PRELIMINARY STUDY ON AIR DISPERSION OF *BEAUVERIA BASSIANA* SPORES FROM FUNGAL GRANULATED BIOPRODUCT

*Mihaela Monica Dinu, Ana-Cristina Fătu, Ana-Maria Andrei**

Research-Development Institute for Plant Protection, Bucharest

**correspondence address*
Research-Development Institute for Plant Protection
8 Ion Ionescu de la Brad
013813, Bucharest, Romania
Phone: + 40 21269 32 31
Fax: + 40 21269 32 39
E-mail: anamaria_111@yahoo.com

Abstract: Given the importance of the spread of spores released in a target habitat, the objective of this study was to monitor the biological control agent *Beauveria bassiana* in soil and air, after application of granulated bioproduct in solanaceous crops. The experiments were conducted over a period of 3 years (2013-2016); samples were collected monthly from May to September in potato and eggplant crops from Valcea County. Isolation of monitored microorganism *B. bassiana* was done on selective culture media: DOB50 for isolation from air and DOC2 for isolation from soil. Density dynamics of entomopathogenic fungus *B. bassiana* in air was similar in treated and untreated variants, when assessments were made at a height of 100 cm and statistically significant ($p<0.0023$) when assessments were made at a height of 4 cm. Air density of fungal conidia was higher at 4 cm above the ground than at 100 cm above or even al soil level. The density in soil of entomopathogenic fungus did not registered statistical differences in treated plots, with a mean conidia density of 2.1×10^3 CFU/g soil.

Key words: *Beauveria bassiana, dispersion, Leptinotarsa decemlineata*

INTRODUCTION

Beauveria bassiana have strong potential for biologically based management of Colorado potato beetle, *Leptinotarsa decemlineata* (Andrei, 1998; Muscalu et al., 2008). The effectiveness of *B.bassiana* treatments for reducing *L. decemlineata* populations depends on ecological valences of this entomopathogenic fungus. This ability of fungus to adapt to varying environmental conditions, characterized by more or less major variations of ecological factors, explains its geographical distribution, its survival and spread in the environment (Andrei, 1998). Changes in *B. bassiana* densities in different habitats were measured to investigate the density dynamics of this fungus (Shimazu *et* al., 2002; Meyling & Eilenberg, 2006).

Air currents, dispersion by water drops and saprophytic grow on substrates inhabited by insects, are recognized as the most important ways of contact between spores and host cuticle. In nature, conidial dispersion (spreading of spores) is essential for the survival of the species.

Conidial dispersion on large areas ensures fungal propagation and its survival. Also, dispersion favours and increases the probability, on the one hand, of infecting a susceptible host insect and, on the other hand, its subsequent colonization in the environment (Andrei, 1999). These attributes were exploited in the technological process in order to improve the use of conidia in products for biological control of insects. Because the conidia represent the infectious stage, the length of time that fungal spores remain viable on a substrate is a critical indicator of that entomopathogenic microorganism potential as biological control agent. Small changes in the period of viability of spores may have a significant impact on susceptible insect's populations.

Given the importance of the spread of spores released in the target habitat, the aim of this study was to monitor the biological control agent *Beauveria bassiana* in soil and in the air, after application of fungal bioproduct in solanaceous crops; considering that aerial conidia are easily dispersible via air currents (Holder & Keyhani, 2005), in our experiments, it was used a bioproduct based on *B. bassiana* conidia obtained on a natural nutritive substrate (autoclaved corn kernels).

MATERIALS AND METHODS

The experiments were conducted over a period of 3 years (2014-2016); samples were collected monthly from May to September in potato and eggplant crops from Vâlcea County (5 experimental plots x 450 sqm) and have been investigated using protocols described by Shimazu et al. (2002) with slightly modifications.

Experimental plots A, D and E have not been treated with *B.bassiana*. Experimental plots B and C have been treated with *B. bassiana* and they were delimited from experimental plots A and D by a buffer zone. *B. bassiana* have been artificially introduced (Figure 1) into experimental plot B (June 2014) and C (May 2015) for biological control of Colorado potato beetle. The density of fungal colonies has been determined monthly in the air and soil from March 2014 until May 2016.

In order to assay the air dispersion of *B. bassiana* conidia, one kg of fungal bioproduct (Figure 1), obtained by inoculation of BbEi2.03 strain on autoclaved corn kernels was manualy spreaded on soil. After 30 minutes, the dispersion was assessed by exposing three Petri dishes with artificial medium DOB50(bactopeptone 0.3%; $CuCl_2$ 0.02%; brilliant green 0.005%; agar 1.5%; pH 10 with Na_2CO_3) on air, for 2 hours. The dishes were placed on plastic trays and held at two different heights (4 cm and 100 cm) for A, B and C variants and at a height of 4 cm for D and E variants and distances between 0-50 m from the centre of the test zone. Exposure of culture media to the air has been done at 10 different points in A, B and C variants, in 9 different points in D variant and one point in E variant. The exposure included three replicate for each point of each variant. The colonies' densities were calculated after incubation at 25^0C for 5 days.

For counting colonies in the soil, soil samples, from the same points in which it has been made outdoor exposure of Petri dishes, have been collected in plastic bags. Soil samples were suspended in an aqueous solution of Tween 80 a rate of 5 g / 100 ml) and vigorously stirred for 1 min. Each suspension was diluted 10 times with the same solution and 0.2 ml of diluted suspension was sprayed on DOC2 medium (bactopeptone 0.3 %; $CuCl_2$ 0.02 %; crystal violet 0.0002%; agar 1.5 %; pH 10 with Na_2CO_3), in Petri dishes (9 cm diameter) followed by incubation at 25^0C for 5 days. When maximum 10 colonies were developed on the plate, they were completely transferred on PDA. When more than 10 colonies were developed on the plate, only 10 colonies, randomly selected, were transferred on PDA.

To correctly estimate the number of fungal colonies, it has been calculated as a ratio of the number of colonies of *B. bassiana* and the number of colonies of other microorganisms.

Figure 1. Granular *B. bassiana* bioproduct before (left) and after application on soil in potato culture (right)

As a measure of density control, the density of fungal colonies was immediately measured after placing in the field the fungal bioproduct.

Statistical analyses were performed using t-test and ANOVA of the AnalistSoft Inc., BioStat v2009.

RESULTS AND DISCUSSION

Density of the entomopathogenic fungus in the air. Average density was evaluated for fungal spores in variants B and C. There were no significant differences, regardless of the height from which analyses were made. The average number of colonies was transformed in number of colony forming units (CFU) per sqm / day. Large fluctuations of the fungal spores' dynamics density were registered in all experimental plots and no trend could be established according to weather conditions or other causes. In the experimental plot treated with granulated bioproduct (Figure 2), although in 2015 additional quantities of fungal spores were released, no proportional increases of fungal densities were recorded.

Figure 2. Granular bioproduct in solanaceous crops (potato, left and eggplants, right) infested with Colorado potato beetle

The results are presented in Figures 3 and 4. In the air, the average density of *B. bassiana* conidia was higher in treated than in untreated plots, and there was a statistically significant difference ($p<0.0023$ in treated variant), in samples from a height of 4 cm from the ground. Conidia density was higher at 4 cm than at 100 cm, especially in treated plots.

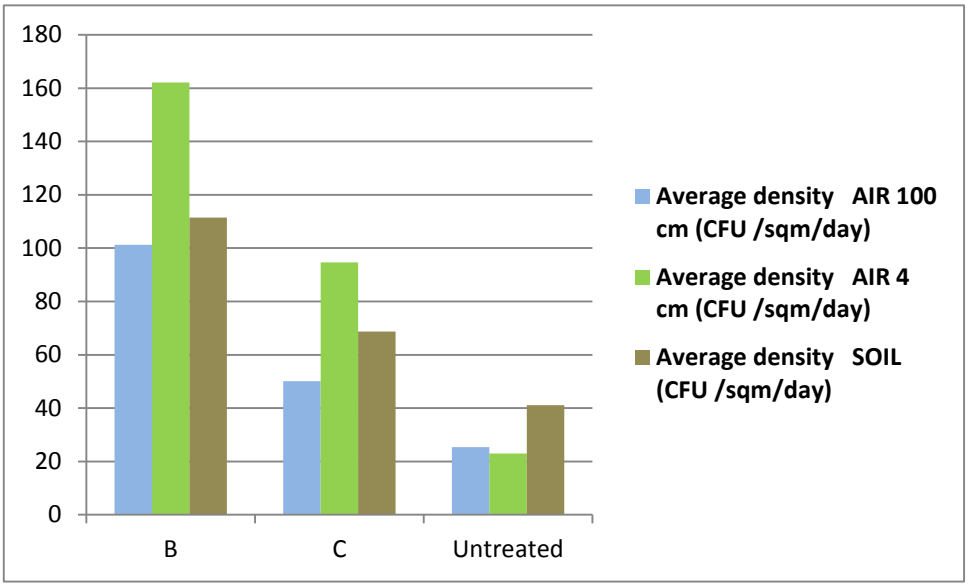

Figure 3. Density in the air of *B. bassiana* in solanaceae crop

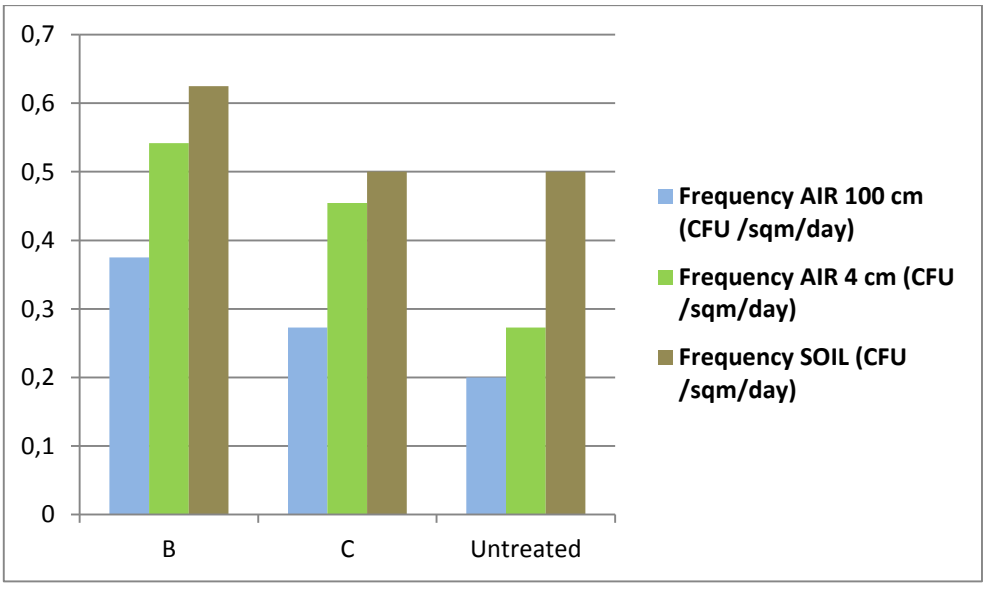

Figure 4. Frequency in the air of *B. bassiana* in solanaceous crop

Mean density of conidia in D plot was 1.8 x 10^3 CFU/sqm/day and frequency was 25/32. In A plot, mean density of conidia was 6.1 x 10^4 CFU/sqm/day and frequency was 12/28. The density dynamic of entomopathogenic fungus was no different from one location to another. We have also isolated fungal spores from E plot, the density of conidia here being 1/10 of D plot density.

The density in soil of entomopathogenic fungus did not registered statistical differences in B and C locations (p=0.324), with a mean conidia density of 2.1 x 10^3 CFU/g soil.

Regarding conidia spreading from the nutrient substrate, it was found that conidia density reached its peak in the middle of field (0 m); as the distance from the midpoint increased, the density of conidia decreased. This observation was interrelated with data on

wind speed and wind direction. The results were compared with those obtained from a similar experiment in tomato crops. During 2006-2009, a granular bioproduct based on *Beauveria bassiana* have been applied in different tomato crops from localities of Ialomita County (Andrei, unpublished data). During 3 years of observations it was found that the results were not different from one locality to another and reveal a decrease by 1.7 x of average conidia density.

The experiment described in this paper represents the first study in Romania on dispersion of entomopathogenic fungi *Beauveria bassiana* colonized on a natural organic substrate, in potato and eggplant crops.

CONCLUSION

Density dynamics of entomopathogenic fungus *B.bassiana* was similar in treated and untreated variants, when assessments were made at a height of 100 cm. Air density of fungal conidia was higher at 4 cm above the ground and the differences between treated and untreated variants were statistically significant. To estimate the density of *B.bassiana* in different habitats - in the air and soil, we recommend quantifying fungal spores in an area as close as possible to the soil surface.

ACKNOWLEDGEMENTS

This study was conducted in the frame of Programme-Nucleu, implemented with the support of ANCSI, project no. PN-16-29-02-01.

REFERENCES

ANDREI, A.-M. (1998). *Beauveria bassiana* – bioinsecticid pentru combaterea gândacului din Colorado. A IV-a Conferinta Nationala de Protectia Plantelor prin mijloace biologice si biotehnice, Brasov, 90 - 96.

ANDREI, A.-M. (1999). Cercetari privind însusirile epizootice ale ciupercii entomopatogene *Beauveria bassiana* (Bals.)Vuill. Analele I.C.P.P., vol. XXX, 143-147.

HOLDER, D.J., KEYHANI, N.O. (2005). Adhesion of the Entomopathogenic Fungus *Beauveria (Cordyceps) bassiana* to substrata. Applied and Environmental Microbiology, 71, 9, 5260-5266.

MEYLING, N.V., EILENBERG, J. (2006). Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation. Mycological research, 110, 188-195.

MUSCALU, A., PIRNA, I., ANDREI, A.-M. (2008). Cercetari privind combaterea ecologica a gândacului din Colorado din cultura de cartof prin metode biologice si fizice. INMATEH, 25, 2, 198-205.

SHIMAZU, M., SATO, H., MAEHARA, N. (2002). Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycetes) in forest air and soil. Applied Entomology and Zoology, 37, 1, 19-26.