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TESTING BioMelCon BIOINSECTICIDE IN SOME FOREST NURSERIES FROM BACAU COUNTY

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Abstract: Promoting biological control method of root pests from forest nurseries is one of the basic requirements of European Community and Forest Stewardship Council (FSC). In this context, forest departments within National Forest Administration (NFA) -ROMSILVA expressed their interest for the use of fungal bioinsecticide BioMelCon, produced by Research Development Institute for Plant Protection, Romania. The results capitalized by beneficiaries, i.e. biological insecticide technology and its application, have been the subject of research and development contract funded by NFA-ROMSILVA. We present our results in 2016. Application of BioMelCon was performed on total area of 1.9 hectares, in forest nurseries belonging to Forest Department of Bacau County. Two repeated treatments consisted in 75 kg/ha each were applied in unplanted high infested fields with *Melolontha melolontha*, *Rhizotrogus aestivus* and *Anomala dubia*. Incorporation into soil was carried out with a disc harrow attached to a tractor. In autumn, the soil results showed a good reduction of larval density and efficacy between 80 and 100% was registered. Also, artificial infection with *B. brongniartii* on two species of white grub, *A. dubia* and *R. aestivus* was reported.

INTRODUCTION

The European cockchafer, *Melolontha melolontha* L. (Coleoptera, Scarabeidae) is a serious pest in some regions of Romania and the most dangerous pest for saplings from forest nurseries in North-East of Moldova (Gradinariu et al., 2012).

The restrictions imposed by FSC regulations determined the use of alternative means like biological treatment and agrotechnical practices for control of white grubs. The entomopathogenic fungus *Beauveria brongniartii* (Sacc.) Petch (Deuteromycetes: Moniliales) is an important natural enemy of *M. melolontha*. Natural *B. brongniartii* infections of *M. melolontha* were reported by Ciornei et al. (2006) in North-East of Moldova.

In Romania, experiments with *B. brongniartii* (BioMelCon) against *M. melolontha* were carried out few years ago in forest nurseries from Botosani, Neamt and Suceava Forest Departments (Fătu et al., 2015).

In this paper, preliminary results of the first year of application of entomopathogenic fungus *B. brongniartii* against white grubs in some forest nurseries from Bacau County are presented. The trials were performed in four FSC certificated forest nurseries at North-East of Moldova, in Forest Directorates of Bacau County. In 2016, the soil surveys prior to saplings plantation showed high infestation with white grubs like *M. melolontha, Anomala dubia* S. and *Rhyzotrogus aestivus* O., so it was decided to be administrated as unplanted field. No natural insects infected with *B. brongniartii* were reported on treated nurseries before bioinsecticide application.

MATERIALS AND METHODS

The bioinsecticide BioMelCon obtained at pilot scale in RDIPP consisted in sterilized barley kernels colonized by autochthonous *B. brongniartii* fungus was used in experiments. The bioinsecticide was applied manually followed by incorporation in soil with a disc harrow attached to a tractor. The treatments were carried out between 18 April and 10 May in four forest nurseries situated at different altitudes (Table 1, Figure 1).

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

Forest District (Forest Department Bacau)	Forest nursery and altitude	Date of first treatment	Treated surfaces (ha)
Caşin	Pietroasa (425 m)	18-19.04.2016	0,7
Comăneşti	Lunca lui Pall (800 m)	29.04.2016	0,3
Moineşti	Ghedeon (465 m)	4.05.2016	0,8
Tg. Ocna	Sărărie (650 m)	10.05.2016	0,1

Figure 1. Field application of BioMelCon and its incorporation in soil with a disc harrow.

The first fungal treatment was applied with 75 kg/ha followed by the second application of the same dose, 30 days later. The final concentration of fungal conidia was $1x10^{13}$ per hectare. The results after treatment were compared with control consisted in non-treated grassy land right next to treated area.

In September, three areas (100x100x30 cm) were examined to determine the relative density of larvae. The efficacy was calculated with formula: $100 \times (D_1 - D_2) / D_1$, where: D_1 - number of larvae/ m^2 before treatment, in spring and D_2 - number of larvae/ m^2 in autumn, after the second treatment.

RESULTS AND DISCUSSIONS

Before the first treatment in April, in Pietroasa nursery, 18 third instar larvae (L_3) and 0.3 adults/ m^2 of *Rhyzotrogus aestivus* was registered. After the first treatment a decreasing of larval density with 18.5% was registered (Table 2). Also 84.6% from larvae founded in soil presented typically signs of fungal infections. This could be determined by mechanical application of fungus injured the larvae that become susceptible to fungus infection. From the literature it is known that *B. brongniartii* mainly attacks *M. melolontha* and *M. hippocastani* but it was also reported on other Coleopteran hosts (Zimmermann, 2007). Because no soil samples were analysed for presence of *B. brongniartii* prior treatment, it is not known if the fungus infecting the white grub is the same strain with the applied one.

In September, only 0.25 second instar larvae (L_2) larvae/ m^2 (Table 2) were observed in treated area but in very low density and a reduction of 99% was assessed. We think that the efficacy of treatment can be due to treatment application considering that in the untreated planted field the number of grubs was much higher.

Table 2. The efficacy of *B. brongniartii* against *R. aestivus, A. dubia* and *M. melolontha* in Pietroasa, Lunca lui Pall, Ghedeon and Sărărie nurseries

Forest district	Pest species present	No. of larvae/ m^2 before treatment, in spring (D_1)	No. of live larvae/ m^2, after the first treatment	No. of larvae/ m^2 in autumn, after the second treatment (D_2)	Efficacy (%)
Pietroasa	*R. aestivus*	18.0 L_3 0.3 A	14.7 L_3	0.25 L_2	99,0
Lunca lui Pall	*A. dubia*	6.3 L_2	2.7 L_2	0.3 L_2 5 L_3	15.8
	M. melolontha	4 L_3	0.3 L_2	0	100.0
Ghedeon	*M. melolontha*	0.37 L_3 0.12 A	2.0 L_2	1.63 L_3 0.10 A	0.0
	A. dubia	0.12 L_2 1.25 L_3	0.25 L_1 0.17 L_2 0.37 L_3	0.27 L_3	80.3
Sărărie	*M. melolontha*	21 L_1 4 L_2 1 L_3	no data	1 L_2 1 L_3 1 A	88.5

In Lunca lui Pall nursery, two species of white grubs were present in high densities of 6.3 larvae/ m^2 *Anomala dubia* and *M. melolontha.* After first treatment, 22.5% from total larvae of *A. dubia* were founded to be infected by *B.brongniartii*. At that time, the number of live larvae was 2.7/ m^2, a reduction of 57.1%. At the same time, in treated plots 0.3 L_2 of *M. melolontha* were observed; in non-treated grassy land 3 L_2 and 1 L_1 were founded. In September, after two treatments with *B. brongniartii*, no larvae of *M. melolontha* was founded in soil, only *A. dubia* at 5.3 larvae/ m^2 (Table 2) meaning an efficacy of 15.8%.

The same species of white grubs like in Lunca lui Pall nursery were observed also in Ghedeon nursery, but in much low density as follows: 0.49 individuals/m^2 of *M. melolontha* and 1.37/m^2 of *A. dubia*, respectively. After the first treatment, 2.0 L_2 larvae of *M. melolontha* were observed, much higher than before treatment. This could be explained by low susceptibility of *M. melolontha* second instar larvae at *B. brongniartii* infections. The results of *M. melolontha* larvae population assessment after second treatment, shows no efficacy of *B. brongniartii*, the relative density being 3.5 times higher than before the first treatment (Table 2), but 5 times less than in non-treated grassy land (data not shown). In contrast, 33% L_2 and 25% L_3 of *A. dubia* founded in soil showed to be infected by *B. brongniartii* after the first treatment. Although no L_1 and L_2 larvae were observed in autumn, it was noticed a 4 time reduction of L_3 larvae in treated plot than before fungus application.

In Sărărie nursery, the initial infestation before treatment was very high (Table 2). The results after the first treatment application were inconsistent because of improper weather condition for soil assessment (torrential rains and floods).This could be the reason of high decreasing of larval density in the following two months meaning an efficacy of 88.5%.

CONCLUSIONS

This is the first article reporting a field artificial infection with *B.brongniartii* on two species of white grub, *A. dubia* and *R. aestivus.*

In first year of applications, two of three forest nurseries infested by *M. melolontha* registered a good reduction of larval density. In the next two years, repeated application of fungus until the dose of 300 kg/ha is needed to considerable reduction of larval density.

The results of our first investigations show that white grubs other than *M .melolontha* can be controlled with *B. brongniartii*.

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