ISSN 1727-1320 (Print),
ISSN 2308-6459 (Online)

ВЕСТНИК ЗАЩИТЫ РАСТЕНИЙ

PLANT PROTECTION NEWS

2021 ТОМ VOLUME 104 ВЫПУСК ISSUE 3

Санкт-Петербург
St. Petersburg, Russia

OECD+WoS: 1.06+RQ (Mycology) https://doi.org/10.31993/2308-6459-2021-104-3-15026

Full-text article

COMPATIBILITY OF THE FUNGUS *LECANICILLIUM MUSCARIUM* AND THE PREDATORY MITE *AMBLYSEIUS SWIRSKII* FOR THEIR COMBINED APPLICATION AGAINST THE GREENHOUSE WHITEFLY *TRIALEURODES VAPORARIORUM*

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The present study evaluated effects of the fungus *Lecanicillium muscarium* (Ascomycota: Hypocreales) and an organic extract from its mycelium on the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and its predator, mite *Amblyseius swirskii* (Acari: Phytoseiidae). Mites were exposed to fungal spores or organic extract prepared from *L. muscarium* mycelium. No negative effect was shown on the predator feeding on *T. vaporariorum* nymphs treated with fungal conidia at a concentration of 5×10^7 spores/ml; by day six the number of mite eggs and nymphs was 18.7% higher than on leaves treated with Tween 80. In contrast, treatment of leaves with a 0.5% alcohol extract derived from *L. muscarium* mycelium caused 35% mortality of *A. swirskii* adults by day two. In a trial conducted in a commercial greenhouse on rose plants, the application of *L. muscarium* conidia followed by the release of *A. swirskii* suppressed *T. vaporariorum* more effectively than each of the control agents applied separately.

Keywords: entomopathogenic fungi, predatory mites, biocontrol agents, side effect, beneficial arthropods

Submitted: 02.06.2021 *Accepted: 31.08.2021*

Introduction

Predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) is widely used in biological programs to control greenhouse whitefly *Trialeurodes vaporariorum*, tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), several species of thrips (Thysanoptera: Thripidae), and phytophagous mites (Tetranychidae) (Nomikou et al., 2001, 2002; Messelink et al., 2006; Cock et al., 2010). *A. swirskii* can be superior to other species of predatory mites because its wider prey range and its resistance to higher air temperatures (up to 25–32 °C) (Lee, Gillespie, 2011). The predator is now used in more than 50 countries, including the Russian Federation (Kozlova et al., 2018). *A. swirskii* selectively feeds on eggs and immature stages of whiteflies, and first instar nymphs of thrips (Swirski et al.1967). To provide effective control of pests at all developmental stages, other biological agents (parasitoids, predatory bugs, soil predatory mites or entomopathogenic fungi) are often considered within a larger IPM program; knowledge on the compatibility of different control agents is required for their efficient use. Numerous studies indicate an often positive nature of the interactions between arthropod natural enemies and fungal pathogens, although in some cases, they can act as antagonists (Roy, Pell, 2000).

Entomopathogenic fungi of the genus *Lecanicillium* are natural pathogens of aphids and whiteflies (Hall, 1981; Goettel et al., 2008; Ansari et al., 2011), and some species have been successfully commercialized for use against sucking pests (De Faria, Wraight, 2007). *Lecanicillium muscarium* (Ascomycota: Hypocreales), for example, is commercially produced and used to control the tobacco whitefly (Cuthbertson et al., 2008; Ali et al., 2017). The activity of these commercial products is based on the fungal spores contained herein. Insecticidal metabolites are also produced by fungal mycelium; extracts prepared from mycelium containing these bioactive compounds have demonstrated efficacy against insects and mites (Mitina et al., 2002, 2012; Wang et al., 2007).

The compatibility of *Lecanicillium* fungi with parasitoids and predators has been well studied (Kanagaratnam et al., 1979; Labbe et al., 2009; Ren et al., 2010; Aqueel, Leather, 2013). However, results of the studies of effects of entomopathogenic fungi on predatory mites are often contradictory. Some authors have established the ability of predatory mites *Neoseiulus barkeri* (Acari: Phytoseiidae), *Typhlodromus pyri* (Acari: Phytoseiidae), and *A. swirskii* to use Ascomycetes, including phytopathogenic species, as an alternative food source (Zemek, Prenerov, 1997; Momen, Abdelkhader, 2010, Ryo et al., 2012). Conidia of an entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales) were not pathogenic when consumed by *N. barkeri* but caused a decrease in the mites' size (Wu et al., 2016). On the other hand, *B. bassiana* adversely affected longevity and fecundity of the mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* (Acari: Tetranychidae) treated with the fungus (Seiedy et al., 2012).

Influence of *L. muscarium* on predatory mites has been explored to a much smaller extent. At high spore concentrations, this species has been found to be pathogenic for *Ph. persimilis* (Donka, Buttner, 2008). According to our preliminary data, when the predatory mites *A. swirskii* were released onto plant leaves with *T. vaporariorum* nymphs treated with conidia or an extract prepared from *L. muscarium* mycelium, they experienced no direct toxic effects. However, their fecundity decreased compared to the mites on untreated leaves (Mitina et al., 2019a).

The aim of this study was to evaluate effects of conidia and mycelial extract of *L. muscarium* on *A. swirskii* and to assess the compatibility and performance of a combined application of both biocontrol agents against the greenhouse whitefly *T. vaporariorum*.

Material and methods

Rearing of mites

Laboratory culture of *A. swirskii* was maintained on the dried fruit mite *Carpoglyphus lactis* (Acari: Carpoglyphidae) at the All-Russian Institute of Plant Protection (VIZR, State Collection of Beneficial Arthropods). Rearing conditions were similar to those described previously for the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) (Krasavina et al., 2009). *C. lactis* was maintained on wheat bran with the addition of 10 % apple flour and was standardized to the density of 120 mites/cm^3 of feed material.

Fungal strains and cultivation

Two strains of *L. muscarium* used in these trials, G-033 VIZR and G-21 VIZR, were obtained from the Collection VIZR WFCC WDCM №760 (Saint-Petersburg). Suspensions of conidia for laboratory and field tests were prepared from G-033 VIZR, which has previously proved to be a highly promising biological control agent for controlling greenhouse whitefly, aphids, and spider mites using its spores (Mitina et al., 2016). The fungus was grown on Sabouraud dextrose agar medium in Petri dishes at 26 °C and conidia were harvested after nine days. Conidia were washed out of the sporulating cultures using a 0.01 % Tween 80 solution and filtered through sterile cloth filter. The concentration of conidia was counted in hemacytometer. Conidial suspension containing 5x10^7 spores/ml 0.01 % Tween 80 were prepared for the assays from the harvested fresh stock suspension.

A crude organic extract was prepared from the biomass of strain G-21 VIZR *L. muscarium* selected for its high levels of insecticidal metabolites in mycelium with high toxicity for *Hemiptera* (Mitina, unpublished data). The fungus was cultivated in a 750 ml Erlenmeyer flasks with 100 ml of Sabouraud medium at 28 °C on a shaker at 200 rpm for 3 days. The flasks with the medium were inoculated by an aliquot of 5 × 10^8 conidia, which were washed from the fresh sporulating cultures with sterile water after fungal growth during 9 days on SDA medium in Petri dishes at 26 °C. The biomass was concentrated by centrifugation and an ethanol extract prepared using previously published procedures (Mitina et al., 2019b). Dried extract was stored at 4 °C and dissolved in water prior to the bioassays to obtain a 0.5 % w/v concentration. The extract in that concentration showed a high insecticidal activity against sucking pests (Mitina et al., 1998) and was safe for a number of beneficials (Mitina et al., 2018).

Laboratory tests of fungi and mites

Greenhouse whitefly, *T. vaporariorum,* was obtained from a laboratory culture maintained on tobacco plants at VIZR. Plants of the common bean *Phaseolus vulgaris* with true leaves (14 days) were artificially infested with whitefly adults and left for two days for oviposition at 25 °C and 18-h light day. Then the adults were removed and plants maintained until appearance of the second and third instar nymphs of the whitefly. Each leaf of approximately the same size (leaf area of 15–17 cm^2) carrying 20–40 nymphs was cut from the bean plants and sprayed with 1 ml of conidial suspension or extract emulsion using a manual household sprayer and allowed to air dry for 20 minutes. The leaves were placed on a sponge in open Petri dishes (9 cm in diameter) filled with water. Treatments with water or with 0.05 % Tween 80 were used as controls. Four adult female mites were placed on each treated leaf and maintained at 24–25 °C. Five replicates of each treatment were prepared, and the experiment was repeated twice. The number of mite adults, eggs, and nymphs, as well as of whitefly nymphs, were counted on each leaf under a stereomicroscope at a 16-fold magnification prior to treatment and on the 2nd, 3rd, 6th and 10th day after treatment. Additional food for the predators was provided by pouring 1 cm^3 of feed substrate (see above) containing 120 prey mites with a measuring spoon on each bean leaf on day five.

Greenhouse experiment on combined application of *L. muscarium* and *A. swirskii*

Combined action of *L. muscarium* conidia and *A. swirskii* and individual action of mites alone and *L. muscarium* alone were tested in commercial greenhouses, located in Leningrad region near Saint-Petersburg on rose plants (var. Peach Avalanche) cultivated using the Dutch technology with automatic control of the main parameters for hydroponic growing method with mineral wool as a substrate (https://www.dutchgreenhouses.com/en/technology). Four experimental plots of 10 m^2, were set up, each containing 15 plants. The plots were located at the distance of 20 m and isolated from each other by two rows of untreated rose plants. Three plants in each plot were selected randomly. Conidial suspensions containing 5x10^7 spores/ml and 0.01 % Tween 80 were applied using a manual sprayer Solo 465 (Germany) until all leaves were coated. The efficiency of applications was checked using water-sensitive paper strips, which revealed 100 % of efficiency. Predatory mites were released onto selected leaves 30 min after the treatment at a ratio of 1:5 (predator to prey). All stages of whiteflies (eggs, nymphs and adults) were counted on three leaves from the lower, middle and upper levels of the plant canopy. The leaves were marked and examined for insect presence directly on the plants (without tearing) before treatment and on days 2, 3, 5, 6, and 11 after treatment. Several leaves from each level were torn off from other plants in the same plot and examined under a stereomicroscope. The air temperature through the trials ranged from 18 °C at night and 27 °C during the daytime with an average of 22.5 °C, and relative air humidity was 60 %.

Statistical analysis

Statistics were performed using SigmaPlot version 12.5 Systat Software. The data were analyzed by one-way ANOVA, using Tukey's test to separate the means (α=0.05). Effectiveness of treatments (control-corrected mortality) was estimated with Henderson–Tilton's formula, which takes into account changes in the number of live individuals in both experimental and control variants.

Results

Laboratory experiments

The number of *A. swirskii* adults did not change during the first six days following their release on the bean leaves treated with *L. muscarium* conidia*,* as well as on the leaves treated with water or Tween 80 (Figure 1). By the 10th day, the number of mites increased 2-, 2.75- and 2.5-fold after treatment with water, Tween 80 and fungal conidia, respectively. On the leaves treated with the mycelial extract,

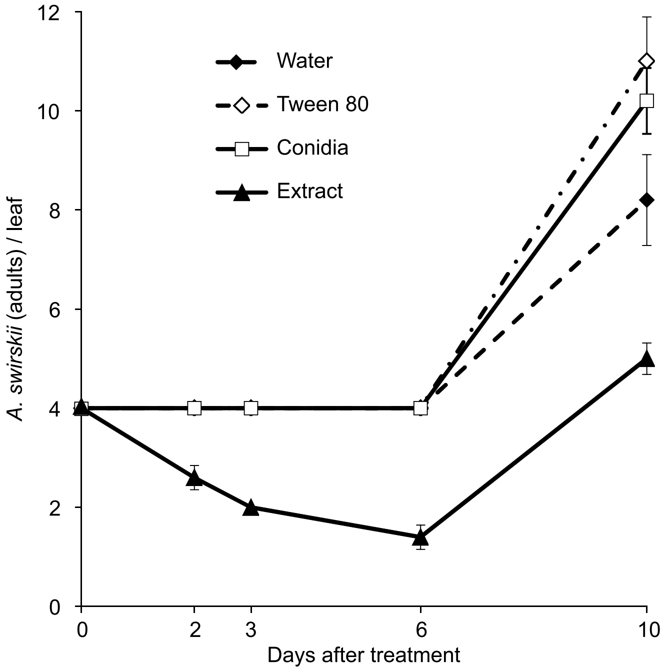

Figure 1. The survival of *A. swirskii* adults released onto bean leaves infested by *T. vaporariorum* nymphs and treated with *L. muscarium* conidia, mycelial extract, water, and 0.05 % Tween 80

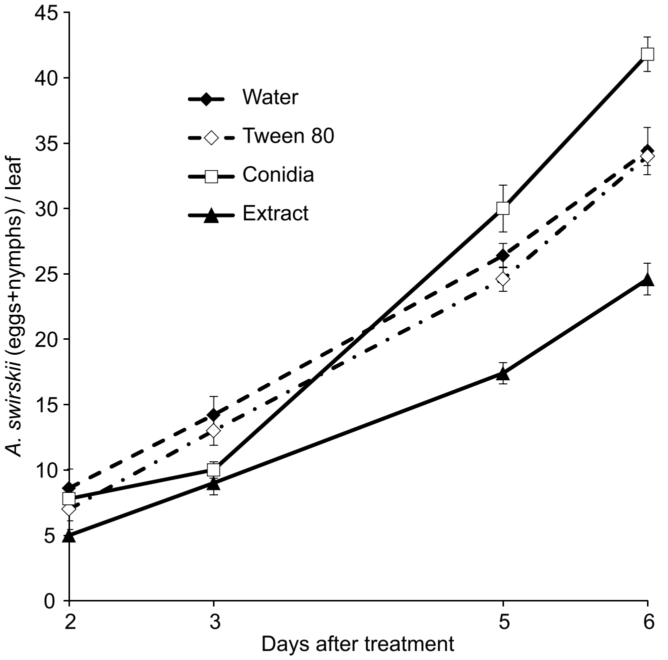

Figure 2. The number of eggs and nymphs of *A. swirskii* on bean leaves infested by *T. vaporariorum* nymphs after their treatment of leaves with *L. muscarium* conidia, mycelial extract, water, and 0.05 % Tween 80

the density of mites decreased to 2.6 individuals per leaf by day two of the experiment, which was significantly lower than either of the control treatments (F = 9.521; p = 0.015). The highest levels of mite mortality (65 %) in the mycelial extract treatment were reached by day six, and by day 10 the number of mites increased 1.25 times only.

Mite eggs were found in all treatments beginning from day two of the test, and their number increased over time. The first nymphs were observed on day five (Figure 2).

On the leaves treated with conidia and mycelial extract, the number of eggs laid by mites on day three was 23 and 36.6 % lower than on leaves treated with Tween 80 (control for conidia) or water (control for extract), respectively. The differences between these treatments and their respective controls were significant (F = 7.230; p = 0.028). Further, by day five and six, the total number of eggs and nymphs was significantly higher on leaves treated with conidia as compared to all other treatments. By day six, the total number of eggs and nymphs on leaves treated with conidia of *L. muscarium* was 18.7 % higher than on leaves treated with Tween 80 only (F = 10.952; $p<0.001$). In contrast, the number of *A. swirskii* eggs and juveniles on leaves treated with the extract were significantly lower than in all other treatments (F = 34.105; $p<0.001$). The mites effectively reduced the number of whiteflies on bean leaves by day two in all experimental treatments (Table 1). Besides, an additional effect was found on leaves treated with the extract. By day two, the highest level of whitefly mortality (50 %) was obtained on leaves treated with the extract, as opposed to an average of 27 % in the control treatments. However, later in the study the mortality of whitefly in the controls increased faster and no whiteflies were found. On the contrary, several whitefly nymphs remained alive in the experimental treatments, although the difference between those treatments and the water control was significant.

Table 1. The number of whitefly *T. vaporariorum* per leaf and its mortality (percentage ± SE) in each treatment and release *A. swirskii*

Treatment, concentration	Number of whiteflies per leaf			Mortality of whiteflies after treatment, %	
	Before treatment	2 days	3 days	2 days	3 days
Water	12.8±1.20	9.2±0.7	0	27.85±1.87 ^{a}	100±0 ^{a}
Tween 80, 0.01 %	22.0±3.8	15.8±3.4	0	27.26±4.63 ^{a}	100±0 ^{a}
Conidia, $5x10^7$ spores/ml	27.6±4.6	19.0±3.3	4.2±2.6	33.42±4.27 ^{a}	88.93±6.81 ^{a}
Extract, 0.5 %	40.0±7.9	21.2±4.8	14.2±3.6	50.27±2.16 ^{b}	63.02±2.74 ^{b}

Within each column, average values followed by the same letter are not significantly different ($p>0.05$; Tukey's HSD multiple comparison test).

Effectiveness of the combined application of *L. muscarium* and *A. swirskii* for control *T. vaporariorum* in commercial greenhouses

The average numbers of whitefly adults, nymphs and eggs on roses in the greenhouse before treatments was about 80, 40, and 30 individuals/leaf, respectively (Figure 3a, 3c, 3e). The effectiveness of predatory mites and conidia of *L. muscarium* was different when applied separately and in a combined treatment against different stages of the whitefly. The mycelial extract was not used in the greenhouse test because it was shown to have a highly toxic effect on *A. swirsii* in the laboratory experiment.

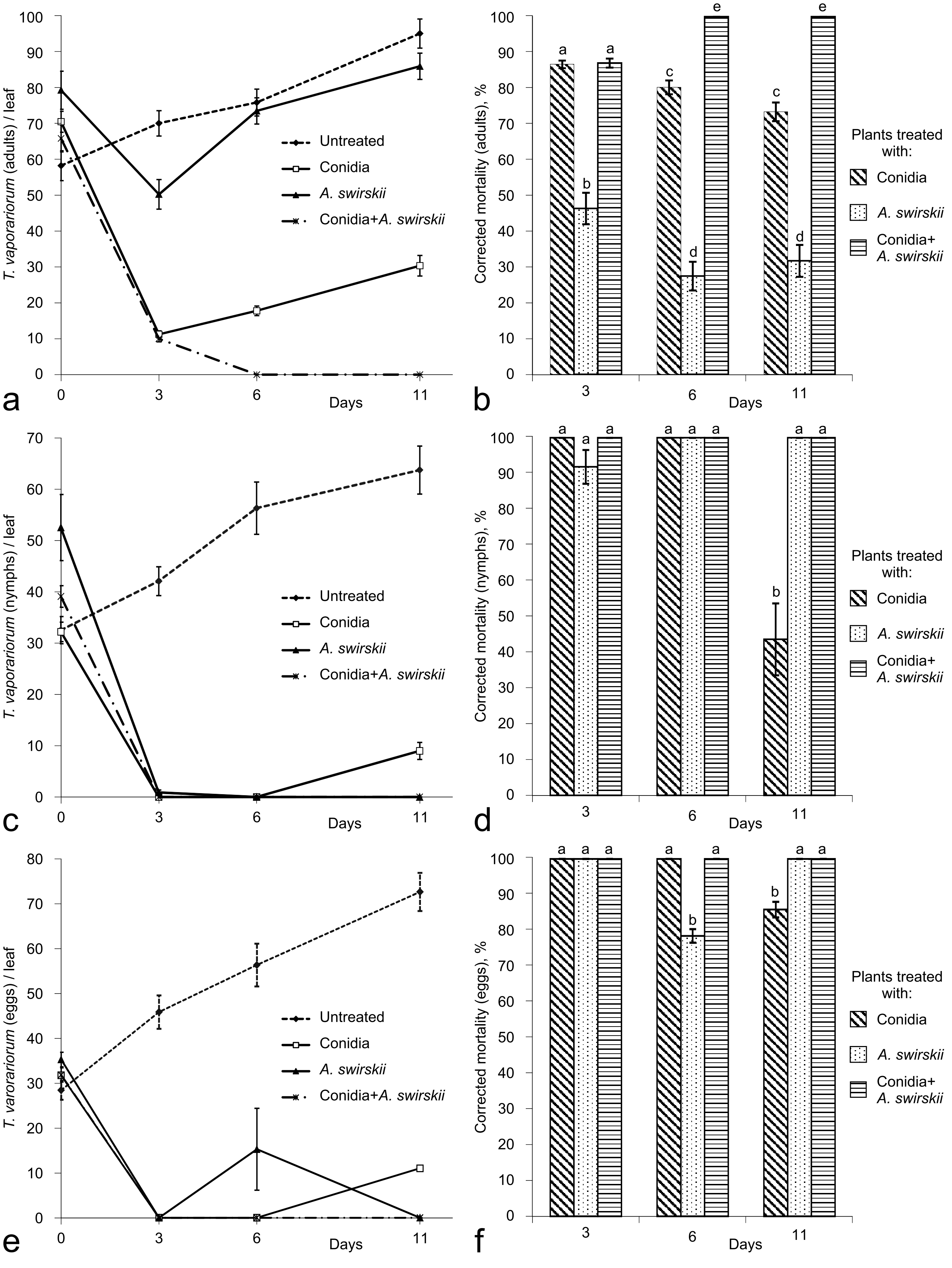

Figure 3. Average density (number ± SE) of *T. vaporariorum* adults (a), nymphs (c), eggs (e) per leaf before and after treatment of plants with conidia of *L. muscarium*, with *A. swirskii*, and with their combination. Corrected mortality of the different stages of whitefly (percentage ± SE) (b, d, f) on day 3, 6 and 11 after treatment. Bars with the same letters were not significantly different from each other

Whitefly adults. By day three, the density of whitefly adults reached 50 individuals per leaf after the release of mites alone and 70 adults per leaf in the control (Figure 3a). The effectiveness (control-corrected mortality) of releasing predatory mites against whitefly adults reached only 46 % (Figure 3b). The number of whitefly adults decreased to 11 individuals per leaf following the application of fungal conidia only (Figure 3a); the effectiveness was 87 % on day three. The same result was obtained when mites were released onto the plants treated with conidia (Figure 3b). The differences between the effectiveness when mites were released alone and when the plants were treated with conidia were significant ($F = 12.869$; $p = 0.001$). By day six, the density of whitefly adults dropped to zero after combined application of conidia and mites; that level remained on day eleven, confirming the 100 % adult mortality. By days six and eleven, the effectiveness of application of conidia alone was 80 % and 73 %, respectively. The effectiveness of releasing mites only against whitefly adults was 28 % and 32 % on the same days of the experiment (Figure 3b).

Whitefly nymphs. By day three, whitefly nymphs were absent in all treatments, except the application of mites alone (there was still 1 nymph left per leaf). In the control, the number of nymphs increased to 42 individuals per leaf by day three of the experiment (Figure 3c). The effectiveness of mites, when conidia were applied separately and combined (mites after conidia), against whitefly nymphs was 100 % by day six (Figure 3d), while the density of nymphs in the control increased. By day eleven, the nymphs were found only in the experiment with conidia alone (9 individuals per leaf), the efficiency of conidia was 86 %. The density of whitefly nymphs increased up to 64 larvae per leaf in the control.

Whitefly eggs. Mortality of eggs was 100 % in all treatments by day six, while the density of eggs in the control increased up to 46 eggs per leaf (Figure 3e, 3f). By day six, whitefly eggs remained only on the leaves where the mites were released alone (14 eggs per leaf); the efficiency of mites was 79 %. The density of whitefly eggs increased up to 57 eggs per leaf in the control. The effectiveness of the combined application of predatory mites and conidia against whitefly eggs reached 100 % by day eleven (Figure 3f). Whitefly eggs remained on the leaves after application of conidia alone (11 eggs per leaf); the efficiency of conidia application was 86 %. The differences were significant when compared to the other treatments ($F = 14.649$; $p = 0.001$).

By the end of the experiment, we observed an increase in the density of whiteflies up to 95 adults, 64 nymphs and 73 eggs per leaf in the control, i.e. 1.6-, 2.0-, and 2.6-fold increase in the number of adults, nymphs and eggs, respectively.

Discussion

Results of the laboratory experiments suggest that *A. swirskii* adults were not affected by *L. muscarium* applied to leaves at a concentration of 5×10^7 conidia/ml. No infected mites were observed, and no mites died during the experiment. Moreover, the number of mites was 18.7 % higher than on leaves treated with Tween 80 by the end of experiment. These results confirmed previous data indicating that *L. muscarium* does not infect *A. swirskii* when the latter is released onto leaves treated with the pathogen's conidia (Mitina et al., 2019a).

The influence of fungal conidia on predatory mites has been studied to a greater extent using *B. bassiana*. The fungus showed low to moderate virulence to *A. swirskii* when applied topically, with the response dependent on the concentration of conidia applied; offspring survival was not affected (Midthassel et al., 2016). Those data led to a conclusion that the two biocontrol agents are compatible. In field experiments, Jacobson et al. (2001) showed that the predatory mite *N. cucumeris* is compatible with *B. bassiana*, but their combined use did not increase the relative effectiveness of a thrips control strategy on cucumber. *Metarhizium brunneum* (Ascomycota: Hypocreales) and *Neozygites foridana* (Entomophthoromycota: Neozygitaceae) did not affect the behavior and feeding of the predator *P. persimilis*, allowing their concurrent use for control of *T. urticae* (Jacobsen et al., 2019).

Our results showed that mycelial extract of *L. muscarium* at a concentration of 0.5 % had a significant toxic effect on *A. swirskii* when the mites were released onto treated leaves. High acaricidal activity of the extract was previously demonstrated against spider mite *Tetranychus urticae* (Mitina et al., 2016). The extract was not toxic to *Encarsia formosa* nymphs when topically applied to parasitized whitefly at a 0.5 % rate; nor did it affect nymphs of the predatory midge *Aphidoletes aphidimyza*, *P. persimilis* adults, and nymphs of the predatory bug *Orius laevigatus* (Mitina et al., 2018). The advantages of the extract from *L. muscarium* mycelium are its fast contact action and high insecticidal activity against greenhouse whitefly, various species of aphids, and spider mites (Mitina et al., 2002). Present work shows that initially the whitefly mortality was significantly higher when using the extract and mites (on day two), but later it became lower compared to the addition of mites alone. The number of mite offspring on leaves treated with the extract was significantly lower than in all other treatments. These results indicate the limited compatibility of the predatory mites *A. swirskii* and the extract from *L. muscarium* mycelium. For its use in IPM together with *A. swirskii*, further studies are needed to determine possible safe concentrations for the mite, safe release times on extract-treated leaves, and repellent and antifeeding properties for the predator.

In the commercial greenhouse trial on roses, higher efficacy against whitefly adults and eggs was obtained following the combined application of *L. muscarium* conidia and *A. swirskii* than from individual applications of each agent. A similar enhanced effect was obtained when *B. bassiana* was applied together with *P. persimilis* against *T. urticae* (Ullah, Lim, 2017), and when *Paecilomyces fumosoroseus* (=*Isaria fumosorosea*, Ascomycota: Hypocreales) and *B. bassiana* were combined with *Neoseiulus californicus* (Acari: Phytoseiidae) against the spider mite (Numa Vergel et al., 2011). Entomopathogenic fungus *Cordyceps javanica* (Hypocreales: Cordycipitaceae) and whitefly parasitoid *Eretmocerus hayati* (Hymenoptera: Aphelinidae) also provided better control of *B. tabaci* when applied together than when applied separately (Ou et al., 2019).

In our greenhouse experiments, the efficacy of each biological agent when applied separately against whitefly nymphs was very high, but the sequential treatment with fungal conidia followed by the release of predatory mites ensured a longer protective effect. Unlike under laboratory conditions, the predatory mites were not confined to a single leaf, and provided effective control of both whitefly eggs and nymphs on the entire plant.

For whitefly adults on rose leaves, conidia alone or in combination with mites induced significantly higher pest mortality as compared with releases of the predatory mites alone, as a result of their selective feeding on eggs and immature stages of whiteflies. Entomopathogenic fungi such as *L. muscarium* are able to infect whitefly adults under favorable conditions, increasing the overall efficacy of combined applications of predatory mite and fungus against all life stages of the whitefly. Infective fungal propagules can also be spread by *A. swirskii* as it moves over leaves, as shown with the predatory bug *Dicyphus hesperus* (Hemiptera: Miridae) when applied against whitefly in a combination with *I. fumosorosea* (Alma et al., 2010).

Overall, the combined use of *L. muscarium* and the predatory mite *A. swirskii* appears to be a highly promising approach for the biological control of the greenhouse whitefly.

This research was supported by a grant of Russian Foundation for Basic Research (project no 20-016-00241).
We thank Ekaterina Kozlova and Vladimir Moor for their assistance throughout the experiment in the commercial greenhouses. Igor Belousov and Yuri Tokarev (All-Russian Institute of Plant Protection) provided valuable advice on improving the earlier version of this manuscript.

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Вестник защиты растений, 2021, 104(3), с. 163–170

OECD+WoS: 1.06+RQ (Mycology) https://doi.org/10.31993/2308-6459-2021-104-3-15026

Полнотекстовая статья

СОВМЕСТИМОСТЬ ГРИБА *LECANICILLIUM MUSCARIUM* И ХИЩНОГО КЛЕЩА *AMBLYSEIUS SWIRSKII* ДЛЯ СОВМЕСТНОГО ПРИМЕНЕНИЯ ПРОТИВ ТЕПЛИЧНОЙ БЕЛОКРЫЛКИ *TRIALEURODES VAPORARIORUM*

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В работе изучено влияние гриба *Lecanicillium muscarium* (Ascomycota: Hypocreales) и органического экстракта из его мицелия на оранжерейную белокрылку *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) и на хищного клеща *Amblyseius swirskii* (Acari: Phytoseiidae). Клещи контактировали с грибными спорами или органическим экстрактом, полученным из мицелия *L. muscarium*. При подсадке клещей *A. swirskii* на листья фасоли, заселенные личинками *T. vaporariorum* и обработанные суспензией конидий в концентрации 5×10^7 спор/мл, не было обнаружено негативного влияния на имаго клеща; на 6-е сутки количество отложенных яиц и личинок клеща было на 18.7% выше по сравнению с контролем. Напротив, обработка листьев 0.5%-ным спиртовым экстрактом, полученным из мицелия *L. muscarium,* вызывала 35% смертности имаго клеща на 2-е сутки. В условиях производственных теплиц обработка конидиями *L. muscarium* с последующим выпуском хищного клеща *A. swirskii* против оранжерейной белокрылки на розах была более эффективна, чем раздельное применение этих биологических агентов.

Ключевые слова: энтомопатогенные грибы, хищные клещи, агенты биометода, побочный эффект, полезные членистоногие

Поступила в редакцию: 02.06.2021 *Принята к печати: 31.08.2021*
