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Brief Report

Biological Control of the Raspberry Eriophyoid Mite *Phyllocoptes gracilis* Using Entomopathogenic Fungi

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Abstract: There is an urgent need to develop biological control methods against the eriophyoid mite, *Phyllocoptes gracilis*, which causes significant losses in organic raspberry production in Europe. The use of entomopathogenic fungi (EF) is a sustainable alternative to conventional chemical pesticides, reducing the risks of pesticide resistance and other negative environmental impacts of agriculture. The objective of this study was to assess the pathogenicity of three strains of EF, two of *Beauveria bassiana* and one of *Metarhizium anisopliae*, on *P. gracilis* under laboratory conditions. Fungal spore suspensions (1×10^7 spores per mL) were sprayed on detached leaves infested with *P. gracilis*. Treated mites were kept under controlled conditions (25 ± 3 °C, 72 ± 10% relative humidity and photoperiod of 16:8 (light/dark)) and mite mortality was assessed three, five and seven days after inoculation. At all three measurement points (days after inoculation), the mortality of *P. gracilis* was highest for *B. bassiana* strain BB 1.1 and *M. anisopliae* strain MA 10.1. Our data demonstrate that EFs are promising candidates for the development of biological control agents against *P. gracilis* in raspberry crops.

Keywords: biological control; Eriophyidae; *Phyllocoptes gracilis*; entomopathogenic fungi; *Beauveria bassiana*; *Metarhizium anisopliae*

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1. Introduction

In recent years, severe infestations of *Phyllocoptes gracilis* Nalepa (Acari: Eriophyidae) in Switzerland have had a negative impact on organic raspberry (*Rubus idaeus* L.) production and resulted in severe economic losses [1]. Due to the small size of *P. gracilis* and its tendency to hide, it is difficult for growers to detect it on crops before the development of symptoms on fruits and leaves [2]. Predatory mites of the Phytoseiidae family are known to feed on eriophyids and to participate in the control of *P. gracilis* [3]. However, under most raspberry growth conditions, these predatory mites do not seem to prevent a rapid increase in *P. gracilis* populations. Entomopathogenic fungi (EF) are a more sustainable alternative to chemical pesticides [4]. EF are used in classical and augmentative biological control strategies because they have no or low impact on natural enemies and biodiversity and are considered safe in terms of human health [5,6]. EF are efficient biological control agents of a wide range of mites, but none have been identified for the control of *P. gracilis* populations.

The objective of this study was to assess the pathogenicity of three strains of EF, two of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and one of *Metarhizium anisopliae* (Metchnikoff) Sorokin, on the eriophyoid mite, *P. gracilis*, under laboratory conditions. The identification of virulent pathogenic fungal strains is of great interest for the development of a potential biological control agent for this phytophagous mite [7].

2. Materials and Methods

Fungal strains were provided by the Plant and Pathogens Group of the Research Institute, Earth, Nature, Environment of Hepia (Geneva, Switzerland). Entomopathogenic fungi (*Beauveria bassiana* (strains BB 1.1 and BB 11.6), *Metarhizium anisopliae* (strain MA 10.1) were isolated from soil samples collected across Switzerland using the "Galleria bait" method [8] and grown on solid medium of potato glucose agar (PGA: potato 4 g/L, glucose 20 g/L, agar 15 g/L, pH 5.4). Subcultures were grown on PGA at 24 °C in the dark for 14 days. Spores were harvested by scraping subcultures and suspended in 10 mL of sterile water [9,10]. Suspensions were then filtered through a sterile aluminum sieve of 100 µm pore size, into a sterile plastic tube. Fungal spore suspensions were mixed for 5 min, and the spore concentration was determined using a counting chamber (Kova® Glasstic® slide 10, Hycor Biomedical Inc., Garden Grove, CA, USA) [9]. Spore suspensions were then adjusted to a concentration of 1×10^7 spores per mL [10]. For each bioassay, spore suspensions were prepared and used on the same day [11]. Spores were left in solution at least two hours before use to allow spore rehydration and swelling. Spore viability was assessed according to [9] 24 h after inoculation. Briefly, spore suspensions were titrated to 1×10^4 spores/mL and plated on Sabouraud dextrose agar (SDA). Spores were observed under a microscope and considered germinated when the germ tube was at least as long as the diameter of the spore [12,13].

Phyllocoptes gracilis were collected from a highly infested organic plantation of raspberry cv. "Tulameen" in Eastern Switzerland. The mite species was identified through microscopic study using the simplified key to the family of Eriophyoidea (Linder 2016, unpublished). Mites were reared on small and healthy raspberry plants (cv. "Tulameen") in a climatic chamber (Conviron Seed germinator G1000, Controlled Environments Ltd., Winnipeg, MB, Canada) at 25 ± 3 °C with a relative humidity of 72 ± 10% and a photoperiod of 16 h:8 h (light/dark). The infestation of new leaves was done according to [14].

According to observations made by [15], eriophyoid mites are sensitive to physical manipulation so the transfer of mites with a microneedle usually results in high mortality. Therefore, our bioassay method was adapted to the small size of eriophyoid mites by pre-counting the mites on infested leaves before application of the treatment.

Leaf discs of 3 cm in diameter were cut from infested leaves of similar maturity and then cut in half [16]. Eriophyoid mites were counted at 70× magnification and dead individuals were removed from the infested leaves. Each individual was counted because it is not possible to differentiate an adult from an immature or a male from a female at 70× magnification. Then, leaf discs were immersed for 5 s in the spore suspension according to [17]. The control was treated with a solution of sterile water as in [16,18].

After treatment, leaves were placed with the abaxial surface upwards on filter paper saturated with sterile water in plastic Petri dishes (55 mm diameter, 17 mm depth). Petri dishes were placed in a climatic chamber (as above) at 25 ± 3 °C with a relative humidity of 72 ± 10% and a photoperiod of 16 h:8 h (light/dark). Each treatment was replicated 10 times.

Mortality was recorded three, five and seven days after the application of the spore suspension [10]. The anterior ends of the mites were gently nudged with a single hair paintbrush to provoke movement and the mites were considered dead if they did not react [17].

Statistical analyses were performed with R software (version 4.0.4). In order to analyze the effects of the two categorical predictors (treatment and day after inoculation) on mite survival 3, 5 and 7 days after inoculation, we fitted generalized linear models (GLMs) to our data with a binomial error distribution and a logit link function [19]. We used the emmeans function [20] for comparing the group means of significant variables ($p < 0.05$).

3. Results

At all three measurement points (days after inoculation), the mortality was highest for BB_1.1 (36%; 66%; 80%) and MA_10.1 (31%; 60%; 76%) and lowest for the control (10%;

28%; 44%) (Figure 1). Inoculation with BB_11.6 triggered a significantly lower mortality than with BB_1.1 and MA_10.1 and a significantly higher mortality than the control at all three time points (24%; 51%; 69%). The mortality increased with days after inoculation.

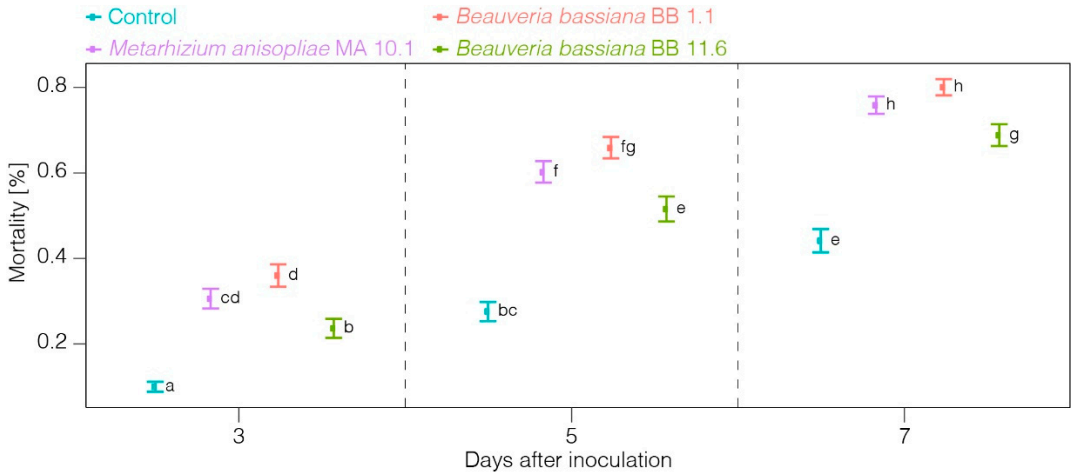

Figure 1. Mortality and asymp. confidence intervals (0.95) of *P. gracilis*, three, five and seven days after treatment application.

4. Discussion

Naturally occurring EF are important regulators of arthropod populations [5] and thus good potential biological control agents for phytophagous mites and insects [6,21]. Several strains of EF are already used in classical and augmentative biological control as well as in conservation strategies [6] and have proved successful against a range of mites, such as *Hirsutella thompsonii* Fisher [22]. In some cases, the use of EF in rotation with chemical miticides is recommended to improve the efficacy of crop protection strategies [6]. The use of EF is considered safe to humans and appears to pose minimal risk to non-target organisms as there are few reports of infection of predatory mites by EF [6].

Eriophyoid mites represent good hosts for EF because they are soft-bodied organisms without hard cuticular barriers (soft cuticle) [22]. In addition, eriophyoid mites often live in humid and dark microhabitats with microclimatic conditions favorable for fungal development [23]. Although no previous study on the pathogenicity of EF to the eriophyoid mite *P. gracilis* has been found, similar experiments on other eriophyoid mites have been performed previously. Alves et al. tested the pathogenicity of *B. bassiana* to the eriophyoid mite, *Phyllocoptruta oleivora*, at different spore concentrations [24]. The mortality of *P. oleivora* was observed two days after treatment application. At a concentration of 1×10^7 spores/mL, the study reached similar results to ours with a mortality of 64.1% ± 11.9% after 5 days. The pathogenicity of the different strains was assessed. Different *B. bassiana* strains applied to the eriophyoid coconut mite, *Aceria guerreronis*, caused mortality ranging between 72.9% and 87.0% five days after treatment application at a concentration of 1×10^8 spores/mL [25].

In this study, the mortality of *P. gracilis* in the control group was relatively high in comparison with the mortality reached in the control of other eriophyoid mite species under similar experimental conditions. Indeed, the mortality of *P. oleivora* in the control reached 6 ± 1.15% while the mortality of *A. guerreronis* amounted to 2.33% five days after treatment application [24,25]. This relatively high mortality in the control may indicate that the conditions of the climatic chamber might not have been optimal for *P. gracilis* development. Further experiments are therefore needed to optimize the climatic conditions for rearing *P. gracilis*.

Follow-up studies should repeat this experiment on different populations of *P. gracilis* to confirm the pathogenicity of tested fungal strains and compare their virulence. A comparison of virulence with commercial formulations of *B. bassiana* and *M. anisopliae* would be of great importance. It would also be necessary to test different concentrations of spores to find the most economically viable strain, as performed in [24], where the lowest concentrations showed the lowest mortality scores but were the most cost-effective, enabling more applications in citrus orchards and therefore provided a better control of

P. oleivora. Finally, it is necessary to investigate the effects of the selected fungal strains on beneficial organisms found in raspberry crops in Switzerland.

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