

RESEARCH ARTICLE

Potential use of the fungus *Beauveria bassiana* against the western flower thrips *Frankliniella occidentalis* without reducing the effectiveness of its natural predator *Orius sauteri* (Hemiptera: Anthocoridae)

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Orius sauteri (Poppius; Hemiptera: Anthocoridae) is an important predator of western flower thrips, *Frankliniella occidentalis* (Pergande; Thysanoptera: Thripidae). *O. sauteri* would be directly exposed to the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin in the field should the fungus be used as a biopesticide. If the fungus were to negatively affect *O. sauteri* in agro-ecosystems, predation of *F. occidentalis* by *O. sauteri* may be limited. The present study was undertaken to evaluate the insecticidal activity of strain B.bassiana-RSB of *B. bassiana*, which is highly virulent to *F. occidentalis*, on the predator under laboratory conditions. Results showed that, regardless of the concentration applied to first instars, Bb-RSB was not insecticidal against *O. sauteri*, nor did direct applications affect the developmental rate of the predator. Significant differences in developmental rates and adult longevity were observed between *O. sauteri* that fed on Bb-RSB-infected *F. occidentalis* cadavers and those that fed on untreated thrips. Developmental time (from first instar to adult) increased from 0.3 to 0.7 days and adult longevity decreased by 0.8 to 1.2 days for predators fed thrips treated with low and high concentrations of strain Bb-RSB, respectively, compared with predators fed on untreated thrips. However, these differences were only 3–13% of mean values for the controls, suggesting that the effects of Bb-RSB on *O. sauteri* are relatively minor. These findings highlight the potential use of *O. sauteri* in combination with *B. bassiana* for the biocontrol of *F. occidentalis*, but field tests must be performed to confirm their compatible use.

Keywords: *Beauveria bassiana*; *Orius sauteri*; *Frankliniella occidentalis*; integrated biological pest control

Introduction

The western flower thrips, *Frankliniella occidentalis* (Pergande; Thysanoptera: Thripidae), is an important pest of ornamentals and vegetables that causes extensive economic losses in greenhouse and open-field plant production (Morse and Hoddle 2006; Reitz, Gao, and Lei 2011). *F. occidentalis* is native to the Western USA, but since the late 1970s it has become a highly invasive, cosmopolitan pest (Kirk and

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Terry 2003). In addition to the quantitative and qualitative damage caused directly by its feeding, this thrips species is also a major vector of tospoviruses, including *Tomato spotted wilt virus* (Reitz et al. 2011). The difficulties in using conventional synthetic insecticides to manage *F. occidentalis* populations (Espinosa, Bielza, Contreras, and Lacasa 2002; Reitz et al. 2011) have led to a growing interest in novel and more effective pest integrated management alternatives (Funderburk 2009).

Many reports have described the detrimental effects of entomopathogenic fungi on target insect pests (Jacobson, Chandler, Fenlon, and Russell 2001; Down, Cuthbertson, Mathers, and Walters 2009; Gao, Reitz, Wang, and Lei 2012). Further, the relative safety and specificity of fungal pathogens may facilitate their acceptance by growers for use in pest management programmes. Indeed, entomopathogenic fungi have been successfully developed worldwide as biological control agents for many agricultural pests. Some are commercially available as biological insecticides for the control of various species of thrips (Ansari, Brownbridge, Shah, and Butt 2008). In China, recent research has identified several isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae) that are highly virulent against *F. occidentalis* and thus have potential as biological control agents (Wang, Lei, Xu, and Gao 2011; Gao et al. 2012). However, the successful use of any biological insecticide not only depends on efficacy against target pests, but also on low virulence against non-target insects, especially beneficial insects (Strasser, Vey, and Butt 2000).

Research has recently focused on the epizootiology and resultant effects of entomopathogenic fungi on predatory arthropods (Traugott, Weissteiner, and Strasser 2005; Labbé, Gillespie, Cloutier, and Brodeur 2009). In theory, if the predatory insect species are susceptible to *B. bassiana* after conidia are directly applied, or the insect is indirectly infected via feeding on contaminated surfaces or infected preys, the potential of the fungal pathogen for integrated pest management (IPM) would be compromised. However, if their effects on natural enemies are low compared with currently used synthetic insecticides, entomopathogenic fungi could be a compatible component with other natural enemies in an IPM programme (Strasser et al. 2000).

Frankliniella occidentalis is attacked by a variety of predatory arthropods (Sabelis and Van Rijn 1997). Among these species, nymphs and adults of *Orius sauteri* (Poppius; Hemiptera: Anthocoridae) are important predators of *F. occidentalis* and other pests in China (Zhang, Yu, Li, Zhang, and Men 2007). Laboratory experiments have demonstrated that *O. sauteri* readily prey upon *F. occidentalis* larvae and adults, suggesting that it might be a key biological control agent of thrips (Zhang et al. 2007). *O. sauteri* nymphs and adults, however, also would be exposed to field-applied entomopathogenic fungi if these are used as a microbial control of *F. occidentalis*. If the fungus kills *O. sauteri*, predation of *F. occidentalis* by *O. sauteri* would be significantly reduced (Zimmermann 2007). Commercial formulations of fungal pathogens are intended as microbial insecticides to provide a relatively rapid knockdown of pest populations but with little residual efficacy (Glare et al. 2012). Alternatively, predatory biological control agents are typically most successful in providing long-term suppression of pest populations rather than therapeutic control (Symondson, Sunderland, and Greenstone 2002). Therefore, if a microbial control is detrimental to predators, overall pest management may be compromised. The

present study was designed to identify the potential hazards to *O. sauteri* of one *B. bassiana* formulation that is being considered for control of *F. occidentalis* in northern China.

Materials and methods

Insect rearing

A colony of *O. sauteri* was established from nymphs and adults collected from cucumber plants (*Cucumis sativus* L. [Cucurbitales: Cucurbitaceae]) at the Changing Greenhouse Experimental Station of the Institute of Plant Protection, CAAS in Beijing. The stock culture of *O. sauteri* was reared in plastic cages (40 cm diameter \times 45 cm height). Each cage was provided with sufficient quantities of soybean leaves (*Glycine max* L. [Fabales: Fabaceae]), infested with pea aphids *Acyrtosiphon pisum* (Harris; Hemiptera: Aphididae) as a food supply for the predators. Bean plants (*Phaseolus vulgaris* L. [Fabales: Fabaceae]) were provided in each cage as an oviposition substrate for *O. sauteri*. A colony of western flower thrips was maintained as described by Liang, Lei, Wen, and Zhu (2010). Briefly, thrips colonies were reared in 0.5 L tube-shaped glass jars containing green bean pods. Rearing jars were kept at $26 \pm 2^\circ\text{C}$ and 60–70% relative humidity (RH) and L13:D11 photoperiod. After 4–5 days, beans were transferred to fresh glass jars. Eggs that were laid within a maximum period of 48 h were used to obtain first instars, which were collected for experimental use.

B. bassiana source

An isolate of *B. bassiana* (Bb-RSB) was originally isolated from the striped stem borer, *Chilo suppressalis* (Walker; Lepidoptera: Pyralidae; Gao et al. 2012) and maintained on Sabouraud dextrose agar at $26 \pm 1^\circ\text{C}$ for 16 days under continuous light conidia were harvested from 1- to 3-week-old cultures by flooding with sterile 0.05% Tween-80. The concentration of conidia was determined with a hemocytometer, equilibrated with sterile 0.05% Tween-80 Viability was determined to be >90%. In all bioassays, *B. bassiana* strain Bb-RSB was tested at two concentrations of conidia, 1×10^4 (Bb-RSB- 10^4) and 1×10^8 spores/mL (Bb-RSB- 10^8), whereas the control was Tween-80 at 0.05% v/v. The concentrations of Bb-RSB used in experiments reflect concentrations sufficient to cause >50 to ~100% mortality in *F. occidentalis* populations (Gao et al. 2012).

Efficacy of direct application of B. bassiana against O. sauteri

Sixty newly eclosed first instars of *O. sauteri* were submerged for 3–5 seconds in the appropriate *B. bassiana* spore suspension, using the aforementioned concentration treatments. Treated nymphs were transferred to Petri dishes (3.5 cm) lined with soybean leaves infested with pea aphids, and were incubated in plastic boxes maintained at $26 \pm 2^\circ\text{C}$ and 75% relative humidity. The presence of fungal mycelia on nymphs was recorded every other day. *O. sauteri* development stages were recorded daily until adult eclosion or mortality. For those individuals that eclosed as adults, adult survivorship was recorded daily.

***O. sauteri* fed on *B. bassiana*-infected thrips**

Groups of 20 *F. occidentalis* larvae were infected with *B. bassiana* by dipping for 3–5 seconds into the appropriate *B. bassiana* concentration or the control. Once infection was confirmed by the presence of aerial mycelia, *O. sauteri* nymphs were provisioned with *B. bassiana*-infected *F. occidentalis* larvae. Similarly, for the control treatment, *O. sauteri* nymphs were provisioned with control larvae. *F. occidentalis* prey was replenished as needed. *O. sauteri* nymphs that died within the first 24 h after the initiation of the trial were not included in data analysis. The entire experiment was replicated three times. *O. sauteri* infection status and development stage were recorded daily until adult eclosion or mortality. For those individuals that eclosed as adults, adult survivorship was recorded daily.

Statistical analyses

The Kolmogorov–Smirnov test was used to ensure that data satisfied the assumptions of the analysis of variance (ANOVA) test. Where the assumptions were satisfied, ANOVA was used to test for differences in developmental times and subsequent adult longevity among treatments. Where data failed to meet ANOVA assumptions, we used the non-parametric Kruskal–Wallis method by ranks, in order to test differences among treatments. The Tukey HSD test was used to compare specific pairs of treatments for these experiments (SAS Institute 1988). The data from the mortality rates of *O. sauteri* feeding on *B. bassiana*-treated *F. occidentalis* prey were evaluated by chi-squared tests for independence.

Results

Efficacy of direct application of B. bassiana against O. sauteri

Testing for potential insecticidal activity of *B. bassiana* strain Bb-RSB by direct application to the beneficial insect *O. sauteri* showed that this strain did not significantly alter the life cycle of the predator (Table 1). Significant treatment differences were not observed in the developmental times for each instar (first instar: $F = 3.21$; $df = 2, 149$; $P > 0.05$; second: $F = 1.13$; $df = 2, 149$; $P > 0.05$; third: $F = 2.46$; $df = 2, 147$; $P > 0.05$; fourth: $F = 1.25$; $df = 2, 147$; $P > 0.05$; and fifth: $F = 3.32$; $df = 2, 143$; $P > 0.05$). Consequently, total developmental times were not significantly different for *O. sauteri* nymphs treated with Bb-RSB-10⁴ or Bb-RSB-10⁸ or the control ($F = 1.76$; $df = 2, 143$; $P > 0.05$). *O. sauteri* nymphs treated with either low or high *B. bassiana* concentrations were able to complete their life cycle as well as control nymphs ($\chi^2 = 3.81$; $df = 2$; $P > 0.05$). Mortality was very low (<5%) in all three treatments. Adult longevity was not affected by *B. bassiana* treatments ($F = 1.56$; $df = 2, 143$; $P > 0.05$).

Effects of B. bassiana infected prey on development and survivorship of O. sauteri nymphs, and subsequent adult longevity

To determine if development and survival of *O. sauteri* nymphs were affected by feeding on Bb-RSB-infected *F. occidentalis* larvae, dead larval thrips showing aerial mycelia after being exposed to *B. bassiana* were offered as prey to *O. sauteri* nymphs.

Table 1. Effects of direct *B. bassiana* application on the survival and development of *O. sauteri* nymphs, and on their adult longevity.

Treatments applied to <i>O. sauteri</i> nymphs ^a	Development time (days)					Total nymphal development time (days)	Survival rate (%)	Adult longevity (days)
	First instar	Second instar	Third instar	Fourth instar	Fifth instar			
Bb-RSB-10 ⁴	2.0±0.03	2.2±0.03	2.1±0.03	2.3±0.04	1.8±0.03	10.4±0.04	95.3±0.05	9.0±0.07
Bb-RSB-10 ⁸	2.0±0.02	2.2±0.04	2.2±0.04	2.3±0.03	1.8±0.03	10.5±0.05	98.2±0.04	9.1±0.08
Control	2.0±0.03	2.3±0.04	2.0±0.05	2.2±0.02	1.8±0.05	10.3±0.08	97.5±0.09	9.1±0.05

^a*B. bassiana* strain Bb-RSB was tested at two concentrations of conidia: 1×10^4 (Bb-RSB-10⁴) or 1×10^8 spores/mL (Bb-RSB-10⁸).

Results showed that the infected prey affected the duration of most nymphal stages of *O. sauteri* (Table 2). Total developmental time was significantly longer for *O. sauteri* nymphs feeding on Bb-RSB-infected *F. occidentalis* than for nymphs feeding on control larvae ($F=178.6$; $df=2, 85$; $P<0.001$; Table 2). However, prey type did not affect survivorship of nymphs to adulthood. *O. sauteri* nymphs were able to complete development as well when feeding on infected thrips prey as when feeding on control prey ($\chi^2=2.54$; $df=2$; $P>0.05$; Table 2). Overall mortality of *O. sauteri* nymphs was very low (<6%) for the three treatments.

In addition to the effect on developmental time, adult longevity of *O. sauteri* was significantly affected by prey type ($F=65.3$; $df=2, 68$; $P<0.001$). Those *O. sauteri* fed infected *F. occidentalis* larvae had significantly shorter adult lifespans by approximately 10–15% than those fed control prey (Table 2).

Discussion

In the present study, direct and indirect effects of *B. bassiana* strain Bb-RSB on the predator *O. sauteri* were examined under laboratory conditions. The bioassays were designed to simulate worst-case scenarios that could occur in field applications by exposing predators and their prey to concentrations of *B. bassiana* sufficient to cause high mortality in a *F. occidentalis* population (Gao et al. 2012), and then monitoring the development and survivorship over the entire life span of *O. sauteri*. Environmental conditions for the bioassays were highly conducive to infection development (Shipp, Zhang, Hunt, and Ferguson 2003); therefore, any deleterious effects on development, survival and adult longevity should have been evident. Direct effects were assessed by applying *B. bassiana* to first instars, which are considered the most susceptible life stage (Van de Veire, Sterk, van der Staij, Ramakers, and Tirry 2002). Indirect effects were assessed by exposing predators to mycosed thrips as prey.

The experiment in which first instars were treated revealed no negative effects on development time or survivorship of immature *O. sauteri*, or on their longevity as adults, regardless of the concentrations of *B. bassiana* formulation used. The lack of lethal infections indicates that *O. sauteri* is not within the physiological host range of this strain of *B. bassiana* (Zimmermann 2007). Other strains of *B. bassiana* have been shown to infect Anthocoridae species but with relatively low rates of successful infection (Ludwig and Oetting 2001; Dunkel and Jaronski 2003). However, Shipp et al. (2003) recommended that *O. insidiosus* not be released at times when *B. bassiana* strain GHA is applied because of high mortality from direct applications to adults. These previous studies were conducted for relatively short periods of time. In our study, *O. sauteri* were monitored over their entire life span and suffered no significant mortality from strain Bb-RSB. Furthermore, potential risks to the predator from direct exposure in field applications of strain Bb-RSB would be minimal, as under natural conditions, predators would likely escape infection as fungal conidia slough off with insect movement through the environment (Quintela and McCoy 1998). Therefore, strain Bb-RSB should not be considered virulent to *O. sauteri* and would be compatible in an IPM programme that includes *O. sauteri*.

Minor effects on *O. sauteri* development rates and adult longevity were observed when predators were given *F. occidentalis* larvae infected with *B. bassiana* as prey. Total nymphal developmental time of *O. sauteri* increased by 3–7% and the adult longevity decreased by 9–13% when feeding on *F. occidentalis* larvae contaminated

Table 2. Effect of *B. bassiana* treated *F. occidentalis* as prey on mortality, nymphal development and survival, and adult longevity of *O. sauteri*.

Prey treatments ^a	Development time (days)					Total nymphal development time (days)	Survival rate (%)	Adult longevity (days)
	First instar ^b	Second instar	Third instar	Fourth instar	Fifth instar			
Bb-RSB-10 ⁴	2.2 ± 0.03a	2.2 ± 0.03a	2.1 ± 0.04a	2.4 ± 0.05a	1.9 ± 0.03a	10.8 ± 0.05a	94.1 ± 0.03a	8.4 ± 0.08a
Bb-RSB-10 ⁸	2.2 ± 0.02a	2.3 ± 0.04b	2.1 ± 0.06a	2.5 ± 0.02b	2.0 ± 0.03a	11.2 ± 0.08a	95.6 ± 0.08a	8.0 ± 0.10a
Control	2.0 ± 0.03b	2.3 ± 0.04a	2.1 ± 0.05a	2.5 ± 0.05a	1.7 ± 0.05b	10.5 ± 0.03b	94.7 ± 0.10a	9.3 ± 0.06b

^a*B. bassiana* strain Bb-RSB was tested at two concentrations of conidia: 1×10^4 (Bb-RSB-10⁴) or 1×10^8 spores/mL (Bb-RSB-10⁸).

^bMeans within columns marked with the same letter are not significantly different, $P > 0.05$.

by *B. bassiana* compared with those fed *F. occidentalis* larvae not treated with *B. bassiana*. The underlying mechanisms for this effect remain unknown. One explanation is that *F. occidentalis* larvae contaminated by *B. bassiana* may be poor quality prey for *O. sauteri* because infection makes the larvae deficient in certain essential nutrients (Simelane, Steinkraus, and Kring 2008) or creates a buildup of fungal toxins or metabolites, which may slow development and shorten adult longevity of *O. sauteri* (Leckie et al. 2008).

Although development of immature *O. sauteri* was slowed by feeding on *B. bassiana*-infected prey, the experiment revealed no significant differences in mortality rates among nymphs given different prey types. This result indicates that *O. sauteri* did not acquire lethal amounts of the pathogen through transmission from the prey. Consequently, the results presented here suggest that the slight negative effects on *O. sauteri* when provided *F. occidentalis* larvae contaminated by *B. bassiana* formulation are most probably prey-quality mediated rather than direct effects of the *B. bassiana* formulation (Sobhy et al. 2010).

Our study used a novel strain of *B. bassiana* that is highly virulent to *F. occidentalis*, but proved not to be detrimental to *O. sauteri*. Overall, our results indicate that *B. bassiana* strain Bb-RSB poses a negligible risk to *O. sauteri* because it did not produce significant amounts of lethal infections, and because neither high spore concentrations nor sporulating prey cadavers retarded development or reduced the life span of the predator substantially. Therefore, *B. bassiana* strain Bb-RSB and *O. sauteri* appear to be compatible and complementary biological control agents for *F. occidentalis* in an IPM programme. *B. bassiana* strain Bb-RSB could be used for rapid, short-term suppression of *F. occidentalis* populations, and *O. sauteri* could be used for longer-term suppression of *F. occidentalis* populations.

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