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Biological Control Potential of Entomopathogenic Fungi Against *Aleurocanthus spiniferus*: Field Trials on *Citrus sinensis* in Agroforestry Ecosystems

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Abstract

The citrus spiny whitefly *Aleurocanthus spiniferus* (Quaintance), recently found in Greece, causes severe damage to the leaves and fruits of tree crops, and treatment against it is urgent. In this work, integrated treatments for the management of the *A. spiniferus* pest on *Citrus sinensis* (L.) trees, which causes intense damage to orange orchards, were studied. The experiment was carried out in an orange orchard on the Aitoloakarnania plain, an agroforestry ecosystem, and three treatments were set up: (i) a combined treatment comprising the entomopathogenic fungi *Beauveria bassiana* and *Cordyceps fumosorosea*, (ii) treatment with the application of a tetramic acid-based formulation, (iii) the control treatment. The damage caused by *A. spiniferus* was estimated by determining the pest stages on the *C. sinensis* leaves, samples of which were collected and examined at the entomology laboratory of the Agriculture Faculty of the University of Ioannina for the calculation of populations. The experimental results of this work encourage us to further investigate the use of the treatments against whiteflies, highlighting the potential of EPF for integrated pest management (IPM) in citrus trees.

Keywords: Aleyrodidae; biological control; entomopathogenic fungi; agroforestry ecosystems

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Academic Editor: Ivo Toševski

Received: 3 October 2025
Revised: 22 October 2025
Accepted: 25 October 2025
Published: 26 October 2025

Citation: Mantzoukas, S.; Papantzikos, V.; Sourouni, T.; Zarmakoupi, C.; Margaritis, A.; Eliopoulos, P.A.; Patakioutas, G. Biological Control Potential of Entomopathogenic Fungi Against *Aleurocanthus spiniferus*: Field Trials on *Citrus sinensis* in Agroforestry Ecosystems. *Agronomy* **2025**, *15*, 2488. https://doi.org/10.3390/agronomy15112488

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1. Introduction

The most important crop of the genus *Citrus* is the orange *Citrus* × *sinensis* (Sapindales: Rutaceae), accounting for 50% of global citrus production. In Europe, orange production amounts to 6.4 million tons [1]. Orange fruits are heavily attacked by various entomological pests during cultivation, notably whiteflies [2], so named for the white powder covering the adults' bodies and wings [3].

Aleurocanthus spiniferus (Quaintance) (Hemiptera: Aleyrodidae) (citrus or orange spiny whitefly) is a polyphagous pest of more than 38 plant families such as Rutaceae, Vitaceae, Lauraceae, Punicaceae, Rosaceae, and Moraceae [4]. According to the European Union, it is considered a quarantine pest [5] that causes serious economic consequences in citrus fruits [5,6]. In September 2016, *A. spiniferus* was observed in Greece, specifically in the northeastern part of Corfu, on orange trees [6]. *A. spiniferus* has six developmental stages:

egg, four nymphal instars, and adult [7]. Reproduction is reduced when temperatures are low [8]. Its biological cycle lasts 2–4 months, it completes 3–6 generations depending on climatic conditions, and females lay more than 100 eggs [5].

Aleurocanthus spiniferus can reduce the production of citrus fruits. Losses are caused on the one hand by the sucking damage they cause to the hosts [9], and on the other hand by the growth of the sooty mold *Capnodium citri* (Capnodiales: Capnodiaceae) on the fruits and leaves due to the honeydew secretions of the Aleyrodidae [10]. If the infestation is severe, the tree acquires a black appearance [11], which is followed by leaf fall and a reduction in the photosynthetic ability of the leaves [12]. In the case of *A. spiniferus*, the consequence of this damage is the limitation of fruit setting, which makes the fruits unmarketable [13]. In more severe infestations by *A. spiniferus*, branch destruction and premature fruit drop can be observed [5]. Sap removal in citrus trees by Aleyrodidae can also affect the amount of nitrogen in the tree, resulting in flowering and fruiting stage reduction [14].

Chemical control of Aleyrodidae is a common practice [15], but in the case of *A. spiniferus*, chemical control is not particularly effective and disrupts natural enemies [12]. For this reason, it is important to develop control methods with the lowest possible environmental footprint. EPF represents an environmentally friendly method to deal with entomological pests [16,17] and can be included in IPM programs [18,19]. Some EPF have been tested for their efficacy on whiteflies [15,20–22], but there are no reports about the application of EPF on *A. spiniferus*. Studies have shown that *Cordyceps fumosorosea* (Hypocreales: Clavicipitaceae) successfully controls *Aleurothrixus floccosus* (Hemiptera: Aleyrodidae) [23] and acts as a biocontrol agent toward the invasive pepper whitefly *Aleurothrixus trachoides* (Hemiptera: Sternorrhyncha) [23]. Several EPF have been reported as pathogenic agents against the citrus blackfly *Aleurocanthus woglumi* (Hemiptera: Sternorrhyncha), such as the *Aschersonia* cf. *aleyrodis* (Hypocreales: Clavicipitaceae) and *Aegerita webberi* (Polyporales: Meruliaceae) [24], *Fusarium volatile* (Hypocreales: Nectriaceae), and *Fusarium proliferatum* (Hypocreales: Nectriaceae) in combined application with an aqueous extract of *Ricinus communis* (Euphorbiales: Euphorbiaceae) [25]. *Isaria fumosorosea* has shown a high mortality rate against greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Sternorrhyncha) [26], and *Beauveria bassiana* (Hypocreales: Cordycipitaceae) has been referred to as an endophytic colonizer of tomato for the control of *T. vaporariorum* [27].

The main objective of this research was to study the concept of the biological control of *A. spiniferus* with an EPF combination of *B. bassiana* and *C. fumosorosea* (Hypocreales: Cordycipitaceae) and treatment with a tetramic acid-based formulation.

2. Materials and Methods

2.1. Field Site and Experimental Design

Infected trees with *A. spiniferus* (Quaintance) were detected in Vonitsa, western Greece. The crop field chosen for the experimental procedure was located in this area and consisted of sweet orange trees, *Citrus sinensis* (L.) Osbeck (variety: Valencia) (Figure 1). Two foliar applications were performed during the 2023 growing season, timed according to the pest's phenology, as follows: first application: 25 May 2023 (targeting early egg and 1st-instar stages); second application: 10 August 2023 (targeting the subsequent generation).

Sprays were applied at night (20:00–23:00) using calibrated Airblast sprayers (Cifarelli, Mist blower L3 evo, Voghera, Italy), which are normally tractor-mounted to ensure thorough canopy coverage, including the underside of leaves. Each treatment was applied to three replicate plots of 50 trees each (total: 450 trees). Plots were separated by at least 15 m buffer zones to minimize spray drift. Randomization was performed at the plot level within orchard rows. In the process of the field experiment, we compared a treatment, a combination of the EPF *B. bassiana* and *C. fumosorosea*, with the application of a tetramic

acid-based formulation and finally with a control treatment. The dose for Treatment A (10^8 conidia per ml) was 100 mL at 100 L H_2O; for Treatment B, it was 100 mL at 100 L H_2O; and finally, for the untreated plots, it was only 100 L H_2O. The final spray volume was 1000 L/ha for all treatments.

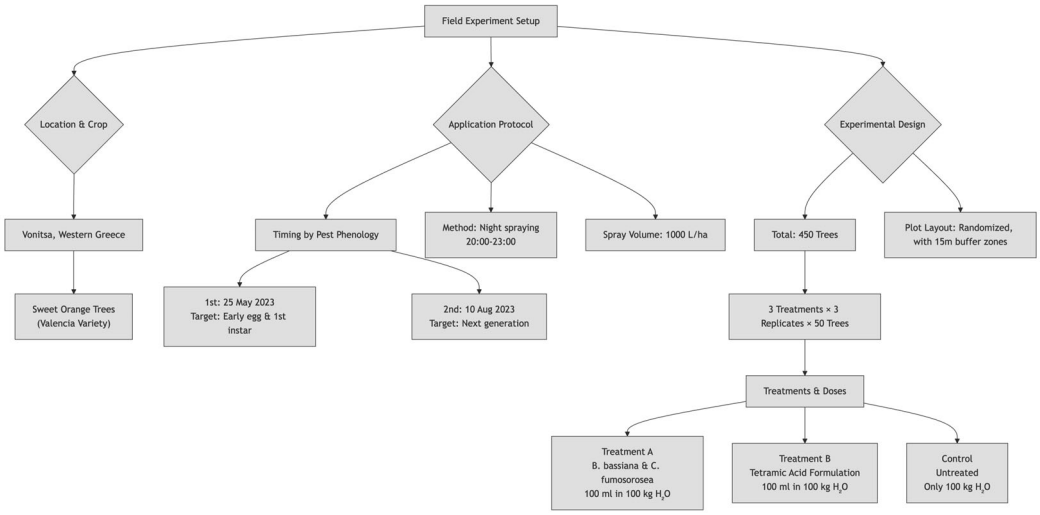


Figure 1. Experimental layout of citrus orchard plots in Vonitsa, western Greece. Each plot consisted of 50 sweet orange trees (Citrus sinensis var. Valencia) and received one of three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

2.2. Monitoring of A. spiniferus

In the field, the developmental stages of *A. spiniferus* were monitored, focusing on the presence and mortality of eggs and nymphal instars (1st–4th) (Figure 2). For this procedure, 1000 eggs of *A. spiniferus* were marked directly onto leaves with a fine brush. To minimize damage and maintain consistency, no more than four eggs were marked per leaf. Furthermore, only one egg was marked per leaf sector to avoid clustering. In a citrus field, each leaf is naturally divided into four sectors by the three major veins radiating from the petiole; this natural division was used to distribute eggs evenly and reduce sector-specific bias. After eggs were marked, a small, lightweight cardboard tag was tied around the petiole of each leaf containing the marked egg(s). Each tag was numbered and annotated with the plot or treatment code, depending on the experimental design, to ensure accurate tracking of leaves across sampling dates. To facilitate the relocation of the sampled leaves in the field, a 1 m length flagging tape was tied in a loose loop around the main stem near the top of each plant. This ensured that the tape could be easily adjusted to maintain visibility throughout repeated visits to the same location. For each marked leaf, the leaf number and positional information were recorded. Positional information was based on the four sectors of the leaf, allowing fine-scale tracking of the egg location within the leaf structure.

In the laboratory, the identity of *A. spiniferus* was verified using both stereoscopic and compound microscopy. General morphology was examined with a stereoscope (SZ51, Olympus, Munster, Germany) at 40× magnification, while detailed diagnostic features were observed using a compound microscope (Primo Star, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) at 100–400× magnification. All bioassay observations were conducted under controlled laboratory conditions (25 ± 2 °C, 65 ± 5% RH, 16:8 h L:D photoperiod). For each treatment, 210 leaves (10 leaves × 7 trees per plot × 3 plots) were randomly selected and collected from tree canopies at weekly intervals. In total, 21 collections were performed between 26 May and 13 October. After collecting, samples were placed in

paper bags and transported to the Laboratory of Productive Agriculture and Plant Health, University of Ioannina, Department of Agriculture (Arta), for examination.

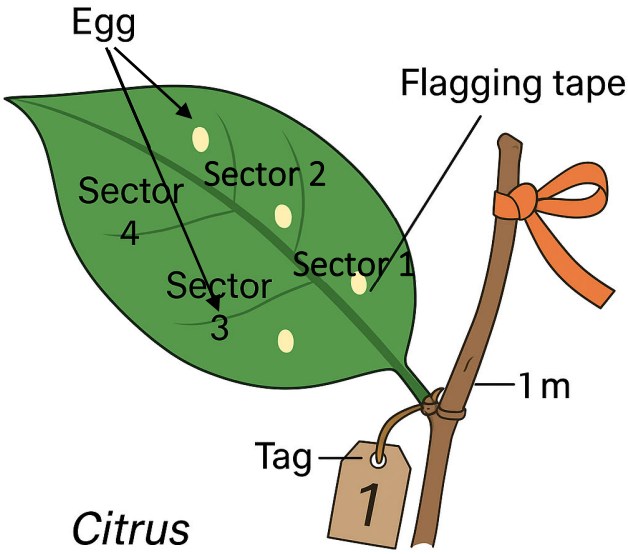


Figure 2. Field procedure for monitoring *A. spiniferus* eggs on citrus leaves. Up to four eggs were marked per leaf (one per sector), with leaf sectors defined by the three major veins. Each marked leaf was tagged at the petiole, plants were flagged for relocation, and egg positions were recorded for repeated observations.

2.3. Mathematical Estimation

For the estimation of stage-specific mortality, we adopted the approach by Southwood and Henderson 2000 [28].

- Actual (or "real") mortality was calculated as dx/l_0, where dx represents the number of individuals dying during stage x and l_0 is the number of insects present at the start of the generation. This measure provides the cumulative proportion lost across all stages when summed over the entire life cycle.
- Apparent mortality (q_x) was defined as the proportion of individuals dying within a given stage relative to those alive at the beginning of that stage. These values can be partitioned into specific factors within each stage, but they can only be combined within that stage rather than across different stages.
- Marginal mortality for a given factor B was obtained using the expression $M_B = d_B/(1 - d_A)$, where d_B is the apparent mortality from factor B and d_A is the cumulative apparent mortality from all other factors. Marginal stage-specific mortalities were further transformed into k-values according to $k = -\ln(1 - M)$, where M is the marginal mortality rate of interest. k-values are additive across factors and stages, simplifying overall analysis. Mortality proportions can be back-calculated from k-values using the relation $1 - e^{-k}$.
- Irreplaceable mortality for a given factor was derived as the difference between the total proportional mortality $\left[1 - e^{-TotalK}\right]$ and the proportional mortality recalculated after removing the k-value of that factor $\left[1 - e^{-(TotalK - k_i)}\right]$.

2.4. Statistical Analysis

Mortality us factor was analyzed using a three-way analysis of variance (ANOVA) in SPSS v.23 (IBM Corp., Armonk, NY, USA). Data on live and dead individuals per stage were analyzed using a three-way analysis of variance (ANOVA) in SPSS v.23 (IBM Corp., Armonk, NY, USA). Fixed factors included treatment, developmental stage, and

sampling date. When significant effects were detected, means were separated using the Bonferroni post hoc test ($\alpha = 0.05$). Prior to analysis, data were checked for normality and homoscedasticity and transformed if necessary.

3. Results

3.1. Observed, Apparent, and Marginal Mortality of A. spiniferus

The mortality of *A. spiniferus* at different developmental stages under field conditions is presented in Tables 1–3. All main effects and interactions were statistically significant with key factor mortality. Treatment ($F = 22.155$, df = 2, 302, $p < 0.001$) and Sample Date ($F = 18.311$, df = 20, 302, $p < 0.001$) each had significant effects. Moreover, strong interactions were observed for Treatment × Sample Date ($F = 43.111$, df = 42, 302, $p < 0.001$). Substantial variation was observed among biological stages, with the egg stage consistently exhibiting the highest mortality at the treatments, while the later instars showed markedly reduced mortality. In the control group (Table 1), mortality was minimal across all developmental stages. The egg stage showed only 1.0% observed mortality, while the first and second instars recorded 0.3% and 0.05%, respectively. No mortality was observed in the third and fourth instars. Apparent mortality was highest during the second instar (0.128), while marginal mortality remained very low across all stages ($\leq$0.026). These results suggest that under untreated conditions, natural mortality is negligible, with survival rates remaining high throughout the development period.

Table 1. Observed mortality, apparent mortality, and marginal mortality under the control treatment on the citrus.

Stage/Factor	Biological Stage			Observed Mortality *		Apparent Mortality **		Marginal Mortality ***	k-Value
	Factor (l_x)	Stage (d_x)	Factor (d_x)	Stage (d_x/l_0)	Factor (d_x/l_0)	Stage (q_x)	Factor (q_x)		
Egg	1000	10		0.010		0.010			
Effect			0		0.000		0.000	0.000	0.000
Unknown			10		0.010		0.010	0.015	0.026
1st Instar	990	3		0.003		0.004			
Effect			0		0.000		0.000	0.000	0.000
Unknown			3		0.003		0.008	0.009	0.018
2nd Instar	987	3		0.0005		0.128			
Effect			0		0.000		0.000	0.000	0.000
Unknown			9		0.009		0.007	0.006	0.012
3rd Instar	984	0		0.000		0.000			
Effect			0		0.000		0.000	0.000	0.000
Unknown			0		0.000		0.000	0.000	0.000
4th Instar	984	0		0.000		0.000			
Effect			0		0.000		0.000	0.000	0.000
Unknown			0		0.000		0.000	0.000	0.000

* Observed mortality refers to the actual number or rate of deaths that occurred in a specific population or group during a defined period. ** Apparent mortality: deaths caused by fungus infection. *** Marginal mortality: the effect on death rates resulting from a very small or "marginal" change in a specific factor rather than a large, or non-marginal, change.

The chemical treatment markedly increased mortality across all developmental stages (Table 2). For the egg stage, 66.5% observed mortality was recorded, with 52.4% attributed to chemical effects and 14.1% to unknown causes. Subsequent instars also showed elevated mortality: 7.2% in the first instar, 10.1% in the second instar, 9.9% in the third instar, and 5.0% in the fourth instar. Apparent mortality values ranged from 0.495 to 0.822, while marginal mortality was highest in the second and third instars (0.698–0.967). These findings indicate that the chemical treatment shows strong efficacy, particularly during the egg and early instar stages.

Table 2. Observed mortality, apparent mortality, and marginal mortality under the chemical treatment on the citrus.

Stage/Factor	Biological Stage			Observed Mortality *		Apparent Mortality **		Marginal Mortality ***	k-Value
	Factor (l_x)	Stage (d_x)	Factor (d_x)	Stage (d_x/l_0)	Factor (d_x/l_0)	Stage (q_x)	Factor (q_x)		
Egg	1000	665		0.665		0.665			
Effect			524		0.524		0.524	0.588	0.801
Unknown			141		0.141		0.141	0.374	0.426
1st Instar	335	72		0.072		0.495			
Effect			52		0.052		0.379	0.385	0.489
Unknown			20		0.020		0.142	0.150	0.232
2nd Instar	263	101		0.101		0.548			
Effect			92		0.092		0.501	0.698	0.964
Unknown			9		0.009		0.028	0.033	0.048
3rd Instar	162	98		0.99		0.668			
Effect			92		0.092		0.596	0.967	0.783
Unknown			7		0.007		0.021	0.029	0.042
4th Instar	64	51		0.050		0.822			
Effect			50		0.050		0.470	0.511	0.711
Unknown			1		0.001		0.003	0.004	0.013

* Observed mortality refers to the actual number or rate of deaths that occurred in a specific population or group during a defined period. ** Apparent mortality: deaths caused by fungus infection. *** Marginal mortality: the effect on death rates resulting from a very small or "marginal" change in a specific factor rather than a large, or non-marginal, change.

Table 3. Observed mortality, apparent mortality, and marginal mortality under the biological treatment on the citrus.

Stage/Factor	Biological Stage			Observed Mortality *		Apparent Mortality **		Marginal Mortality ***	k-Value
	Factor (l_x)	Stage (d_x)	Factor (d_x)	Stage (d_x/l_0)	Factor (d_x/l_0)	Stage (q_x)	Factor (q_x)		
Egg	1000	678		0.678		0.678			
Effect			570		0.570		0.570	0.666	0.828
Unknown			108		0.108		0.108	0.274	0.226
1st Instar	322	58		0.258		0.547			
Effect			48		0.048		0.379	0.485	0.589
Unknown			10		0.010		0.042	0.050	0.032
2nd Instar	264	92		0.092		0.628			
Effect			81		0.081		0.491	0.598	0.901
Unknown			9		0.009		0.038	0.043	0.028
3rd Instar	172	103		0.103		0.709			
Effect			89		0.089		0.596	0.707	0.813
Unknown			14		0.014		0.290	0.305	0.254
4th Instar	69	45		0.045		0.552			
Effect			42		0.042		0.374	0.478	0.674
Unknown			3		0.003		0.058	0.073	0.093

* Observed mortality refers to the actual number or rate of deaths that occurred in a specific population or group during a defined period. ** Apparent mortality: deaths caused by fungus infection. *** Marginal mortality: the effect on death rates resulting from a very small or "marginal" change in a specific factor rather than a large, or non-marginal, change.

Biological treatment also induced significant mortality, though generally at slightly lower levels than the chemical treatment ($F = 11.221$, $df = 2, 302$, $p < 0.001$). Egg mortality reached 67.8%, with 57.0% caused by biological factors and 10.8% by unknown causes. For the first instar, 25.8% observed mortality was observed, higher than that under the chemical treatment. Mortality continued in the second and third instars (9.2% and 10.3%, respectively), while the fourth instar showed 4.5% mortality. Apparent mortality values ranged from 0.347 to 0.709, with marginal mortality between 0.478 and 0.901. These results suggest that the biological treatment was particularly effective during the egg and first nymph stages of *A. spiniferus* ($F = 11.221$, $df = 2, 302$, $p < 0.001$).

3.2. Alive and Dead Biological Stages of *A. spiniferus*

All main effects and interactions were statistically significant with the key factor Alive. Treatment (F = 18.355, df = 2, 302, $p < 0.001$), Biological Stage (F = 20.111, df = 2, 302, $p < 0.001$), and Sample Date (F = 8.396, df = 2, 302, $p < 0.001$) each had significant effects, indicating that responses differed across experimental conditions, developmental stages, and sampling times. Moreover, strong interactions were observed for Treatment × Sample Date (F = 60.135, df = 7, 302, $p < 0.001$) and Treatment × Biological Stage (F = 68.125, df = 7, 302, $p < 0.001$). The average number of *A. spiniferus* eggs per treatment leaf is shown in Figure 3. In the control treatment, egg numbers remained consistently high, with negligible natural mortality. By contrast, both chemical and biological treatments caused sharp reductions in egg numbers. The *A. spiniferus* under the chemical treatment produced the highest egg number at the end of the experiment, whereas in the biological treatment, the egg number was also significantly reduced but at a slightly lower rate. The main effects and interactions for all factors proved to be significant with the factor Dead (Mortality) (Treatment: F = 25.232, df = 2, 302, $p < 0.001$; Biological Stage: F = 30.844, df = 2, 302, Sample Date: F = 5.232, df = 2, 302, $p < 0.001$; Treatment × Sample Date F = 44.435, df = 7, 302, $p < 0.001$, Treatment × Biological Stage F = 36.105, df = 7, 302, $p < 0.001$).

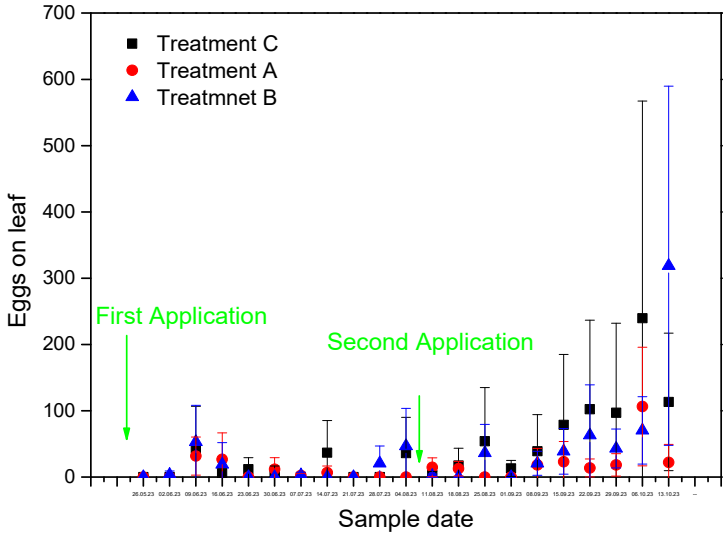


Figure 3. The average number of *A. spiniferus* eggs on treated leaves under three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

After the first treatment (May–June), the population of nymphal instars (1st–4th) was reduced to zero across all treatments, whereas the egg density on 9 June averaged 74.9 ± 11.2 in the control, 53.2 ± 9.1 in the chemical treatment, and 31.6 ± 8.1 in the biological treatment. In July, a slight increase in eggs was observed in control but not in the chemical or biological treatments. Following the second application on 10 August, egg density in the biological and chemical treatments remained significantly lower than in the control. In September, egg density peaked on 15 September at 78.7 ± 23.1 under control, 36.3 ± 13.9 under the chemical treatment, and 23.8 ± 6.1 under the biological treatment. The egg densities of control populations reached their highest levels at 239.6 ± 38.9 on 6 October, and the treated egg densities were 71.8 ± 4.9 in the chemical treatment and 66.4 ± 2.3 in the biological treatment, respectively.

The dynamics of first-instar nymphs are presented in Figure 4. In the control, survival was high, and only a small fraction of natural mortality was observed. In the chemical treatment, the mortality of first instars increased although survival remained higher than

in eggs. Following the second application on 10 August, nymph populations in biological and chemical treatments remained significantly lower than in control. Biological treatment exerted the greatest impact at this stage, resulting in a substantial reduction in live individuals and higher average mortality compared with chemical treatment. These results reinforce the importance of the first instar as a key target stage for biological control agents.

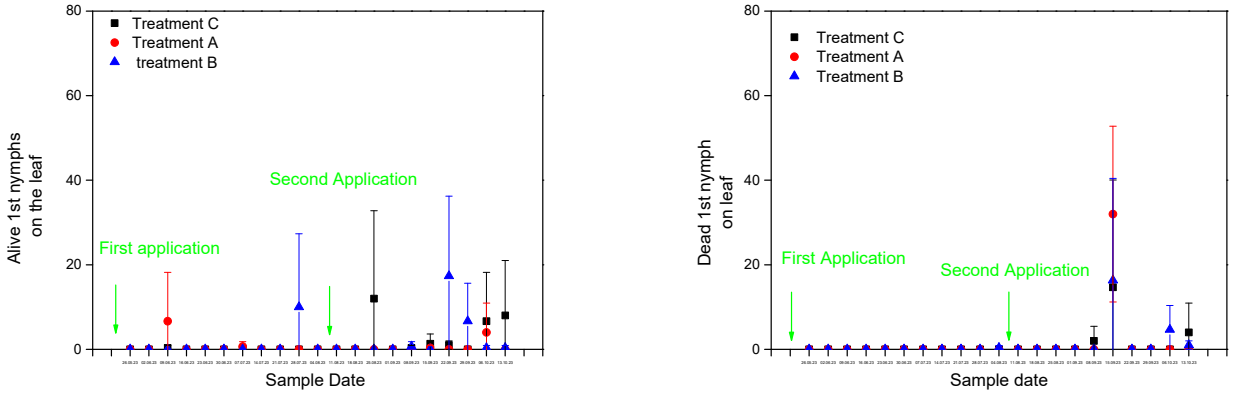


Figure 4. The average number of *A. spiniferus* 1st-instar nymphs (alive and dead) under three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

Figure 5 shows the numbers of second-instar nymphs alive and dead under the different treatments. In July, a slight increase in nymphs was observed in the control but not in the chemical or biological treatments. On 18 August, the mean density of second-instar nymphs was 4.3 ± 0.6 in the control, compared with 1.33 ± 1.52 in the chemical treatment and 1.15 ± 0.33 in the biological treatment. Mortality in the control remained minimal, while chemical treatment caused pronounced reductions in survival, with higher average mortality than in the biological treatment. However, biological treatment also suppressed this stage, albeit less sharply than the chemical treatment. The second instar thus represents a stage of intermediate susceptibility, with greater responsiveness to chemical intervention.

The average number of alive and dead third-instar nymphs (Figure 6) highlights a shift in susceptibility. Mortality in the chemical treatment remained significant, while the biological treatment had a weaker effect compared with earlier instars. In the control, survival was almost complete, confirming negligible natural mortality. By October, control populations reached their highest levels across all nymphal instars, with third-instar density at 63.3 ± 17.3. These results suggest that the third instar is less susceptible to biological agents and requires chemical inputs for effective suppression. The fourth instar (Figure 7) exhibited the lowest mortality across all treatments. Both chemical and biological interventions resulted in only limited reductions in live individuals, while, in control, almost all individuals survived. This supports the mortality tables, which consistently showed the lowest mortality rates for the fourth instar. On 6 October, nymphal density in the chemical and biological treatments remained low (20.6 ± 5.7 and 12.3 ± 3.9, respectively). The persistence of this stage across treatments highlights it as the most tolerant developmental stage and a potential bottleneck for management strategies.

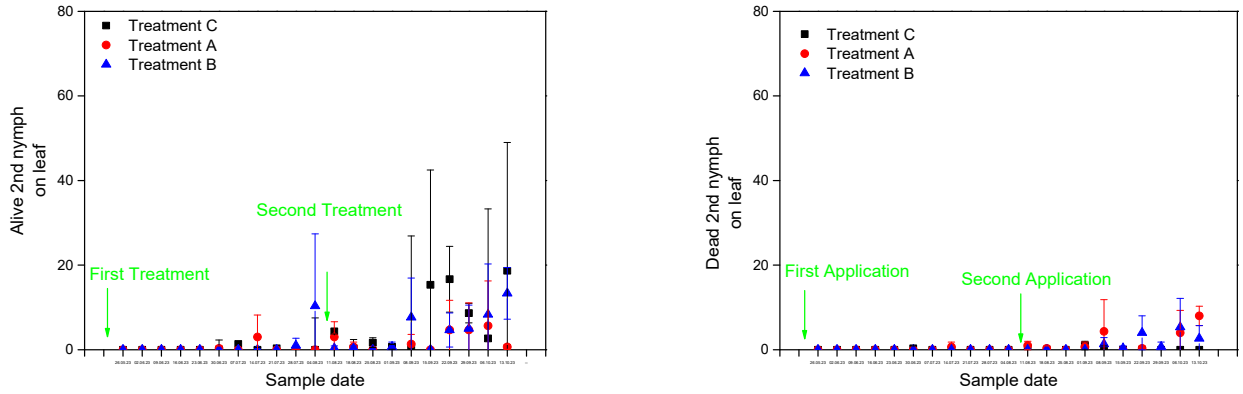


Figure 5. The average number of *A. spiniferus* 2nd-instar nymphs (alive and dead) under three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

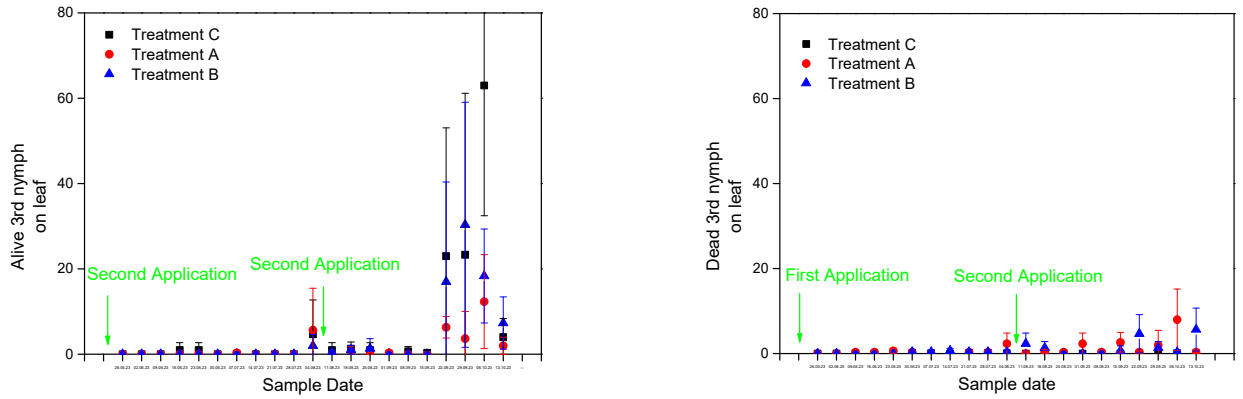


Figure 6. The average number of *A. spiniferus* 3rd-instar nymphs (alive and dead) under three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

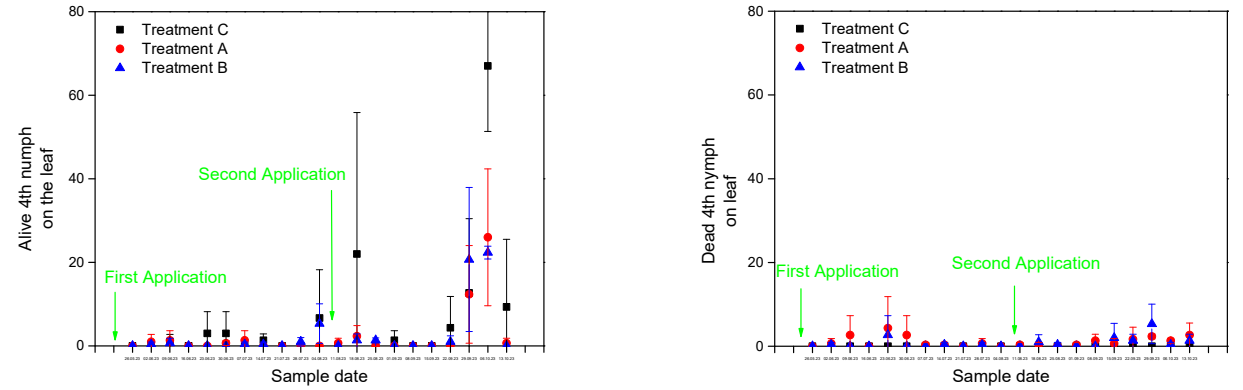


Figure 7. The average number of *A. spiniferus* 4th-instar nymphs (alive and dead) under three treatments: A = *B. bassiana* + *C. fumosorosea*; B = tetramic acid–based formulation; C = untreated control. Applications were carried out on 25 May and 10 August 2023 at night.

4. Discussion

Developing EPF with enhanced efficacy against insect pests is now a central focus of biological control research. These fungi infect hosts by penetrating the cuticle, proliferating within the hemocoel, and ultimately causing death through nutrient depletion and toxin production. This unique mode of action, combined with their high specificity and safety

for non-target organisms, makes EPF attractive and environmentally sustainable alternatives to chemical pesticides. Recent advances in strain selection, molecular improvement, formulation technologies, and application strategies have increased EPF persistence and reliability under diverse field conditions. These developments strengthen their role in IPM programs. Several fungal genera, including Beauveria, Metarhizium, Cordyceps, Isaria, Paecilomyces, Lecanicillium, and Aschersonia, have shown pathogenicity against various whitefly life stages, with early-instar nymphs particularly susceptible [29,30].

Our results are consistent with previous research demonstrating the high efficacy of entomopathogenic fungi (EPF) against whiteflies. Numerous studies have reported mortality rates exceeding 80–90% in *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), and related species following treatment with *B. bassiana*, *M. anisopliae*, or *C. fumosorosea* under both laboratory and field conditions [23,29–35]. These studies, together with our findings, confirm that EPF can effectively suppress whitefly populations through direct infection and secondary epizootic spread. Such high virulence levels demonstrate their potential as reliable biocontrol agents, offering efficacy comparable to that of chemical insecticides. Moreover, the greater susceptibility of early developmental stages observed across studies highlights the importance of application timing in maximizing control efficiency and minimizing pest resurgence.

The efficacy of these fungi is influenced by a variety of factors, such as strain virulence, inoculum concentration, host plant characteristics, and environmental factors (temperature, relative humidity) [36]. In the control treatment, mortality remained negligible at all stages, suggesting that natural mortality factors alone exert little influence on population suppression. This result underscores the pest's high survival potential under untreated conditions, which may contribute to rapid population buildup in citrus orchards. The application of chemical treatment significantly increased mortality across all stages, with the most pronounced effects observed at the egg stage and during the second and third instars. The high values for apparent and marginal mortality during these stages suggest that chemical agents effectively disrupt development and reduce survival beyond the early instars. However, mortality in the fourth instar was comparatively lower, implying greater tolerance or reduced exposure in older nymphs. The biological treatment also induced significant mortality, particularly during the egg and first-instar stages. Notably, mortality in the first instar was higher under biological treatment than under chemical treatment, suggesting that EPF are particularly effective against newly hatched nymphs. Nevertheless, mortality in later instars (2nd–4th) was slightly lower compared with that under chemical treatment, which may reflect the limitations of EPF in penetrating protective coverings or overcoming the defenses of later instars. Taken together, the results indicate that eggs are the most susceptible to both chemical and biological mortality factors, whereas fourth instars are consistently the most tolerant. The high mortality observed in the second and third instars under chemical treatment compared with the biological treatment suggests that chemicals may be more effective against intermediate instars, whereas biological treatments provide stronger suppression of early instars. In addition to direct pathogenic effects, EPF may confer indirect benefits by colonizing host plants endophytically, thereby reducing insect feeding and reproduction and enhancing plant resistance. Consequently, the integration of EPF into whitefly management strategies, particularly in combination with selective insecticides or cultural practices, represents a promising approach to sustainable pest suppression [37].

The impact of fungal epizootics on host insect populations can be profound, and considerable efforts have been directed toward exploiting this potential for pest management. Successful strategies have included both classical biological controls, involving the permanent introduction and establishment of fungal agents, and augmentative approaches based

on periodic releases [38]. Because EPF can modulate or suppress components of the host immune system, understanding these interactions is critical for predicting and optimizing their effects on pest population dynamics [39]. Achieving substantial suppression of target pests requires precise timing of applications, as the efficacy of fungal treatments is strongly influenced by the developmental stage of the host and prevailing environmental conditions.

The combined application of EPF in the present study is consistent with the findings of Yun et al. [40], who investigated the dual activity of *B. bassiana* and *M. anisopliae* against *Myzus persicae* (Hemiptera: Aphididae). Their results demonstrated that the use of culture filtrates in combination with blastospores produced the highest mortality in *M. persicae*, whereas the combination of conidia and filtrate resulted in lower efficacy compared to filtration alone, highlighting the strong virulence potential of fungal metabolites. Furthermore, EPF are regarded as safe and environmentally compatible alternatives to chemical insecticides [41], strengthening their value as biocontrol agents against destructive pests such as *A. spiniferus*.

5. Conclusions

From an applied perspective, these findings highlight the importance of stage-targeted management strategies. For effective suppression of *A. spiniferus* populations, interventions should ideally be timed to coincide with the egg and early-instar stages, when susceptibility to both chemical and biological mortality factors is highest. In summary, our results demonstrate that EPF shows promising results against Aleyrodidae and should therefore be further investigated as potential biological control agents and as a valuable component of citrus IPM. Although this was a preliminary investigation, the fungal isolates tested showed encouraging insecticidal effects that warrant extensive follow-up work. Future research should address biosafety for non-target organisms, evaluate performance under diverse field conditions, and develop formulations with improved persistence, shelf life, ease of application, and consistent virulence.

Author Contributions: Conceptualization, S.M.; methodology, S.M.; V.P., C.Z., T.S. and A.M.; software, S.M.; validation, S.M. and P.A.E.; formal analysis, V.P.; investigation, S.M., V.P., T.S., C.Z. and P.A.E.; resources, G.P. and S.M.; data curation, S.M. and P.A.E.; writing—original draft preparation, S.M. and V.P.; writing—review and editing, S.M., V.P. and P.A.E.; visualization, S.M.; supervision, G.P. and S.M.; project administration, S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author S.M.

Conflicts of Interest: The authors declare no conflicts of interest.

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