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Experimental Field Application of *Beauveria bassiana* (Bals.) Vuill. for Control of the Invasive Sawfly *Aproceros leucopoda* Takeuki, 1939 (Hymenoptera: Argidae) in Romania

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Abstract: Fungal treatment with a native strain of *Beauveria bassiana* isolated from *Aproceros leucopoda* larvae and prepared as a blastospore suspension (2.7 x 10^{12} bl./l) was applied by a foliar spraying of elm trees in forests in the eastern part of Romania. The trees were naturally infested by L1-L5 larvae of *A. leucopoda* from the G2, G3, G4 generations, as well as with eonymphs in the summer cocoons. The treatment was applied in June – July at three different sites. Using a backpack sprayer, doses of 100 to 200 l/ha of bioinsecticide were dispersed on surface of 20 ares. The assessment of larval density was performed before and after the repeated biological treatment by choosing three sample trees. The efficacy was assessed 7–14 days after the treatment. The insecticidal effect of the entomopathogenic fungus *B. bassiana* was expressed as a decrease of infestation by *A. leucopoda*, as well as a decrease of defoliation of trees where the biological treatment was applied. The population decreasing percentage was positively correlated with the dose of the bioinsecticide. The best results were obtained using the dose of 200 l/ha and the efficacy ranged from 60% to 90%. The recorded defoliation showed values negatively correlated with the treatment dose, ranging from 5% to 23%.

Key words: Entomopathogenic fungus, *Beauveria bassiana*, *Aproceros leucopoda,* elm forest.

Introduction

The invasive alien sawfly *Aproceros leucopoda* Takeuki, 1939 (Hymenoptera: Argidae) is an important defoliator pest on the elm trees (*Ulmus* sp.) in Romania that was first reported in 2006 in the Banat and Moldavian regions (Blank et al. 2010) and then in 2016–2017 in the Oltenia region (Nețoiu et al. 2018). High infestation of *A. leucopoda* was recorded in most of the broadleaved forests with elm in the Moldavian region, and the most severe impact was registered in the southern part of the region (Cardas et al. 2011), where repeated defoliations have caused dieback of trees of at least 37.5% (Cardas 2012). In the Oltenia region the species was detected on isolated trees in the urban areas and in the forests (Nețoiu et al. 2018).

In Romania, the chemical treatment based on metaflumizone experimented by Cardas (2012) has been approved as an effective mean of controlling the larvae. However, due to the restrictive measures laid down by the requirements of EC Directive 91/414 (EU 1991) and the forest certification system introduced by the Forest Stewardship Council

(FSC), no insecticide for the sawfly larvae has been in use. In the context of the forest protection, the use of natural enemies like entomopathogenic fungus is an alternative method for the control of population size of the dangerous insect pests (Augustyniuk-Kram & Kram 2012)

Beauveria bassiana (Bals.) Vuill. is a well-known entomopathogenic fungus with a worldwide distribution in insects, and also in different habitats (Zimmermann 2007), capable of infecting more than 700 host species (Faria & Wraight 2007). In Romania, isolates of *Beauveria* were reported from different species of forest pests (Ciornei et al. 2006, Dinu et al. 2012, Andrei et al. 2013), including *A. leucopoda* (Fătu & Andrei 2017). In a laboratory bioassay, eonymphae (pupae) and larvae of *A. leucopoda* have proved to be highly susceptible to the native strains of *B. bassiana* applied as blastospores (Fătu & Andrei 2017). The treatment with blastospores appears to be more appropriate for the control of the soft bodied insects (Mascarin 2015). The fast germination rate of blastospores compared to aerial conidia increases the probability of penetration in the insect's body by reducing the need of moisture and UV radiation. Furthermore, the rapid germination rate of blastospores increases the probability of infecting the moulting or social insects that groom nest-mates (Jackson et al. 2010). A biocontrol agent, which was commercially developed as blastospores is *Isaria* (*Paecilomyces*) *fumosorosea* (Wize) (Hipocreales: Clavicipitaceae), is used for the control of several glasshouse pests (Jaronski 2014).

In the present study we evaluated the insecticidal ability of *B. bassiana* applied as blastospores suspension against *A. leucopoda* larvae and eonymphs, and assessed the feasibility of using *B. bassiana* blastospores as an active ingredient in the sprays, based on the data obtained by field experiments.

Materials and Methods

The trials were conducted in 2010–2011 in mixed deciduous forests (more than 10% represented by *Ulmus glabra* Huds) highly infested with *A. leucopoda*, located in the Forest Management Units in the eastern part of Romania. The treatment was applied at three different sites in June – July.

One of the trial sites was set in Trușești (Botoșani County), where trees with field elm *U. glabra* were identified. They are located in the northeast of the country, in the low-hill area of the Moldavian Plain, characterised by temperate continental climate, with an annual average temperature of 8.6°C and precipitation of 488.1 mm. The other two trial sites were set in the Vaslui Forest Department (in the eastern part of the country) in an area which is characterised by an average annual temperature of 10°C and precipitation of 525 mm (Cardas 2012).

The elm trees were naturally infested by *A. leucopoda* in all developmental instars from the second and third generations (G2 and G3, respectively). The fungal treatment was performed using a native strain of *B. bassiana* isolated from *A. leucopoda* larva and prepared as a blastospore suspension (2.7×10^{12} bl./l). The treatment was applied by foliar spraying using a backpack sprayer of three different doses (100 l/ha, 150 l/ha and 200 l/ha, respectively).

At each site, four plots (one plot per treatment) with a surface of 500 m^2 each, with three *U. glabra* trees per plot, were established. The trees were examined and their individual infestation level and defoliation rate assessed before and after the treatment according to Blank et al. (2010). Each site was homogenous in terms of the relative density and developmental instars of the insect. The efficacy was assessed 7–14 days after the treatment.

The first trial was conducted at Vaslui (UP V Ivănești, u.a.40 A, Vaslui County) on 15 June 2010, when the defoliator was in G2. On that date the insect was in the L1–L5 (L3–L4 mostly) instar (Table 1), with an average relative density of 41% at all experimental plots. During this application the weather was sunny and without rainfall during the following seven days. The average temperature recorded was 17°C. The efficacy was evaluated 10 days after the treatment.

The second trial was performed at Trușești (UP III Cozancea, u.a. 33 B, Botoșani County) on 22 July 2010, when the defoliator was in G3. The biological product was applied in the same way as in the previous trial. On that date the insect was represented mostly by eonymphae instars in the summer cocoons on the leaves, the rest of the population being in the L4–L5 instar, with an average relative density of 50.5% at all experimental plots (Table 2). During this application the weather was also sunny and without rainfall during the following seven days. The average temperature recorded was 20°C. The efficacy was evaluated 7 and 14 days after the treatment.

The third trial was conducted at Brodoc (UP U.P. III Negrești, u.a. 52, Vaslui County) on 12 July 2011, when the defoliator was at the beginning of the G3. On that date, the insect was mostly in the egg and L1 instar (Table 3). In a very small

Table 1. *Aproceros leucopoda* G_2 infestation level at Vaslui (U.P. V Ivănești, u.a.40 A). E – eggs; L – larvae; P – pupae.

***B. bassiana* applied dose (l/ha)**	**The level of infestation at the time of treatment (%)**							
	E	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**	**P**	**Σ living insects**
100 l/ha	–	0.3	5.7	16.3	11.0	7.3	–	40.6
150 l/ha	–	0.2	5.9	16.0	10.7	7.2	–	40.0
200 l/ha	–	0.4	5.5	16.7	11.3	7.4	–	41.3
Control	–	0.6	4.4	15.1	14.0	8.3	–	42.4

Table 2. *Aproceros leucopoda* G_3 infestation level at Trușești (UP III Cozancea, u.a. 33 B). E –eggs; L – larvae; P – pupae.

***B. bassiana* applied dose (l/ha)**	**The level of infestation at the time of treatment (%)**							
	E	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**	**P**	**Σ living insects**
100 l/ha	–	–	–	–	7	3	47	57
150 l/ha	–	–	–	–	7	6	37	50
200 l/ha	–	–	–	–	7	3	33	43
Control	0.8	1.6	2	2	5	3.7	36.9	52

Table 3. *Aproceros leucopoda* G_2 infestation level at Brodoc (U.P. III Negrești, u.a. 52). E –eggs; L – larvae ; P – pupae.

***B. bassiana* applied dose (l/ha)**	**The level of infestation at the time of treatment (%)**							
	E	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**	**P**	**Σ living insects**
100 l/ha	33	95		–	–	–	2	128
150 l/ha	41	88	2	–	–	–	1	131
200 l/ha	28	75	3	–	–	–	–	106
Control	30	45	23	1	–	–	1	98

proportion (G2), eonymphs from the previous generation affected by fungi were observed on the leaves. The site was chosen for its high level of infestation (115%). The treatment was applied in a very hot weather (25°C at 9 a.m.). During the next seven days no precipitation was recorded. The effect of the treatment was established 10 days after the treatment.

In each trial the efficacy of the different kind of treatment in reducing insect density percent was calculated according to the formula of Henderson & Tilton (1955). The significance of the difference between the mean defoliations in different time intervals was calculated with a t-test. The correlation (Pearson) between the defoliation percentage and the applied doses of *B. bassiana* was performed with GraphPadPrism v7.05 for Windows (GraphPad Software, San Diego California USA).

Results

At the first trial site, in Vaslui, the results showed that the values regarding the percentage share of the population of *A. leucopoda* was positively correlated with the treatment dose (r=0.998; correlation coefficient after Pearson), without exceeding the value of 61% at the maximum dose (Table 4). The pre-treatment defoliation in all variants was moderate and was maintained at the same level even 10 days post-treatment, except during the control test when extensive defoliation (75.8%) was registered. The defoliation percentage was negatively correlated (r=-0.985) with the applied doses of *B. bassiana*. The application of the highest dose resulted in defoliation three times lower than in the control test (Table 4).

In this trial, there were no larvae or eonymphs of *A. leucopoda* with the characteristic symptoms of

Table 4. Efficacy and mean defoliation percentage (%) after 10-day treatment with different doses of *B. bassiana* blastospores at Vaslui (U.P. V Ivăneşti, u.a.40 A).

***B. bassiana* applied dose (l/ha)**	**Efficacy after 10 days (%)**		**Mean defoliation after 10 days (%)**
	Mean ± SD	**Coefficient of variation, s%**	
100 l/ha	44.7±4.04	9.05	23.0
150 l/ha	53.3±3.49	6.54	15.5
200 l/ha	60.6±2.09	3.46	11.5
Control	–	–	37.3

Table 5. Efficacy and mean defoliation percentage (%) after 7- and 14-day treatment with different doses of *B. bassiana* blastospores in Truşeşti (UP III Cozancea, u.a. 33 B).

***B. bassiana* applied dose (l/ha)**	**Efficacy (%)**		**Mean defoliation**	
	after 7 days	**after 14 days**	**after 7 days**	**after 14 days**
100 l/ha	100.0	48.17	2.6	2.9
150 l/ha	100.0	57.92	2.6	5.2
200 l/ha	100.0	81.18	6.2	6.5
Control	–	–	12.0	16.0

Table 6. Efficacy and mean defoliation percentage (%) after 10-day treatment with different doses of *B. bassiana* blastospores in Brodoc (U.P. III Negreşti, u.a. 52).

***B. bassiana* applied dose (l/ha)**	**Efficacy after 10 days (%)**		**Mean defoliation after 10 days (%)**
	Mean ± SD	**Coefficient of variation, $s_{\%}$**	
100 l/ha	75.0±3.97	5.29	12.6
150 l/ha	82.0±2.00	2.43	10.2
200 l/ha	90.0±1.48	3.60	8.4
Control	–	–	43.4

the *B. bassiana* fungus infection observed. Infected insects by different species of the entomogenic fungi, which naturally intervene in regulating the numerical density of the *A. leucopoda* populations, of which *Isaria* (*Paecylomyces*) *farinosa* (Holmsk) Fr. and *Aspergillus flavus* Link were the most common, were recorded both in and outside the treated areas at the end of the next generation (L5 instar larva and G3 pupa).

At the second trial site, in Truşeşti, seven days after the treatment only 38.5% insect density was registered in the control. There were no larvae on foliage of the treated variants recorded. This can be explained by the transformation of the eonymphs, which prevailed in variants at the time of treatment, into adults and by the simultaneous presence of L1 and L4 larvae in the control. Later on, 14 days after the treatment, young larvae appeared, with densities of 13%, 26% and 27% in the treated variants. The efficacy values were positively correlated with the applied doses of *B. bassiana* (r=0.973). The application of the highest dose resulted in a percentage of defoliation two times lower than in the control (Table 5). No significant differences (t=0.668) of the defoliation on the 7th and 14th days were registered. In the control, the insect density recorded 14 days after the treatment was found to be 2.3-fold higher than the density of the treated variants.

At the third trial site, in Brodoc, the mean efficacy of *B. bassiana* treatment ranged from 75% to 90% and was higher than that of the other two sites. The efficacy values were positively correlated with the applied doses of *B. bassiana* (r=0.999). The application of the highest dose resulted in a percentage of defoliation five times lower than in the control (Table 6). At this site, although the pre-treatment insect density was very high, the defoliation was maintained at the same level even 10 days post-treatment in the treated variants, and was moderate compared with the control, in which severe defoliation was registered (89.8%).

Discussion

The treatment performed under field conditions in 2010 and 2011 with the biological product based on the blastospores of *B. bassiana* indicated a decrease in density of *A. leucopoda* population in the three trials coducted under different seasonal and infestation conditions.

In all three experiments significant dose-response effect was observed. The best results were obtained when applying a dose of 200 l/ha and the efficacy ranged between 60% and 90%. No observations were made on the post-mortem evolution of mycosis after the biological treatment. The highest insect density was observed in the control treatment compared to that in the treated variants.

At both experimental sites in the Vaslui region, the maximum degree of defoliation was maintained at moderate levels in the treated variants (58 and 60%, respectively) compared with the controls, in which severe degrees of defoliation were registered after 10 days (76% and 90%, respectively). In the Botoșani region, although a high insect density was registered, very weak defoliation during the pre-treatment (8.1–9.9%), and weak defoliation during the post-treatment, including the control (13–24.5%), was recorded after 14 days. Different correlations between the insect density and the defoliation degree in those two regions (counties) were reported by Cardas (2012). The author explained these correlations with the temperature as a primary factor that has contributed to stronger *A. leucopoda* gradations. The annual averages increase from the northern to the southern part of the analysed area, showing values of 8.6°C at Botoșani and 10°C at Vaslui. Cardas (2012) found very high insect density (101%, 195% and 239%) but weak defoliation (0.9%, 10.1% and 24.1%) at Botoșani in 2009 and 2010, when the defoliator is in G1, G3 and G4, respectively. However, at Vaslui to the insect density of 103.2% corresponds defoliation of 41.8%.

From practical point of view, the treatment with blastospores to control forest pests is not recommended when large forest areas are to be treated. The utilisation of blastospores has many advantages related to the mass production (as short fermentation time, reduced production costs) (Mascarin et al. 2015) and virulence (Niranjana et al. 2008). However, the blastospores in liquid cultures are not stable compared to dry conidia, and this has been a problem in several biocontrol programmes using *Beauveria* spp. (Lane et al. 1991). This was the case with the first attempt to control *Dendrolimus* spp. (Lepidoptera: Lasiocampidae), a serious defoliator pest of the Masson's pine in China. The dry blastospores treatment has resulted in average mortality of 82% of the insects on the 8th day. The necessity for large quantities and short shelf life of the blastospores in liquid cultures and the impractical value has justified the application of *Beauveria* as aerial conidia (Zengzhi 2007). Moreover, the first attempts to control *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) by contamination of the breeding sites using *Beauveria brongniartii* (Saccardo) Petch blastospores spread by helicopter have been successful (Keller et al. 1997). However, the high costs of this technique and the extensive logistic requirements have led to the application of another method based on the introduction of sterilised barley kernels colonised by *B. brongniartii* (Keller 1992). The low viability of the blastospores after the drying process and the transformation of the fungal propagules into a powder is another challenge. Stephan & Zimmermann (1998) found that the adding of protectors such as skimmed milk powder and sugar beet syrup in the blastospore suspension can minimise the damage on the propagule viability after the spray drying process. Recently, Mascarin et al. (2015) have analysed some promising fungal isolates of *B. bassiana* and *I. fumosorosea* that produce high yield of blastospores with high desiccation tolerance and shelf stability. It was found that the blastospores are more virulent than aerial conidia when tested against *Bemisia tabaci* white fly nymphs in laboratory conditions. The desiccation tolerance is intrinsic to the isolate and influenced by nutritional aspects in the liquid production medium (Mascarin et al. 2015).

Conclusions

The biological treatment based on *B. bassiana* applied in forests infested with *A. leucopoda* (larvae and eonymphs) led to the reduction of *A. leucopoda* densities. The blastospore suspension applied at three different sites induced the biological efficiency percentage levels in the range from 60% to 90% after 10 days. As a result, the defoliation of the elm trees registered at the treated plots was 2–5 lower than in the untreated plots.

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