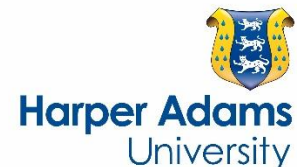


# Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents

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# Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents

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**Abstract** The two biocontrol agents *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) have the potential to complement one another as part of an integrated pest management programme. While both can suppress whitefly and thrips infestations in protected crops, *A. swirskii* is primarily used preventatively whereas *B. bassiana* can be used as a curative treatment at higher pest levels. With this concomitant use in mind, the research presented here aimed to identify potential negative effects of the commercial *B. bassiana* strain GHA on *A. swirskii* in a laboratory study. Adult *A. swirskii* were found to be susceptible to *B. bassiana* infection with slight to moderate virulence ( $20.74 \pm 3.89$  to  $48.33 \% \pm 3.07$ ) depending on the type of exposure and with a reduction in fecundity over time. There was however no negative effect on juvenile survival neither on dry residue nor on

the offspring of infected mites. Thus, these two biocontrol agents do have the potential to be complementary. Further trials in the field are still required before a final conclusion can be reached.

**Keywords** Integrated pest management · Mites · Phytoseiidae · Entomopathogens · Sublethal effects

## Introduction

Augmentative biocontrol in protected crops often involves multiple biological control agents (BCAs) targeting the same or different pest species (Wittman and Leather 1997; Shipp et al. 2003; Calvo et al. 2009; Labbe et al. 2009; Chow et al. 2010; Messelink et al. 2011). These BCAs may also be used in conjunction with biorational chemicals in integrated pest management (IPM) programmes where the focus is to combine crop protection strategies with the overall aim to reduce the use of broad spectrum pesticides and chemical residues on produce (Cock et al. 2010). Combining several BCAs may allow a holistic biological pest management programme but interactions between different BCAs are complex and one species can potentially disrupt the functions of another. Biocontrol programmes can be compromised due to competition or intraguild predation (e.g. hyperpredation, hyperparasitism and pathogenicity) from other introduced BCAs or naturally occurring

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species (Momen and Abdel-Khalek 2009; Chow et al. 2010; Buitenhuis et al. 2010; Messelink et al. 2011; Shipp et al. 2012; da Silva et al. 2015). In order to develop efficient and successful pest management programmes compatibility between the different BCAs must be established.

Phytoseiid mites are important components of pest management programmes in protected crops and one of the most widely used species is *Amblyseius* (= *Typhlodromips*) *swirskii* Athias-Henriot (Acari: Phytoseiidae) (van Lenteren 2012). Marketed primarily for whitefly (Hemiptera: Aleyrodidae) and thrips (Thysanoptera: Thripidae) control, both of which are major pests in tomatoes, peppers, cucumbers and ornamentals (Malais and Ravensberg 2003), this polyphagous predator can suppress pest outbreaks on its own (Messelink et al. 2006; Chow et al. 2010; Messelink et al. 2010; Calvo et al. 2011). Combining its use with other BCAs can enhance pest control particularly when the BCAs do not compete directly but exploit different life stages of the same pest (Calvo et al. 2009; Dogramaci et al. 2011). Conversely, *A. swirskii* can interfere with other BCAs by inter- and intraguild predation (hyperpredation) (Buitenhuis et al. 2010; Messelink et al. 2011). In the field, *A. swirskii* is primarily used preventatively to avert pest establishment and for controlling light whitefly and thrips infestations (Messelink et al. 2010; Calvo et al. 2011). At high pest pressure, curative control by predatory mites is often insufficient and corrective applications with chemical pesticides or biopesticides may be required (Calvo et al. 2009; Medd and GreatRex 2014). Ideally, the products used for corrective treatments should have minimal impact on other BCAs present in the crop.

The fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) exhibits a wide host range and several strains have been developed as commercial mycoinsecticides (de Faria and Wraight 2007; Zimmermann 2007). Commercial formulations of *B. bassiana* are used curatively to suppress larger outbreaks of arthropod pests (Wraight et al. 2000; Faria and Wraight 2001; Inglis et al. 2001; Jacobson et al. 2001; Shipp et al. 2003). As both *A. swirskii* and *B. bassiana* target whitefly and thrips, it is natural to consider them as potentially complementary intraguild BCAs: *A. swirskii* for preventative control and *B. bassiana* for curative control.

Due to its wide host range, *B. bassiana* may have detrimental effects on beneficial arthropods but this will depend on pathogen strain and host species (Seiedy et al. 2015). For instance, *B. bassiana* has been found to be infectious to hymenopteran parasitoids (Ludwig and Oetting 2001; Shipp et al. 2003) and may potentially disrupt their biocontrol functions. Despite the wide host range of *B. bassiana*, pathogenicity has only been reported to relatively few species of mites (Chandler et al. 2000).

Interactions between arthropods and entomopathogens are however complex (Agboton et al. 2013; Aqueel and Leather 2013) and the impact of sublethal effects is poorly understood (Pozzebbon and Duso 2010; Seiedy et al. 2012; Shipp et al. 2012). The aim of this study was to establish the potential of the commercially available *B. bassiana* strain GHA to infect and kill *A. swirskii* under optimal conditions for the pathogen and to investigate the effects on specific population parameters when subjected to topical application of *B. bassiana* conidial suspensions and/or dry residues on leaf discs.

## Materials and methods

### Mite cultures

*Amblyseius swirskii* were acquired from a commercial culture at BCP Certis (Kent, UK) and reared on 50 mm French dwarf bean, *Phaseolus vulgaris* L. leaf discs at 25 °C, 70 % RH and 16:8 L:D. Cattail pollen (*Typha* sp.) was provided as a food source ad libitum.

### Fungal cultures

Laboratory cultures of *B. bassiana* strain GHA were established from the commercial product BotaniGard® 22 WP (Laverlam International Corporation, USA) by spread-plating 100 µl of a 0.0625 % suspension onto Sabouraud Dextrose Agar (SDA) in 90 mm diameter Petri dishes. Cultures were incubated in darkness at 25 °C and 75 % RH for 14 days. Sub-cultures were prepared by spread-plating 100 µl conidial suspensions.

### Preparation of conidial suspensions

Following 14 days of incubation, the *B. bassiana* cultures were dried by removing the Petri dish lid and

placing the cultures in 25 °C and 40 % RH for 18 h (Baxter 2008). Conidia were dislodged and collected by tapping the Petri dish containing the culture into a sterile glass funnel (100 mm diameter) leading to a sterile 50 ml glass bottle. The conidia collected from 10 plates were suspended in 5 ml sterile water with 0.02 % Tween<sup>®</sup> 80, and vortex mixed for 2 min (Mascarin et al. 2013). Conidial suspensions were prepared from dried cultures to represent the conidia from the dry formulation of the commercial product.

The concentration of conidia was assessed using a Neubauer Haemocytometer 0.1 mm (Hawksley, UK). Conidial suspensions were diluted to  $2.5 \times 10^7$  and  $1 \times 10^8$  conidia ml<sup>-1</sup>, representing the lowest and highest recommended rates, for whitefly and thrips respectively, according to the BotaniGard<sup>®</sup> 22 WP label. Six replicate conidial suspensions were prepared per treatment for each experiment.

To estimate viability of the conidia, 10 µl of the conidial suspension was applied onto a thin layer of SDA on one microscope slide per replicate conidial suspension (n = 6) placing a cover slip on top (Bugeme et al. 2008). The slides were incubated in darkness at 25 °C, 75 % RH for 18 h. Germination rate was estimated by assessing 100 conidia in four different fields of view per slide (Liu et al. 2003). Viability was >95 % in all experiments.

### Bioassays

Female adults of *A. swirskii*, age 2–4 days, were exposed to *B. bassiana* by topical application, dry residue on *P. vulgaris* leaf discs or a combined exposure with topical application followed by dry residue exposure, inspired by the methods of Dennehy et al. (1993) and Pozzebon and Duso (2010) as described below. The trial arenas (leaf discs in 50 mm Petri dishes) were incubated at 25 °C, 90 % RH and 16:8 L:D to maximise the likelihood of fungal infection.

Topical application of *A. swirskii* females was conducted by immersing 10 mites individually in 1 ml of *B. bassiana* conidial suspensions of  $2.5 \times 10^7$  or  $1 \times 10^8$  conidia ml<sup>-1</sup> in a Petri dish for 30 s. The mites were then placed on filter paper to drain off excess liquid prior to transfer onto leaf discs using a fine brush. This procedure was replicated six times, hence 60 mites were tested per treatment. Control mites were immersed in a 0.02 % Tween<sup>®</sup> 80 solution.

Pollen (*Typha* sp.) was provided as a food source in all treatments throughout the experiments.

Dry residues of *B. bassiana* were prepared by immersing 50 mm leaf discs in 10 ml conidial suspensions of  $2.5 \times 10^7$  or  $1 \times 10^8$  conidia ml<sup>-1</sup>, and 0.02 % Tween<sup>®</sup> 80 for the control, for 30 s and air drying them for 1 h. The mites in the dry residue and combined exposure trials were allowed to forage on *B. bassiana* residues for four days (Bugeme et al. 2008), representing the persistence of conidia on the top leaves of a plant in the field (Inglis et al. 1993).

Survivorship and fecundity were monitored once per day for 10 days, removing the eggs from the leaf discs each day. On day 1, 4, 7 and 10 post-treatment, eggs were collected from each leaf disc using a fine brush and placed onto untreated leaf discs. The offspring were monitored for six days recording egg viability, juvenile survival and sex ratio.

Dead mites were transferred to Petri dishes with moistened filter paper to encourage sporulation. Death by mycosis was confirmed by sporulation or red colouration of cadavers. The identity of the fungal growth on cadavers was confirmed by microscopic investigation of lactophenol blue stained conidiphores and conidia at magnification  $\times 400$ , according to Humber (1997).

The effect of *B. bassiana* residues on juvenile *A. swirskii* was studied to simulate the impact on new founding populations of mites dispersing into a newly treated crop. Ten gravid *A. swirskii* were allowed to oviposit on dry residue of the conidial suspensions, or 0.02 % Tween<sup>®</sup> 80 as a control, for 16 h. The eggs deposited were corrected to 15 per replicate leaf disc (n = 6) and reared to adulthood for six days on the dry residue, recording egg viability and juvenile survival. Six days was sufficient for all mites to have reached maturity and to have mated. The surviving adult females were transferred to clean leaf discs and monitored for a further 10 days for survivorship and fecundity.

### Data analysis

Survival rates were estimated by parametric survival analysis with the Weibull distribution, due to non-constant hazards, in the R 3.1.2 statistical package (R Core Team 2012), which generates *z* values as a result of Wald tests. Multiple comparisons were conducted

using Tukey's post-hoc in the multcomp package in R (Hothorn et al. 2008).

Fecundity rates were analysed using generalized linear mixed models (GLMM) with binomial errors, which uses the logit link function, in the lme4 package in R, where the proportion is the total number of eggs on a given day in a replicate leaf disc to the total population (number of surviving female mites + number of eggs) in that leaf disc on that day. Treatment was set as the fixed effect and replication and time (days numerated 1–10) as random effects. GLMM analysis allows comparisons of change in fecundity among treatments over time. Trend lines were plotted using generalised additive models (GAM).

Egg viability, juvenile survival and sex ratio of offspring was analysed by generalized linear models (GLM) with binomial errors, which uses the logit link function to transform the data. This generates  $z$  values as a result of Wald tests. Tukey's post-hoc analysis was conducted to identify significant differences between treatments.

## Results

### Survivorship

The *B. bassiana* treatments had a significant effect on the survival rates of adult *A. swirskii* compared with the control treatment. On dry residue the suspension with  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  significantly reduced survival compared with the control treatment ( $z = 2.97$ ,  $p < 0.01$ ). There was no significant difference between this treatment and the  $2.5 \times 10^7$  conidia  $\text{ml}^{-1}$  treatment ( $z = 1.77$ ,  $p = 0.18$ ), nor between the latter and the control ( $z = 1.76$ ,  $p = 0.18$ ) (Fig. 1a).

Topical application of *B. bassiana* significantly reduced the survival rates of adult *A. swirskii* for both  $2.5 \times 10^7$  ( $z = 2.92$ ,  $p < 0.01$ ) and  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  ( $z = 3.70$ ,  $p < 0.001$ ) compared with the control treatment. There was no significant difference ( $z = 1.53$ ,  $p = 0.27$ ) between the two *B. bassiana* treatments (Fig. 1b).

Combined exposure to topical application and dry residue of *B. bassiana* significantly reduced the survival rates of adult *A. swirskii* for both  $2.5 \times 10^7$  ( $z = 3.39$ ,  $p < 0.01$ ) and  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  ( $z = 3.68$ ,  $p < 0.001$ ) compared with the control treatment. There was no significant difference

between the two *B. bassiana* treatments ( $z = 0.92$ ,  $p = 0.61$ ) (Fig. 1c).

*Beauveria bassiana* did not have a significant effect on survival rates of juvenile *A. swirskii* reared from egg to adult on dry residue and monitored for a further 10 days after final ecdysis (Fig. 1d). There was no treatment effect on egg viability and juvenile survival was similar in all treatments.

The scale parameter was  $< 1$  in all experiments indicating that the risk of dying decreases with time (Crawley 2007). This suggests the time frame of observation (10 days) was sufficient to capture the majority of the mites dying as a result of the treatment.

Mites with confirmed mycosis were observed in all *B. bassiana* treatments except with juveniles reared on the dry residue of the conidial suspension with  $2.5 \times 10^7$  conidia  $\text{ml}^{-1}$ . Dead mites exhibited pink to dark red colouration after death followed by sporulation. No mycosis was observed in the control treatments.

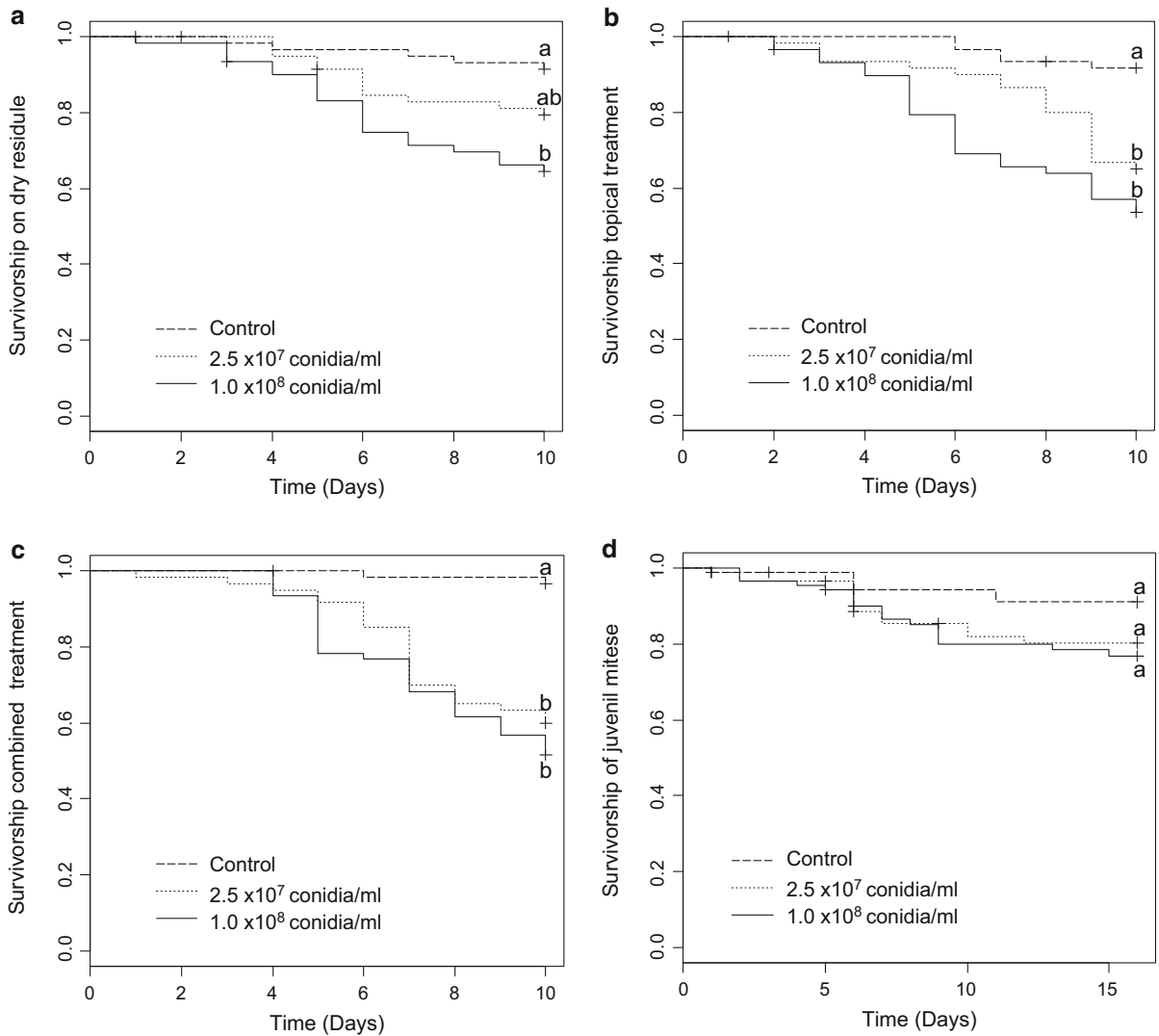
### Fecundity

The oviposition rate decreased with time in all treatments and the number of eggs deposited per surviving female per day decreased at a higher rate for *A. swirskii* treated with *B. bassiana* than control mites (Fig. 2a–d). Exposing adult *A. swirskii* to dry residue of *B. bassiana* did not have a significant effect on fecundity (Fig. 2a). Juvenile *A. swirskii* exposed to dry residues however exhibited a significant decrease in fecundity in the subsequent mature life stage for  $1.0 \times 10^8$  ( $z = 3.90$ ,  $p < 0.001$ ) and  $2.5 \times 10^7$  conidia  $\text{ml}^{-1}$  ( $z = 2.62$ ,  $p < 0.05$ ) (Fig. 2d).

For adult mites exposed to topical and combined treatments there was a significant effect on fecundity at  $2.5 \times 10^7$  ( $z = 2.38$ ,  $p < 0.05$ ) and  $1.0 \times 10^8$  conidia  $\text{ml}^{-1}$  ( $z = 4.49$ ,  $p < 0.001$ ), respectively. Treatments with  $1.0 \times 10^8$  and  $2.5 \times 10^7$  conidia  $\text{ml}^{-1}$  did not exhibit a significant effect on fecundity for topical ( $z = 2.27$ ,  $p = 0.06$ ) (Fig. 2b) and combined ( $z = 1.76$ ,  $p = 0.18$ ) exposure (Fig. 2c) respectively, due to within-group variation in the data.

### Offspring

Egg viability, juvenile survival and sex ratio of the offspring from *B. bassiana*-treated adult *A. swirskii* are summarised in Table 1. As there were no differences in any of the measured parameters between sampling



**Fig. 1** Survivorship of *Amblyseius swirskii* adults exposed to **a** dry residue, **b** topical treatment, **c** combined treatment, and **d** juveniles on dry residue of *Beauveria bassiana* conidial suspensions (n = 6). Different letters indicate significant

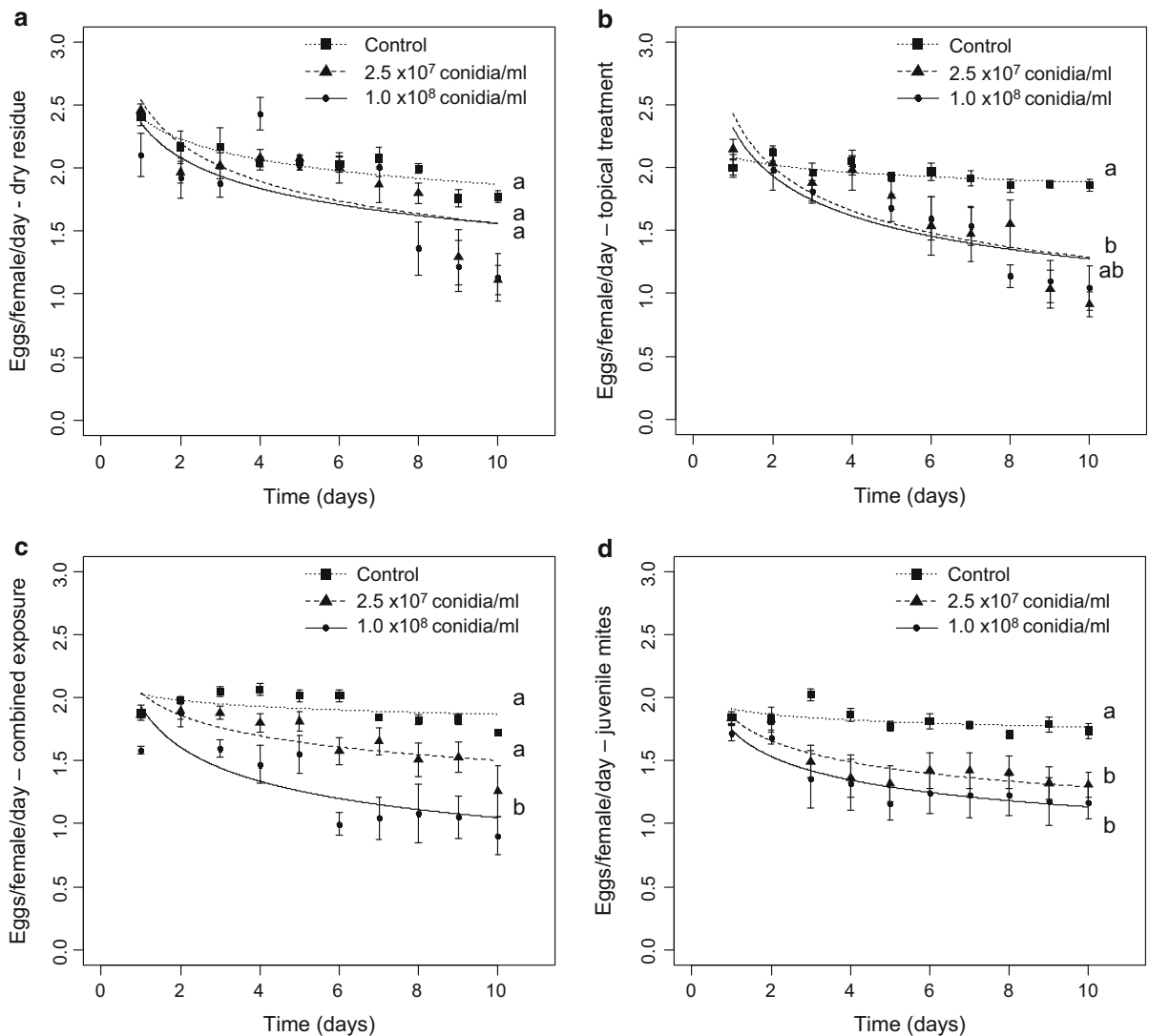
days of the same treatment these have been combined. Treating mites with *B. bassiana* had no observable or measurable effect on their offspring and there were no significant differences between the offspring of treated mites and the control (Table 2a–c).

**Discussion**

*Beauveria bassiana* strain GHA was found to be infectious to *A. swirskii* when exposed to topical

difference in survival rate as analysed by the Weibull distribution and compared using Tukey’s post-hoc (p < 0.05). The ‘+’ signs indicate censoring, i.e. mites that are missing or survived the observation period

application and/or dry residues under laboratory conditions with slight to moderate virulence (according to the IOBC toxicity classes). The topical treatment was intended to simulate the effect on a mite population in the crop directly exposed to a *B. bassiana* application followed by dispersal to untreated leaves or new growth. The dry residue exposure simulated mites dispersing into a recently treated crop whereas the combined exposure simulated the effect on mites exposed directly to the spray application and remaining on the treated leaves.



**Fig. 2** Mean daily fecundity of *Amblyseius swirskii* adults exposed to **a** dry residue, **b** topical treatment, **c** combined treatment, and **d** juveniles on dry residue of *Beauveria bassiana* conidial suspensions ( $n = 6$ ). Error bars represent SE. Different

letters indicate significantly different trend in oviposition rates as analysed by generalized linear mixed models and compared using Tukey's post-hoc ( $p < 0.05$ ). Trend lines were plotted with generalised additive models

Exposure of adult *A. swirskii* to *B. bassiana* at both the highest and lowest recommended dose according to the BotaniGard® 22 WP label significantly reduced survivorship of *A. swirskii* compared with the control treatment, indicating a clear treatment effect. There was no significant difference between the two doses applied in terms of survivorship, which is consistent with the results of Numa Vergel et al. (2011) who found no difference in mortality of *Neoseiulus*

*californicus* and *Phytoseiulus persimilis* treated with three different doses of *B. bassiana*.

The survival rates of *A. swirskii* exposed to topical treatments of *B. bassiana* strain GHA corresponds to the mortality rates of *A. swirskii* exposed to *B. bassiana* strain F, as reported by Seiedy et al. (2015). The authors found the virulence of *B. bassiana* to *A. swirskii* to be dependent on pathogen strain indicating the importance of studying compatibility

**Table 1** Egg viability, juvenile survival and sex ratio (proportion  $\pm$  SE) of the offspring from *Amblyseius swirskii* adults following different treatments of *Beauveria bassiana* conidial suspensions

Experiment	Treatment	Egg viability	Juvenile survival	♀ sex ratio
Dry residue	Control	1.00 $\pm$ 0.00	0.95 $\pm$ 0.01	0.68 $\pm$ 0.01
	$2.5 \times 10^7$	0.99 $\pm$ 0.01	0.93 $\pm$ 0.01	0.69 $\pm$ 0.02
	$1.0 \times 10^8$	0.99 $\pm$ 0.00	0.93 $\pm$ 0.02	0.64 $\pm$ 0.02
Topical	Control	1.00 $\pm$ 0.0	0.96 $\pm$ 0.01	0.69 $\pm$ 0.01
	$2.5 \times 10^7$	0.99 $\pm$ 0.01	0.95 $\pm$ 0.01	0.69 $\pm$ 0.02
	$1.0 \times 10^8$	0.98 $\pm$ 0.01	0.94 $\pm$ 0.01	0.73 $\pm$ 0.02
Combined	Control	1.00 $\pm$ 0.00	0.96 $\pm$ 0.01	0.69 $\pm$ 0.01
	$2.5 \times 10^7$	1.00 $\pm$ 0.00	0.95 $\pm$ 0.02	0.68 $\pm$ 0.01
	$1.0 \times 10^8$	0.99 $\pm$ 0.01	0.95 $\pm$ 0.01	0.68 $\pm$ 0.02

with specific strains, particularly commercially available strains such as GHA.

*Beauveria bassiana* has been found to be infectious to other phytoseiid mites under laboratory conditions. Castagnoli et al. (2005) reported slight mortality of the spider mite predator *N. californicus* when exposed to topical treatment of *B. bassiana*. However, mortality was assessed after three days, whereas fungal pathogenesis may require several days to kill its host (Inglis et al. 2001; Shah and Pell 2003). Nevertheless, a significantly higher mortality of *B. bassiana*-treated *N. californicus* compared with the control was reported, with similar survivorship as reported for *A. swirskii* in the present study. Numa Vergel et al. (2011), on the other hand, reported that although *N. californicus* is susceptible to *B. bassiana*, virulence is low (<30 %) and not different to the control treatment over a 12-day period.

Several studies have found the spider mite predator *P. persimilis* to be susceptible to *B. bassiana* topical treatment and exposure to dry residues under laboratory conditions. The studies reported susceptibility with slight to moderate virulence to *P. persimilis* after observation periods of three days (Duso et al. 2008), six days (Pozzebon and Duso 2010) and 12 days (Numa Vergel et al. 2011). Only Ludwig and Oetting (2001) reported high virulence to *P. persimilis* exposed to topical treatment of *B. bassiana* with 100 % mortality, although time of death and sporulation was not mentioned. With the exception of the latter, these studies report mortality rates comparable to the effects of *B. bassiana* to *A. swirskii* presented here.

Sublethal effects of an entomopathogen on non-target predators can be manifested by a change in fecundity, predation rates, longevity and fitness of subsequent offspring (Duso et al. 2008; Seiedy et al.

2012; Shipp et al. 2012). There was an overall trend of reduced fecundity in *B. bassiana*-treated *A. swirskii* in the present study, except for adult mites exposed to dry residue only, indicating an effect on the fitness of the mites.

Pozzebon and Duso (2010) reported no effect on the fecundity of *P. persimilis* when exposed to dry residue and/or topical treatment of *B. bassiana* but a significant reduction in eggs deposited was discovered when the mites were exposed to a combination of topical treatment, dry residue and feeding on *B. bassiana* treated prey. Furthermore, Numa Vergel et al. (2011) demonstrated that *B. bassiana* topical treatment had no negative effect on the fecundity of *N. californicus* and *P. persimilis* at three different concentrations. It is therefore reasonable to conclude that *B. bassiana* can reduce the fecundity of phytoseiid mites, including *A. swirskii*, but primarily when experiencing a combination of exposure routes with high conidial concentrations.

Apart from egg viability, the effect of *B. bassiana* on the offspring of treated individuals have not previously been studied for phytoseiid mites. Offspring of arthropods infected with *B. bassiana* may exhibit reduced survival and fecundity, moulting problems and malformations (Torrado-Leon et al. 2006). Egg hatching success can be significantly reduced for gravid *B. bassiana*-treated *N. californicus* (Castagnoli et al. 2005) and *P. persimilis* (Pozzebon and Duso 2010), but, contrary to these findings, treatment of *P. persimilis* with *B. bassiana* has also been reported to have no effect on egg hatching success (Duso et al. 2008; Seiedy et al. 2012). The results of the current study are consistent with the latter: no significant effect was observed on egg viability, juvenile survival or the sex ratio of offspring



**Table 2** Overview of results from GLM analyses with binomial errors and Tukey's post-hoc for multiple comparison for **a** egg viability, **b** juvenile survival and **c** sex ratio of offspring from adult *Amblyseius swirskii* exposed to treatments of *Beauveria bassiana*

Treatments	Control		2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	
	z	P	z	P
<b>a</b>				
Dry residue				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.68	0.21	0.78	0.71
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	1.10	0.51		
Topical treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.59	0.25	0.12	0.99
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	1.74	0.19		
Combined treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.25	0.42	1.30	0.39
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.12	0.99		
<b>b</b>				
Dry residue				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	2.21	0.07	1.61	0.24
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.72	0.75		
Topical treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.67	0.22	0.70	0.76
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.96	0.61		
Combined treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.34	0.37	1.45	0.32
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.20	0.98		
<b>c</b>				
Dry residue				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	1.04	0.55	0.79	0.71
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.22	0.97		
Topical treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	0.49	0.88	0.37	0.93
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.96	0.60		
Combined treatment				
1.0 × 10 <sup>8</sup> conidia ml <sup>-1</sup>	0.24	0.97	0.04	0.99
2.5 × 10 <sup>7</sup> conidia ml <sup>-1</sup>	0.31	0.95		

from *B. bassiana*-treated *A. swirskii*. Moreover, no deformation or moulting problems in the offspring was observed (A Midthassel pers. obs) suggesting that the fitness of the eggs produced by an infected adult female is not compromised by the pathogen.

The study on juvenile *A. swirskii* developing from egg to adult on dry residue of *B. bassiana* was intended to simulate the effect on a founding population of *A. swirskii* inoculated or dispersing into a recently treated

crop. There was no significant effect on survivorship of juvenile *A. swirskii* on *B. bassiana* dry residue compared with the control treatment and mycosis was observed in a small proportion of dead mites at 1 × 10<sup>8</sup> conidia ml<sup>-1</sup> only. This indicates that, although adult mites dispersing into a treated crop may succumb to treatment residues of *B. bassiana*, their offspring will not be affected and are therefore capable of establishing new populations. The subsequent reduced fecundity of these mites however suggest that *A. swirskii* juveniles on dry residue were infected by *B. bassiana*, but with a lighter infection, revealing sublethal effects but not causing mortality. This would most likely be due to fewer inoculum successfully invading the host.

Immature stages are often less susceptible to fungal infection due to germinating conidia being shed during ecdysis (Vey and Fargues 1977; Ugine et al. 2005). Short immature life stages, and therefore frequent ecdysis, combined with relatively low persistence of *B. bassiana* conidia on leaf surfaces can explain the low susceptibility of juvenile *A. swirskii* to this pathogen. Viability of *B. bassiana* conidia decreases gradually on leaves and persistence on top leaves has been reported to be four days (Inglis et al. 1993). As *A. swirskii* eggs, which were not affected by *B. bassiana* dry residue, hatch 1–2 days post-oviposition and the nymphs moult to the next instar every 1–2 days under optimal conditions (Lee and Gillespie 2011; Midthassel et al. 2013) the juvenile stages are able to evade pathogen invasion during the first few days post-application and therefore survive until the conidial viability has been reduced. The effect of *B. bassiana* residue on juvenile phytoseiid has not previously been studied and these results offer the first insight into the effect of *B. bassiana* on the population-founding individuals in a treated crop.

Field studies of *B. bassiana* applications with concomitant inoculations of phytoseiid mites have largely concluded that there is no detrimental effect on the phytoseiid and deemed these BCAs as compatible (Jacobson et al. 2001; Chandler et al. 2005; Numa Vergel et al. 2011). Compatibility however does not warrant an additive or synergistic contribution to pest control. Jacobson et al. (2001) found that combining *B. bassiana* with *Neoseiulus cucumeris* did not improve nor disrupt thrips control in a cucumber crop and *B. bassiana* treatments in conjunction with *N. californicus* or *P. persimilis* did not improve nor disrupt

*Tetranychus urticae* Koch (Acari: Tetranychidae) control in roses (Numa Vergel et al. 2011). Enhanced *T. urticae* control has been reported on tomato with concomitant use of *B. bassiana* and *P. persimilis* compared with either one on their own (Chandler et al. 2005) suggesting that improved control by combined treatments may be crop-specific or dependent on pest level and age distribution.

Although concomitant pest control with *A. swirskii* and *B. bassiana* may not be additive or synergistic, these two BCAs may complement each other in an IPM programme: *A. swirskii* for preventative control and *B. bassiana* as a curative treatment. When pest populations build up beyond the level where reasonable control can be expected from predatory mites, corrective applications with *B. bassiana* can be conducted followed by re-introduction of *A. swirskii* if needed. The effect of *B. bassiana* on other arthropod BCAs in the IPM programme must still be considered, and the potential detrimental effect of other components, such as fungicides, on *B. bassiana* must be taken into account. IPM programmes are complex and inconstant and there is much further work to be conducted to fully understand and optimise these control strategies.

Based on the results from the current study the susceptibility of *A. swirskii* as a physiological host for *B. bassiana* GHA is confirmed with slight to moderate virulence depending on exposure type. Despite the sublethal effects of *B. bassiana* on the fecundity of *A. swirskii*, these two BCAs have good potential for concomitant use in the field due to the following points: (1) no significant effect of dry residue on juvenile survival; (2) no significant effect of dry residue on fecundity; (3) no detrimental effect on the offspring of infected *A. swirskii* with respect to egg hatching, juvenile survival and sex ratio; and (4) the mortality in field conditions are generally expected to be lower than in the laboratory. Further work should focus on field compatibility of these two BCAs.

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