later. In september the evaluation of insect density had resulted in a good reduction of larval density in some forest nurseries compared with non treated surfaces and new species of white grub other than *Melolontha* were reported as infected by *B.brongniartii* (Fătu et al., 2016). The treatments with the same bioinsecticide were repeated in 2017 in the same locations as in 2016.

The purpose of this paper was to identify biotic and abiotic factors that influence the effectiveness of biological treatments; results on the biological efficacy of an experimental entomopathogenic bioinsecticide, after the second year of application of the biological treatment are presented.

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MATERIALS AND METHODS

In the second year of experimentation, the bioinsecticide BioMelCon was applied between 28.04 and 04.05.2017 in forest nurseries administrated as unplanted field (Figure 1). Before and after the bioinsecticide administration, the soil was tilled. The bioinsecticide, barley kernels colonized by *B. brongniartii*, was manually and uniformly distributed on the surface of the experimental area. Imediately after distribution, the fungus kernels were tilled into the soil at a depth of 10-15 cm using a disc harrow attached to a tractor.

Figure 1. Soil and BioMelCon preparation to apply biological treatment in Ghedeon nursery (28.04.2017)

The treatments in 2017 were carried out between May and April in three forest nurseries situated at different altitudes (Table 1).

The fungal treatment was applied in a single dose of 150kg/ha at a concentration of $1x10^{13}$ conidia per m^2. In order to determine the relative density of larvae, soil excavations (100x100x30 cm) were performed in three different dates, before treatments and after two and five months from bioinsecticide application (Figure 2).

Figure 2. Carrying out soil surveys to determine the efficacy of the biological product
a. Forest District Comăneşti (12.10.2017), b. Forest District Caşin (10.10.2017), c. Forest District Moineşti (24.08.2017)

The relative density of larvae are presented as the mean of three excavations in surfaces up to 0.4 ha and the mean of five excavations in surfaces up to one ha. In control, only one excavation was performed. The identification of white grub species and larval stage was based on the raster pattern on the last abdominal segment and by analyzing cephalic width, respectively according to identification keys previously reported by Simionescu et al. (2000).

The population reduction after 2 and 5 months was calculated with formula 100 x (D1 -D2 or D3)/D1, where: D1 - density of individuals before treatment, in spring; D2 - density of individuals at two months, and D3 - density of individuals at five months. The dinamic of infestation after two years of application was expressed as an index of infestation by the formula $I = I_1/I_2$, (I_1- mean number of white grub per m^2 in autumn 2017, I_2 - mean number of white grub per m^2 in spring 2016, before treatments).

Table 1. Location of nurseries treated against white grubs, date of first treatment and surface

Forestry Department Bacau	**Forest nursery/altitude**	**Treated surfaces (ha)**	**Date of first treatment in 2017**	**Date of observation (2017) after ….months of treatment**	
				2	**5**
Forest District Caşin	Pietroasa/425 m	0.7	02.05	13.07	11.10
Forest District Comăneşti	Lunca lui Pall/800 m	0.3	04.05	11.07	12.10
Forest District Moineşti	Ghedeon/465 m	0.8	28.04	28.06	13.10

RESULTS AND DISCUSSIONS

Before the first treatment in 2017, Lunca lui Pall nursery from F.D. Comănești presented no infestation with larvae in plot previously treated with fungal bioinsecticide. At that time, the control plot was highly infested with pupae of *Phyloperta horticola* and adults of *M. melolontha* (Table 2). In the second survey in July (Table 1), a reinfestation with second and third larval stage of *M. melolontha* and *Anomala* sp. was registered. *M. melolontha* population was greatly reduced during the two years of fungal applications (I = 0.05), being currently at a level of weak infestation. Instead, *Anomala* sp. population remained at levels relatively constant (I = 1.04), although in 2016 after the first treatment, 22.5% infected larvae by *B. brongniartii* were registered. No infected larvae by entomopathogenic fungus have been identified.

Before the first treatment in aprilie 2017, in Ghedeon nursery a very high infestation with third instar larvae of *M. melolontha* and *Anomala* sp. was registered. The population of *M. melolontha* was slightly reduced during two years of fungal application (I = 0.81) (Table 3). Although in the fall of 2016 the *Anomala* population registered a decrease probably due to fungal infection following treatments, in the spring of 2017, there was a reinfestation. However, the results obtained in the autumn of 2017 showed a population decrease of 69.2% for *M. melolontha* and 94.6% for *Anomala* sp. During the two years, the population of *Anomala* was reduced (I = 0.14). In Figure 3 we present insects collected from experimental plots in 2017, before or after treatment application.

We suppose that the spring reinfestation with *M. melolontha* could be due to the application of treatments in 2016 to the second instar larvae known to be less susceptible to fungal infection than the third instar once (Ferron, 1967). Moreover, the susceptibility varies during the L_3 stage (Ferron, 1985). This could also be the case of the *Anomala* population that suffered decreases in 2016 due to treatments that probably affected third-instar larvae more than the second instar onces. No infected larvae by entomopathogenic fungus have been identified. Pietroasa nursery presented on 02.05.2017 a high infestation with second and third instar larvae consisted in *M. melolontha, Anomala* sp. and *Rhyzotrogus aestivus*. It is mentioned that *Melolontha* and *Anomala* were not registered in 2016. In Control plot only

Rhizotrogus was identified at very high infestation level. Soil surveys performed in the autumn 2017 showed a weak infestation due to the decrease in the *Rhizotrogus* population of 58% after the treatment application in 2017. During the two years, the population of *R. aestivus* recorded a very large decrease (I = 0.02). Because a decreasing population was registered in control plot, we can assume that abiotic factors contributed to this reduction. No larvae of *M. melolontha* and *Anomala* sp. was founded at two and five months after treatment application.

Figure 3. Insects collected from experimental plots in 2017

M. melolontha and *Anomala dubia* larvae (Lunca lui Pall Nursery, 12.10.2017)

Diseased *M. melolontha* pupa and larva (L_3)

Healthy *M. melolontha* larvae

(Gheodon Nursery, 24.08.2017)

Table 2. The population reduction of *Rhizotrogus aestivus*, *Anomala* sp. and *Melolontha melolontha* in Lunca lui Pall, Pietroasa and Ghedeon forest nurseries

Forest Nursery	**Variant**	**Pest species**	**D_1**	**D_2**	**D_3**	**Population reduction (%) after… months**	
						2 (#)	**5 (##)**
Lunca lui Pall	Treated area	*M. melolontha*	0	0,25$L_2$0,25L_3	0,2 L_3	0	0
		Anomala sp.	0	2,5L_2 2,5 L_3	6,6 (0,2 L_1, 0,2 L_2, 6,2 L_3)	0	0
	Control	*M. melolontha*	2 A	0	0	-	-
		Phyllopertha horticola	19 P	0	0	-	-
Pietroasa	Treated area	*M. melolontha*	1.6 L_2	0	0	100,0	100,0
		R. aestivus	1L_2	0,43 L_1	0,42 (0,28 L_2, 0,14 L_3)	57	58
		Anomala sp.	0,3 L_3	0	0	100,0	100,0
	Control	*R. aestivus*	19,5 L_2	1,0 L_2	2,0 (1 L_2, 1 L_3)	94,9	89,7
Ghedeon	Treated area	*M. melolontha*	1,3 L_3 0,5 A	0	0,4 (0,1P, 0,3A)	100,0	69,2
		Anomala sp.	3,7 L_3	0	0,2 L_3	100,0	94,6
	Control	*M. melolontha*, *Anomala* sp.	0	0	0	-	-

D_1: density in May-April 2017
D_2: density 2 months after tratment
D_3: density 5 months after tratment
(#) $100 (D_1 - D_2) / D_1$
(##) $100 (D_1 – D_3) / D_1$

Table 3. The index of infestation after two years of succesive application of *Beauveria brongniartii* in Lunca lui Pall, Pietroasa and Ghedeon forest nurseries

Forest nursery	Pest species	Index infestation		
		I_1	I_2	I
Lunca lui Pall	*M. melolontha*	0.2	4	0.05
	Anomala sp.	6.6	6.3	1.04
Pietroasa	*R. aestivus*	0.42	18	0.02
Ghedeon	*M. melolontha*	0.4	0.49	0.81
	Anomala sp.	0.2	1.37	0.14

I: Index of infestation
I_1: Mean number of white grub per m^2 in autumn 2017
I_2: Mean number of white grub per m^2 in in spring 2016 before treatments

CONCLUSIONS

The results registered after second year of *Beauveria brongniartii* treatments show important reduction of scarabs population, especially of *Melolontha* and *Rhyzotrogus* larvae, who registered very high initial density (>4 insect/square meter) before the first application of *B.brongniartii* bioinsecticide, in 2016. This can be due to cummulative effect of fungal application and soil tillage.

The weak reduction of *Anomala* population can be attributed to low susceptibility of second instar larvae to fungal infection.

The results obtained demonstrate the need for annual study of repeated application of the *B. brongniartii* based bioinsecticides.

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