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ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana* (Bals-Criv.) Vuill. AS A PROMISING AGENT FOR THE RASPBERRY CANE MIDGE *Resseliella theobaldi* (Barnes) BIOCONTROL

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Abstract

Red raspberry *Rubus idaeus* L. is a widespread crop including Eurasian continent. This berry crop is often damaged by the dangerous pest, the raspberry cane midge *Resselliella theobaldi* (Barnes). This insect pest commonly destroys 30-80 % of raspberry canes and decreases berry yield 5-6-fold. Chemical insecticides predominate in raspberry plant protection against the pest. However, the trend of recent years lays in replacement of chemicals for ecologically safe biological agents, especially for soft fruit plant protection. The entomopathogenic fungus *Beauveria bassiana* (Bals-Criv.) Vuill. is a well-known agent for biocontrol of *Diptera* insects, including the cane midge. In this paper for the first time we have used a Siberian isolate of *B. bassiana* as a biological agent for regulation of Siberian population of this dangerous pest of the red raspberry. The aim of the research was to demonstrate capability of Siberian *B. bassiana* strain to suppress the number of raspberry cane midge in the laboratory and in the field conditions for two red raspberry cultivars. *B. bassiana* strain IC-1480-25 was isolated from Colorado beetle dead larvae in Novosibirsk region. In the laboratory the influence of *B. bassiana* was studied by counting of adults flying from soil where larvae of last instar were treated with entomopathogenic fungus at 10^6-10^7 CFU/ml concentration. Laboratory test efficacy was more than 80 %. In 2015-2016 the impact of *B. bassiana* on *R. theobaldi* was studied in the field conditions (plantation of Siberian Garden, Novosibirsk region). Raspberry cultivars Altai Zorenka (cv. 1) and Yellow Giant (cv. 2) differed in the susceptibility to the insect were field tested. In order to provide a reliable occupancy of ecological niche by phytophagous insect and to obtain reliable results, the artificial cane splits were created before the strain application. The results of field test were in line with the laboratory experiments. The *B. bassiana* activity depended on weather conditions, particularly on humidity. In the more humid 2015, the contrast between two varieties in response to *B. bassiana* was more pronounced. The efficacy of treatment with 10^7 CFU/ml fungal suspension was less than 60 % for cv. 1 and 100 % (all larvae died) for cv. 2. The results of this research evidence that the suppression of raspberry cane midge by the potential biocontrol agent depends on berry cultivar, weather conditions and *B. bassiana* dose. Larvae number and their mortality as well as degree of bark splits occupancy is mainly determined by the differences in raspberry variety and their response to weather changes, and also by the impact of weather on insect population. These results confirm the data of Russian and foreign researchers that 10^7 CFU/ml concentration of *B. bassiana* provides a marked effect on *Diptera* pests.

Keywords: red raspberry, raspberry cane midge, fungus *Beauveria bassiana*, pest number regulation, biological efficacy

Red raspberry *Rubus idaeus* L. is a widespread crop [1, 2], including in Eurasia [3-6]. This berry crop is often damaged by a dangerous pest, the raspberry cane midge *Resseliella theobaldi* (Barnes) [7-10]. Females lay eggs in fissures on raspberry canes, whether naturally occurring or resulting from damage, after which the hatched larvae destroy the stem tissue. The phytophage can destroy up to 30-80% of raspberry shoots, resulting in 5-6 times lower yield. The pest migrated to Western Siberia from the European regions of the former USSR on plantings; this happened in the first half of the 1970s at the latest. Here, the midge adapted successfully and formed a local population, further spreading across the region; it was not detected immediately since the imago is small, and the larvae are well-capable of hiding. In the Novosibirsk Region, the midge was first discovered in 1983; scientists further studied its harmfulness and researched into the opportunities to suppress its population [11].

Raspberries are conventionally protected against the midge by chemical insecticides [12-14] based on a variety of compounds [15, 16]. However, the use of chemicals causes an increasing concern [17] and a discussion of the need to minimize pesticide loads while keeping high product quality [18], which draws attention to eco-friendly biologicals, especially for the treatment of plantings [19, 20]. The feasibility of controlling the population of raspberry cane midge was first shown for the Russian biological based on *Bacillus thuringiensis* subsp. *israelensis*, which had been developed to control the populations of dipterans [20]. However, for the order *Diptera*, which includes raspberry cane midge, the most appropriate biological population control agent is the entomopathogenic fungus *Beauveria bassiana* (Bals-Criv.) Vuill. [21-24]. Earlier laboratory tests prove three strains of *Beauveria bassiana* isolated in different geographical locations to have a >90% efficacy against raspberry cane midge [25]. Since the midge affects the inner cortex of canes (primary cortex parenchyma and the non-corked periderm), the ability of *B. bassiana* to colonize the insides of plants is crucial [26-29].

This paper is the first to demonstrate the effectiveness of a local *B. bassiana* isolate as a bioagent for controlling the Siberian population of raspberry cane midge.

The goal hereof was to test the siberian strain of *beauveria bassiana* as a suppressor of raspberry cane midge larvae in laboratory and field tests on two red raspberry varieties.

Techniques. Experiments were carried out in 2015 and 2016. *B. bassiana* strain IC-1480-25 from the collection of Issledovatelsky Tsentr LLC was isolated from dead Colorado beetle larvae in the Novosibirsk Region. To culture the strain, the team prepared various nutrient media (pH 6.5), poured 500 ml of each in a 2,000-ml Erlenmeyer flask, closed the flasks with cotton and gauze plugs, and sterilized in an autoclave at 121 °C over 40 minutes. Post-sterilization pH was 6.3. Nutrient media were cooled to 25 °C and inoculated with a culture grown in potato glucose agar, and then incubated in a thermostatic shaker at 25 °C over 8 to 10 days.

To assess the effects of the bioagent on raspberry cane midge larvae, a suspension of fungal conidia (10^6 and 10^7 CFU/ml) was placed in 125-ml plastic cups, each pre-filled with 100 g of soil sampled from under raspberry plants. Experiments were run four times. For control, the soil in cups was treated with 25 ml of water. During the experiment, the soil was moisturized systematically with 10 ml of water per cup by manually spraying it on the surface [24]. After 14 to 20 days of room-temperature incubation, 20 ready-to-pupation third-instar *R. theobaldi* larvae were placed onto each sample; the larvae had been extracted with a brush from raspberry cane fissures of the same plantation where the soil had been sampled prior to the experiment. The cups were covered with nylon smeared with Polyfix entomological glue by ITs Khimtek LLC, Russia; this would cause mature insects born from the pupae to stick to the nylon surface; 20 days later, the team counted the imagoes that had left the soil.

Biological efficacy (*BE*, %) was calculated by Abbott's formula: $BE = (K_1 \cdot K_c) \cdot (K_0 \cdot K_2)^{-1} \cdot 100$, where K_0 is the number of living specimens before treatment (experiment), K_1 is the number of living specimens after treatment (experiment), Kc is the number of living specimens before treatment (control), K_2 is the number of living specimens after treatment (control).

In-field effects of *B. bassiana* on midge larvae were evaluated at experimental sites of the Siberian Garden, Novosibirsk Region, in 2015 and 2016 for two cultivars: Zorenka Altaya, which is relatively resilient to midge, and Zheltyi gigant, which is not. The fungal suspensions were applied at concentrations of 10^6 and 10^7 CFU/ml. For reference, the team used the Iskra-M chemical by Tekhnoexport, Russia, while untreated raspberry canes were evaluated as controls. Larvae populations per fissure and larvae mortality rates were counted to assess the efficacy of the bioagent.

To enable the phytophage to fill the niche more reliably for more significant results, fissures had been made in canes on purpose. To that end, a 10-cm cut was made in each cane at 30 to 50 cm above ground with a dissecting needle, which would attract midge females. A 1 to 2-mm epidermis strip was exfoliated from the fissure to make a pocket for imagoes to lay eggs; canes were treated with the fungal suspension on the same day. For treatment, the experimenters used Orion-Kwazar back-carried sprayers (Kwazar Corporation Sp. z o.o., Poland); the application rate was 500 to 1,000 l/ha. Five or 6 labeled canes were cut two weeks after treatment to count larvae populations per fissure as well as their mortality rates.

Statistical processing was done in ANOVA and Microsoft Excel 2010 with means (*M*) and standard errors of the mean (±SEM) calculated. Significance was assessed by Student's *t*-test at $p < 0.05$ [30].

Results. Laboratory experiments run to test *B. bassiana* effects on raspberry cane midge at two concentrations revealed a statistically significant ($p < 0.05$) reduction in the flown-out imago numbers against the controls. Thus, the biological efficacy of both concentrations (10^6 and 10^7 CFU/ml) was at least 80%, see Table 1.

1. Hatching of *Resseliella theobaldi* (Barnes) imago when exposed to the entomopathogenic fungus *Beauveria bassiana* (Bals-Criv.) Vuill. (lab test)

Group	Imago hatching		Biological efficacy, %
	average number per group (*M*±SEM)	against the original larvae population, %	
Comttol	14.3±0.7		
B. bassiana, $\times 10^6$ CFU/ml	2.8±0.6	13.8	80.5
B. bassiana, $\times 10^7$ CFU/ml	1.3±0.5	6.3	91.0

These results are consistent with the data on the laboratory performance of *Beauveria* (10^7 CFU/ml) isolated by Borisov [25] in three remote locations in Russia and Ukraine.

Laboratory test results were confirmed in field tests in 2015 and 2016, see Table 2. In 2015, treating Zorenka Altaya canes with *B. bassiana* at two concentrations resulted in a statistically significant drop in larvae populations ($p < 0.05$), with a maximum of 1 living larva per fissure. Biological efficacy thus reached 54.0% to 57.6%. The chemical Iskra M reduced the living population to 0.4 specimens per fissure with a 94% mortality rate and a 70.0% biological efficacy. Zheltyi gigant contained living larvae only if treated with *B. bassiana* at 10^6 CFU/ml. The higher concentration of 10^7 CFU/ml killed all the larvae. The biological efficacy of this concentration (10^7 CFU/ml) was high, although still below that of the chemical. Experiments were repeated in 2016; for Zorenka Altaya, the results were nearly the same. Zheltyi gigant did not demonstrate as drastic difference between the two concentrations as it had done a year earlier, see Table 2. It seems that cultivars might differ in attractiveness for the pest, which affects the effects of *B. bassiana*. Fungal agents are known to act differently depending on weather, in particular on the humidity. The heavier rainfall in 2015 might have boosted the efficacy of the fungal agent for Zheltyi gigant plants.

2. *Resseliella theobaldi* (Barnes) specimens per cortex fissure when exposed to *Beauveria bassiana* (Bals-Criv.) Vuill. and to a chemical insecticide (*M*±SEM, SKhA Siberian Garden, Novosibirsk Region)

Group	Larvaes				Mortality, %		Biological efficacy, %	
	total number		alive					
	2015	2016	2015	2016	2015	2016	2015	2016 год
Cv. Zorenka Altaya								
Control	6.0±0.9	14.6±0.1	4.3±0.1	10.4±0.2	28.3	28.8		
B. bassiana, $\times 10^6$ CFU/ml	5.1±0.7	5.2±0.2[ab]	0.8±0.2[a]	1.0±0.3[ab]	84.3	80.8	54.0	52.0
B. bassiana, $\times 10^7$ CFU/ml	5.7±0.7	5.5±0.2[ab]	0.7±0.3[a]	0.9±0.2[ab]	87.7	83.6	57.6	54.9
Iskra-M, 0.2 % (st)	6.8±1.0	7.4±0.1[ac]	0.4±0.2[a]	0.1±0.1[ac]	94.1	98.8	70.2	70.0
Cv. Zheltyi gigant								
Control	9.1±2.8	16.8±0.2	8.2±0.1	13.0±0.2	9.9	22.6		
B. bassiana, $\times 10^6$ CFU/ml	11.9±3.3[b]	3.4±0.4[ab]	6.1±0.2[a]	1.0±0.3[a]	48.5	70.6	39.5	48.0
B. bassiana, $\times 10^7$ CFU/ml	7.7±2.9	2.7±0.5[ab]	0	0.7±0.3[a]	100.0	74.1	77.1	51.5
Iskra-M, 0.2 % (st)	5.0±3.7	8.0±0.4[ac]	0	0	100.0	100.0	90.1	77.4

Note. a — difference from control is statistically significant at $p < 0.05$, b — difference from standard (st) is statistically significant at $p < 0.05$, c — difference from the preparation of lower is statistically significant at $p < 0.05$.

The results are consistent with those of other studies [22, 24, 31-33], which prove that Beauveria must be applied at a minimum of 10^7 CFU/ml to be effective against *Diptera*. Notably, these papers only present laboratory tests. Thus, in vitro infestation of Mexican fruit fly *Anastrepha ludens* (Loew) larvae, pupae, and imagoes with Brazilian, Mexican, and Ecuadorian strains of *B. bassiana* killed only the mature insects in numbers [22]. In laboratory tests, contact and oral transmission of Beauveria (10^8 CFU/ml) to mature olive fruit fly *Bactrocera oleae* (Gmelin) killed 50% of the specimens [24]. Three *B. bassiana* strains were lab-tested on the pupae of onion fly *Delia antiqua* (Meigen) (*Diptera*: *Anthomyiidae*). At 10^7 CFU/ml, the fungus killed 35.4% to 52.5% of the insects. In vitro tests also demonstrated the effects of *B. bassiana* on the puparia and imago of *Ceratitis capitata* (Wiedemann) (*Diptera*: *Tephritidae*). *B. bassiana strain* Bb-1333 caused a >50% mortality [31]. Beauveria has been isolated from the same insect species in Brazil [32]. The authors have not found any publications on the effects of this fungal biocontrol agent on raspberry cane midge, whether in vivo or in vitro. *B. bassiana* is known for its ability to spread under the cortex [34], which likely contributes to its action against raspberry cane midge as shown by this study.

Thus, the paper presents lab and field test results to demonstrate that the population of raspberry cane midge *Resseliella theobaldi* can be controlled by the Siberian strain of *Beauveria bassiana*. It shows that the suppressive effects depend on the raspberry cultivar, the weather, and the concentration of *B. bassiana*, which must be at least 10^7 CFU/ml in suspension. The difference in larvae populations and mortality, as well as in their presence in cane cortex fissures, mainly depends on the cultivar, its reaction to weather, and the weather effects on raspberry cane midge population.

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