Biological Control of Adult Populations of Grape Phylloxera with Entomopathogenic Fungus

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ABSTRACT

In order to analyze the efficacy of the 4 types of fungus preparation based on the *Beauveria bassiana* spores on the adult populations of grape phylloxera an experiment arranged in a randomized complete block design with three replications was organized in the greenhouse conditions. There were taken into study two grapevine varieties, Cabernet Sauvignon and Feteasca alba. The efficiency of bioproducts was determined based on the results regarding the period of the persistence and the viability of conidia on the leaves after the treatments, the period of the virulence of bioinsecticides and their insecticidal capacity. The conidia of *Beauveria bassiana* persisted on the leaves surface and infected the larvae and adults of phylloxera for a period of minimum six days, depending of the bioinsecticide composition. The chemical insecticides caused a stronger and faster lethality in comparison with the fungal biopreparations, the high larval mortality of 90.7% being recorded during the first two days from the treatment. After six days, the rate of lethality caused by the variant of bioinsecticide represented by conidial suspension added with 1% glycerol and 1% canola oil was similar to that produced by chemical preparation. Repeated application of the bioinsecticides based on the *Beauveria bassiana* can cause a significant reduction of the Phylloxera population and it could replace the chemical treatment.

Keywords: *Beauveria bassiana*, bioinsecticide, grape phylloxera.

INTRODUCTION

Phylloxera (*Phylloxera vastatrix*) is considered the most important pest of vineyards worldwide. One of the most effective ways to control the damage is based on the use of the *Beauveria bassiana* entomopathogenic fungus biopreparation (Daoust and Pereira, 1986).

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has shown potential for the management of insects (Feng *et al.* 1994) and its pathogenicity has been demonstrated in a field environment against insect populations, belonging to various orders: Coleoptera (Liuel Bauer, 2008, Todorova *et al.*, 1996; Todorova *et al.*, 2002), Homoptera (Dara *et al.*, 2007), Lepidoptera (Nguyen *et al.*, 2007) and hemiptera (McGuire *et al.* Leland, 2006).

In order to be used as biocontrol agents, the conidia, which are the main component of the bioinsecticides based on *Beauveria bassiana* have to be stable (Sabbahi *et al.*, 2008).

There are very few reports concerning the persistence of the *Beauveria bassiana* conidia on the leaves under natural conditions and also, regarding the efficacy of the products based on *Beauveria bassiana* on the adult populations of *Phylloxera vastatrix*, under controlled conditions, in the greenhouse and field.

The present study aimed to establish the optimum composition of the bioinsecticid that could determine the maintaining of the viability of conidia for a longer period of time while maintaining their virulence ability for reducing of phylloxera population.

MATERIALS AND METHODS

A conditioning liquid product of *Beauveria bassiana* with fungitoxic effect was used in the biological control. The studies concerning the efficiency of the bioproducts based on *Beauveria bassiana* on the grape phylloxera (*Dactylosphaera vitifoliae*) populations were achieved in the greenhouse conditions using Cabernet Sauvignon and Feteasca alba as biological material.

The efficiency of bioproducts was determined based on the results regarding the period of the persistence and the viability of conidia on the leaves after the treatments, the period of the virulence of bioinsecticides and their insecticidal capacity.

The data were compared with those recorded in the case of the chemical treatments.

An experience with 3 factors, using the method of subdivided plots was performed. 16 experimental variants were afforded:

Factor A: the variety with two graduations ($a = 2$):

a_1 – Cabernet Sauvignon;

a_2 – Fetească albă.

Factor B: conidial concentration, with two graduations ($b = 2$):

b_1 – $2,5 \times 10^7$ /ml;

b_2 – $3,2 \times 10^9$ /ml.

Factor C: the type of fungal formulation, with four graduations ($c = 4$):

c_1 – conidial suspension in Coral modified liquid medium;

c_2 – conidial suspension added with 1% glycerol;

c_3 – conidial suspension added with 1% glycerol; 1% canola oil;

c_4 – conidial suspension added with 1% glycerol; 1% canola oil and 0,5% clay.

The treatments were performed in the greenhouse conditions, in open space. A hand sprayer with flow rate of two liters was used to carry out the treatments. The treatments were performed in the morning, in order to limit the unwanted effects caused by UV radiation.

Aspects concerning the organization of the experimental device are presented in Fig.1.

The persistence of the insecticidal activity and viability of the conidia were performed by analysis of leaves after the treatment. Six leaves for each experimental variant were collected every three days, for nine days. Three leaves were used to determine the period of virulence of bioinsecticides, and three days for establishing the degree of the conidia viability.

Evaluation of the duration of insecticidal activity of the preparations based on *Beauveria bassiana* on the grape phylloxera population was performed every two days after treatments.

Three leaves per each variant were placed in the Petri dish on filter paper moistened with sterile distilled water. Leaf petiole was kept in a bowl of water in order to maintain the leaf turgency. Each leaf was infested with 20 larvae

Fig. 1. Aspects concerning the organization of the experimental device

of grape phylloxera. Microscopic observations on larval mortality were made for six days. True mortality was taken to occur for those cadavers on which sporulation were visible.

The mortality assay was calculated according to the Abbot's Mortality corrected % using formula:

$$\text{Abbot's Mortality corrected \%} = (1 - n \text{ in T after treatment} / n \text{ in Co after treatment}) \times 100.$$

where: n = number of live insects, T = treated, Co = control.

RESULTS AND DISCUSSION

CONIDIA VIABILITY

A significantly decrease in the number of conidia on the leaves, during the time was put into evidence.

The highest number of conidia reported to the leaf area was recorded after two days of the treatment. The values ranged between 56.0×10^3 CFU/cm² (conidial suspension in Coral modified liquid medium) and 83.3×10^3 CFU/cm² (conidial suspension added with 1% glycerol; 1% canola oil) considering the application of the bioinsecticides in concentration of 2.5×10^7 CFU/ml and between 58.3×10^3 CFU/cm² (conidial suspension in Coral modified liquid medium) and 85.3×10^3 CFU/cm² (conidial suspension added with 1% glycerol; 1% canola oil and 0,5% clay) in case of the application

of the bioinsecticides in concentration of 3.2×10^9 CFU/ml.

After 6 days of treatment, the number of conidia decreased up to 97.6% in case if the application of the bioinsecticides in concentration of 2.5×10^7 conidia/ml and up to 98.3% at the concentration of 3.2×10^9 conidia/ml (Fig. 2).

The analysis of variance concerning the conidia viability after the first day of treatment, for the whole experience, indicated a lack of statistical significance in the case of the simple action of Factor A (variety), and Factor B (concentration of bioinsecticide) and the presence of the significance for factor C (composition of bioinsecticide) (Fig.1.).

The effect of these reactions partially extended on the binary interactions involving Factor A (A x B) and (A x B x C) which have not statistically significance.

The existence of statistical significance for factor C and binary interactions between factors B x C indicates the great influence exercised by these two factors from the first day of treatment.

The analysis of variance concerning the conidia viability after six days of treatment indicated a lack of statistical significance in the case of the simple action of Factor A (variety), and of combined action A x B, the presence of an weak statistical significance for Factor B (concentration of bioinsecticide) and the maintaining of the presence of the significance for factor C

Note: c₁ – conidial suspension in Coral modified liquid medium; c₂ – conidial suspension added with 1% glycerol; c₃ – conidial suspension added with 1% glycerol; 1% canola oil; c₄ – conidial suspension added with 1% glycerol; 1% canola oil and 0,5% clay.

Fig. 2. Evolution of the conidia viability related to the composition and concentration of bioinsecticide

Tab.1. Analysis of variance regarding the viability of the conidia after the first day of the treatment

Cause of variability	SP	GL	s ²	S _d	F Probe		t-Test		
					0.5%	1%	5.0%	1.0%	0.1%
The variability of large plots									
SP large plots	1.34	5	0.27						
SPR	1.33	2	0.67		18.51	98.5	4.3	9.93	
SPA (A- Variety)	0.003	1	0.003	0.011	2.29		0.05	0.11	0.35
SP Error (a)	0.003	2	0.001						
Variability of medium plots									
SP large and medium plots	3.51								
SP medium plots	2.18	6	0.36		7.71	21.2	2.78	4.6	8.61
SPB (B - Concentration of bioinsecticide)	0.56	1	0.56	0.176	1.51		0.49	0.81	1.52
SP combination AxB	0.69				7.71	21.2			
SP AxB	0.12	1	0.12	0.249	0.32		0.69	1.15	2.15
SP Errors (b)	1.49	4	0.37						
Variability of small plots									
SPT	67.2								
SP small plots	63.7	36	1.77		3.01	4.72	2.06	2.8	3.75
SPC (C- Compozition of bioinsecticide)	61.6	3	20.52	0.097	365**		0.20	0.27	0.36
SP combination AxC	61.6				3.01	4.72			
SP A x C	0.1	3	0.02	0.137	0.38		0.28	0.38	0.51
SP combination B xC	62.8				3.01	4.72			
SP B xC	0.7	3	0.23	0.137	4.015*		0.28	0.38	0.51
SP combination abc	63.1				3.01	4.72			
SP A x B x C	0.1	3	0.02	0.194	0.396		0.40	0.54	0.73
SP Error (c)	1.3	24	0.06						

(composition of bioinsecticide) and for binary (A x C; B x C) and ternary (A x B x C) interactions.

The two vinifera varieties, Cabernet Sauvignon, and Feteasca alba had a similar reaction to the same concentration of about bioinsecticides, this aspect being maintained throughout the duration of the experience as evident from analysis of variance which put into evidence that the variety (Factor A) is not a significant factor influencing the viability of conidia.

The simple action of factor C (mean C) after 6 days of treatment remained very significant compared with the average of the experience. The simple conidial suspension without protection, in Coral liquid medium and that with the addition of 1% glycerol induced significant differences in a negative way, the conidia viability being with

67.47% and 60.24% respectively lower than the mean of experience.

The other two variants, conidial suspension added with 1% glycerol; 1% canola oil (c₃) and conidial suspension added with 1% glycerol; 1% canola oil and 0.5% clay (c₄) caused significant differences in a positive way, the conidia viability being higher with 39.76%, and 26.51% respectively.

The comparison between these last two variants showed a greater efficacy with 9.48% of the variant c₃.

Compared with the mean of experience, regarding Cabernet Sauvignon variety a lower viability of conidia with 60.49%, respectively with 48.15% was registered for the types of c₁ (conidial suspension in Coral modified liquid medium) and c₂ (conidial suspension added with 1% glycerol)

formulations and with 81.48%, respectively with 29.63% higher for c_3 (conidial suspension added with 1% glycerol; 1% canola oil) and c_4 (conidial suspension added with 1% glycerol; 1% canola oil and 0.5% clay) formulations.

Concerning Feteasca alba variety, a similar response was observed but the differences from the mean are much higher (Fig. 3).

Establishing the period of the virulence of the bioinsecticides

A decrease of the insecticidal activity of the formulations based on *Beauveria bassiana* was registered depending of the treatment

time. After two days from the treatment, a lethality rate between 11.7% and 21.7% at the concentration of 2.5×10^7 conidia/ml and double at the concentration of 3.2×10^9 conidia/ml was recorded.

After 6 days from the treatment the rate of lethality increased up to 70% in case of 2.5×10^7 conidia/ml and up to 94.7% at the concentration of 3.2×10^9 conidia/ml. The chemical insecticides can determined a more intense and rapid lethality in comparison with the fungal biopreparations, 88.3% on average after two days and 95% after four days of treatments (Fig. 4).

Note: c_1 – conidial suspension in Coral modified liquid medium; c_2 – conidial suspension added with 1% glycerol; c_3 – conidial suspension added with 1% glycerol; 1% canola oil; c_4 – conidial suspension added with 1% glycerol; 1% canola oil and 0.5% clay.

Fig. 3. The viability of conidia after 6 days of treatment depending on the type of fungal formulation and variety

Note: b1 – 2.5×10^7 conidia/ml b2 – 3.2×10^9 conidia/ml; c_1 – conidial suspension in Coral modified liquid medium; c_2 – conidial suspension added with 1% glycerol; c_3 – conidial suspension added with 1% glycerol; 1% canola oil; c_4 – conidial suspension added with 1% glycerol; 1% canola oil and 0.5% clay.

Fig. 4. Insecticidal activity of the bioproducts during a week

Our data do not contradict those obtained by Daoust and Pereira, (1986), which showed a persistence of the insecticidal activity of *Beauveria bassiana* conidia on leaves around a week. The treatments were carried out under exposure to sunlight with full protection, with partial protection and without protection. In terms of total exposure to sunlight, the viability of conidia was reduced at half in 1-2 days and the insecticidal activity lasted up to 7 days. Conidia protected from sunlight survived much longer, lasting insecticidal activity of approximately 3 weeks.

Similar results were obtained Sabbah et al., 2008, in terms of applying treatments with a bioinsecticide based on *Beauveria bassiana* in order to protect strawberries crops against *Lygus lineolaris*.

CONCLUSION

The formulations based on *Beauveria bassiana* caused a gradual reduction of populations of phylloxera, while the chemical treatment acted more intense and rapid.

Conidia of *Beauveria bassiana* can persist and infest the larvae and adults of phylloxera for at least 6 days. The repeated application of bioinsecticides based on *Beauveria bassiana*, every 6 days, can cause a significant decrease in the phylloxera population and can replace, partial or total, the chemical treatments.

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