J Pest Sci
DOI 10.1007/s10340-012-0476-4

ORIGINAL PAPER

Joint action of *Beauveria bassiana* and the insect growth regulators diflubenzuron and novaluron, on the migratory locust, *Locusta migratoria*

N. Bitsadze · S. Jaronski · V. Khasdan · E. Abashidze · M. Abashidze · A. Latchininsky · D. Samadashvili · I. Sokhadze · M. Rippa · I. Ishaaya · A. R. Horowitz

Received: 19 September 2012 / Accepted: 22 December 2012

Abstract *Beauveria bassiana* (Balsamo) Vuillemin and sublethal concentrations of the insect growth regulators (IGR) diflubenzuron and novaluron were applied simultaneously and sequentially to second-instar *Locusta migratoria migratorioides* (Sauss.) to determine the interaction between these materials and an entomopathogenic fungus. Nymphs were fed on corn leaf disks treated with several concentrations of each IGR, and a constant dose of *B. bassiana* was applied topically. Additive interaction was demonstrated in all instances when second-instar nymphs were exposed to diflubenzuron or novaluron simultaneously with *B. bassiana* treatment, and when the fungus was applied first and IGR after 48 h. Additive interaction was also exhibited when novaluron was applied first and *B. bassiana* after 48 h. Antagonism was demonstrated when nymphs were fed diflubenzuron-treated corn leaves first, then after 48 h were treated with *B. bassiana*. The additive interaction that was observed in our experiments could still be useful to achieve efficacious levels of acridid control at low rates of an IGR along with a biological control agent such as an entomopathogenic fungus.

Keywords Entomopathogen · IGR · Beauveria · Diflubenzuron · Novaluron

Introduction

In many countries, especially in the tropics, locusts and grasshoppers have been considered major pests in agriculture since biblical times, causing significant damage to many field crops (Nevo 1996). Under favorable conditions, certain species exhibit gregarious and migratory behavior, leading to the formation of spectacular swarms (Lomer et al. 2001). Locusts and grasshoppers cause significant

Communicated by M. Traugott.

N. Bitsadze · E. Abashidze · D. Samadashvili
Department of Entomology and Applied Zoology, Kanchaveli L. Institute of Plant Protection, Tbilisi, Georgia

S. Jaronski (✉)
Northern Plains Agricultural Research Laboratory, USDA ARS, Sidney, MT, USA
e-mail: stefan.jaronski@ars.usda.gov

V. Khasdan
Department of Life Science, Ben-Gurion University, Beer-Sheva, Israel

E. Abashidze
Department of Phytopathology, Kanchaveli L. Institute of Plant Protection, Tbilisi, Georgia

M. Abashidze
National Academy of Science, Tbilisi, Georgia

A. Latchininsky
Department of Renewable Resources, Entomology Section, University of Wyoming, Laramie, WY, USA

I. Sokhadze
Javakhishvili State University, Tbilisi, Georgia

M. Rippa · A. R. Horowitz
Department of Entomology, Gilat Research Center, ARO, Negev, Israel

I. Ishaaya
Department of Entomology, Agricultural Research Organization (ARO), The Volcani Center, Beit Dagan, Israel

Published online: 15 February 2013

damage throughout Africa, the Middle East and Australia, parts of Asia, and North and South America (Bullen 1966).

Control strategies for locusts and grasshoppers rely almost exclusively on the use of chemical insecticides (Prior and Streett 1997). However, recognition of such associated problems as non-target effects, environmental pollution, and the high economic costs involved have prompted the development of alternative control strategies and environmentally "softer" chemicals such as insect growth regulators (IGRs). IGRs are "low risk" insecticides, which have a relatively minor detrimental effect on the environment and its inhabitants, rendering them important components in IPM programs (Horowitz and Ishaaya 2004). They have been found to be sufficiently useful in the control of locust pests by barrier treatment (Tingle 1996). On the basis of the mode of action, IGRs are grouped into three categories: juvenile hormones (JHs) and their analogs (JHAs), also called juvenoids; ecdysone agonists (EA); and chitin synthesis inhibitors (CSIs) or molt inhibitors (MIs) (Mondal and Parween 2000).

Among the CSIs are the benzoylphenyl ureas (BPUs) such as diflubenzuron (Dimilin®, Chemtura AgroSolutions) and novaluron (Rimon®, Chemtura AgroSolutions). BPUs act on insects by inhibiting chitin formation (Ishaaya and Casida 1974) and are thus selective for immature stages (Ishaaya and Horowitz 1998). In general, compounds that act on insect hormonal receptors or on particular insect processes, not present in mammals, are considered important insecticides for controlling insect pests with minimal harm to humans, beneficial arthropods, and the environment. However, some natural enemies (especially predators) can be affected by IGRs. In addition, there is some concern regarding BPUs with respect to their potential effects on crustacean species (Ishaaya 1990; Ishaaya and Horowitz 1998).

Fungal biological control agents (BCAs) have also been shown to offer alternative and rapid prospects for implementation against grasshoppers and locusts (Prior and Greathead 1989; Prior and Streett 1997; Lomer et al. 2001). Among these entomopathogenic fungi is *Beauveria bassiana* (Balsamo) Vuillemin, which has demonstrated considerable potential for the management of a variety of insect pests, including Acrididae (Johnson et al. 1992; Reuter et al. 1993; Jaronski and Goettel 1997). One strain of this fungus, GHA, has been commercialized in the US for the control of a wide range of insects, including grasshoppers on rangeland and improved pastures.

A criticism of the entomopathogenic fungi is that they act too slowly (e.g., Lomer et al. 2001). The combined use of chemical insecticides, ideally at a low, sublethal rate, and entomopathogenic fungi has been an attractive approach to counter this criticism. Purwar and Sachan (2006) evaluated the effect of combining *B. bassiana* or *Metarhizium anisopliae* (Metschnikoff) with endosulfan, imidacloprid, lufenuron, diflubenzuron, dimethoate, and oxydemeton methyl for use against 10–11 day old larvae of *Spilarctia obliqua* (Walker). In some cases the combination treatments showed a higher dose mortality response than treatment with only fungal conidia or insecticide. Quintela and McCoy (1997) observed that *B. bassiana* and *M. anisopliae*, combined with sublethal doses of imidacloprid as a contact or oral treatment, synergistically increased the mortality of *Diaprepes abbreviatus*. In another study Purwar and Sachan (2006) found similar results with *Lipaphis erysimi*. Thus, application of the insecticide and entomogenous fungus in combination was more deleterious to the insect than application of either alone.

The effect of insecticides on an entomopathogenic fungus cannot be generalized. The sensitivity of different fungal isolates of the same species to a particular insecticide may differ greatly (Olmert and Kenneth 1974). The interaction effects may vary from isolate to isolate and the chemical nature of the pesticides. The synergistic effect of dual treatment was reported by several authors, among them Serebrov et al. (2003), Bahiense and Bittencourt (2004), Mohan et al. (2007), Morales-Rodriguez and Peck (2009), and Russell et al. (2010). A range of effects were observed from synergism to neutral effect. The addition of diflubenzuron (Dimilin) to the *B. bassiana* formulation increased the speed of mortality in laboratory treated, fourth instar grasshoppers (Foster et al. 1995).

The general objective of our study was to determine the effect of two IGRs (novaluron and diflubenzuron) either used simultaneously with *B. bassiana*, or used sequentially, against locust nymphs. Laboratory tests were conducted in order to determine the required concentrations of the IGRs and *B. bassiana* when applied against locust pests and to define the type of interaction, in other words whether the two control agents (chemical and biological) act additively, synergistically, or antagonistically.

Materials and methods

Locusta migratoria capito (*Locusta migratoria migratorioides*) (Sauss.) (Orthoptera: Acrididae) were obtained from a laboratory colony of the Gilat Research Center (Department of Entomology, ARO, Israel) maintained on greenhouse-grown corn foliage (*Zea mays* sp.) and wheat bran. Insects were reared in special cages according to the methods of Harvey (1990) at 30–38 °C ambient temperature and a photoperiod of 14:10 (L:D).

The formulations of the IGRs diflubenzuron and novaluron were diluted with distilled water to the required concentration. Fresh leaves of corn were treated with different concentrations of each IGR by dipping the leaves in the IGR solution for 20 s and then air-drying the leaves for 2 h before administration to the locusts.

In all the tests described herein, *B. bassiana* (GHA strain, Emerald BioAgriculture, now Laverlam International, technical grade, Lot 03–04) was used against 5-day-old second instars *L. migratoria migratorioides* as a conidial suspension in sterile aqueous 0.01 % Triton-X100 as a wetting agent. Viability of the *Beauveria* conidial powder was determined once every three months during the course of our study by plating an appropriately diluted conidial suspension on potato dextrose agar (PDA) solid medium supplemented with 100 μg ml^{-1} ampicillin; the number of colony forming units (CFU) was determined after incubating the plates at 27 °C for 48–72 h, at that time the colonies were identified as *Beauveria* by visual observation under magnification. The CFUs were then used to calculate conidial viability.

Initial multiple dose bioassays of the *Beauveria* were first conducted to select the appropriate dose for interaction studies. A series of conidial concentrations (10^2–10^9 viable conidia ml^{-1}) were prepared in sterile aqueous 0.01 % Triton X100. Locust nymphs were placed within small plastic tubes with net bottoms and dipped 6 times into the different concentrations, and then the insects were removed and excess liquid was removed with a paper towel. The nymphs were then transferred to 500 ml Styrofoam cups with perforated lids. Nymphs were incubated at 27 ± 2 °C, 50 ± 5 % relative humidity, 14:10 L:D photoperiod, and fed corn leaves and wheat bran daily. The cumulative mortality was then determined after 10 days.

To study the interaction between *B. bassiana* and IGRs (novaluron and diflubenzuron), four treatment combinations were employed: *B. bassiana* alone; treatment with either novaluron or diflubenzuron only; treatment with both *B. bassiana* and each of the IGRs; and an untreated control.

Conidia of *B. bassiana* were applied topically to second-instar nymphs by dipping the insects in a conidial suspension of 0.01 % Triton-X100 as described earlier. Corn leaves treated with the IGR were cut into pieces (35 ± 2 cm^2) and placed in Styrofoam cups (500 ml) along with one locust. There were ten insects per IGR concentration (novaluron 0.5, 1, and 2 ppm); diflubenzuron 0.1, 0.5, and 1 ppm). Each assay was repeated three times independently.

A series of assays were also conducted to evaluate the effect of simultaneous and delayed applications of IGR and *B. bassiana* on second-instar locusts. Second-instar locusts were treated topically by dipping into a *B. bassiana* suspension and were then fed corn leaves treated with different concentrations of novaluron and diflubenzuron. In the first assays, treated leaves of corn were cut and placed in 500 ml Styrofoam plastic cups containing moistened filter paper along with a single second-instar nymph for 48 h; a fresh untreated leaf was then added. Cumulative mortality was determined for 10 days. In other tests, insects were first treated with *B. bassiana* as described earlier and placed in cups containing fresh corn leaves. After 48 h the insects were treated with either IGR for 2 days (by being fed IGR-treated corn leaves). The locusts were then transferred into individual 500 ml plastic cups with lids and fed untreated for an additional 8 days, at which time mortality was determined. Each concentration and combination was tested with ten replicates on three separate days. Insects were incubated under the conditions described earlier.

Median lethal concentrations (LC_{50}) of the *B. bassiana* and the IGRs were determined by probit regression analysis (Polo plus, version 1, copyright © 2002–2009 LeOra software). Two-way ANOVA and Bonferroni post-test comparisons were used for analysis of the interaction between IGRs and *B. bassiana*. Statistical analysis was done using Graph Pad Prism 5™ software. χ^2 analysis, based on Nishimatsu and Jackson (1998) and written in Excel®, was used for synergism calculation.

Results

Bioassay with *B. bassiana* strain GHA

The bioassays with *B. bassiana* strain GHA demonstrated a dose–response effect. Dose mortality regression analyses of data of second-instar nymphs treated with of *B. bassiana* were significant (heterogeneity $\chi^2 = 24.4$, Table 1). The median lethal concentration for a 10-day observation period was 5×10^5 (1.5×10^5–1.8×10^6) viable conidia ml^{-1}. A dose of 10^4 viable conidia ml^{-1} was chosen for the subsequent interaction experiments.

Bioassay with IGRs

Second-instar nymphs fed with corn leaves treated with either IGR (diflubenzuron and novaluron) exhibited the typical effects of IGRs (Fig. 1), i.e., trembling and deformation of the cuticle and hind legs after molting. The size of treated nymphs was smaller than that of untreated nymphs. Probit regression analyses of dose–response data in the case of novaluron and diflubenzuron were significant (heterogeneity, $\chi^2 = 7.48$, 3.38, respectively (Table 1). Mortality caused by the IGRs was statistically not significantly different from each other ($P > 0.05$) but at several individual concentrations diflubenzuron caused higher mortality than novaluron.

IGR—*B. bassiana* interactions

Statistical analyses (Bonferroni post-test comparison) were done to compare the effect of application of each IGR

Table 1 Effect of *B. bassiana* and IGRs (novaluron and diflubenzuron) on second-instar locusts

Compounds*	Slope (±SE)	LC_{50} (±95 % fiducial limits)**	χ^2
B. bassiana GHA	0.32 (0.04)	5×10^5 (1.5×10^5–1.8×10^6)	24.4 (20)
Diflubenzuron (10 % ai)	1.73 (0.37)	0.4 (0.26–0.6)	3.38 (7)
Novaluron (25 % ai)	1.38 (0.22)	0.9 (0.58–1.35)	7.48 (12)

* Novaluron and diflubenzuron were applied only to foliage, *B. bassiana* conidia were applied to nymphs topically

** Concentration of *B. bassiana* in spore suspension, ml^{-1}; the IGRs in mg (ai) l^{-1}

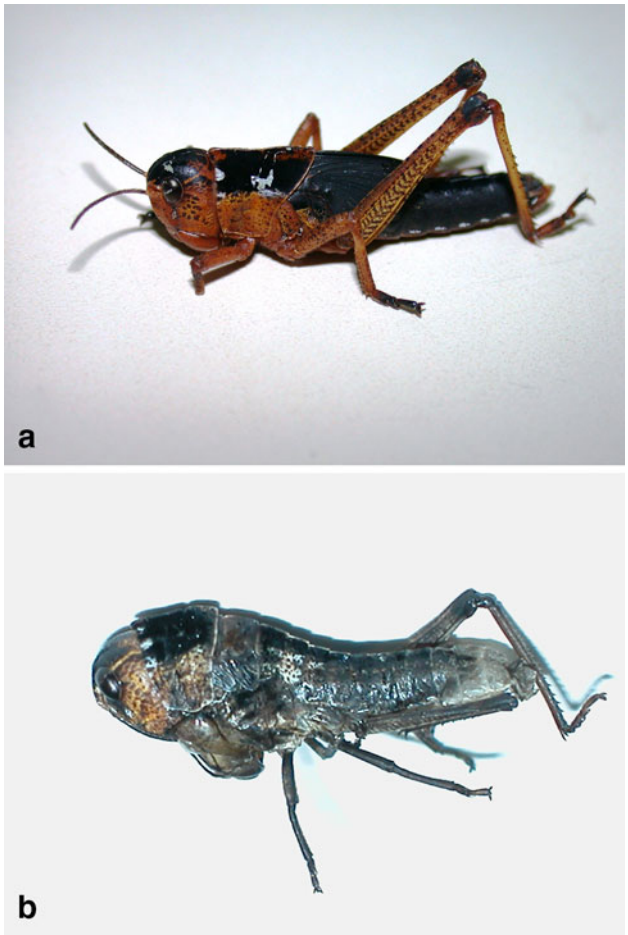

Fig. 1 Effect of IGRs on nymphs of *L. migratoria*: **a** normal and **b** deformed nymph

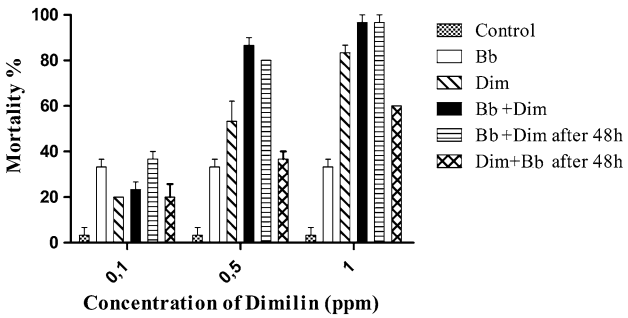

Fig.. 2 Bioassay with different concentrations of diflubenzuron ('*Dim*') and *B. bassiana* ('*Bb*' at approx. the LC_{30}); application of combinations (diflubenzuron alone, simultaneous application of *B. bassiana* and diflubenzuron, application of *B. bassiana* first and diflubenzuron after 48 h, application of diflubenzuron first and *B. bassiana* after 48 h)

alone versus simultaneous applications of both agents (IGR plus fungus). When diflubenzuron was applied at the same time with *B. bassiana,* significant difference was found only at IGR concentration of 0.5 ppm ($P < 0.001$) (Fig. 3). When *B. bassiana* was applied first and then diflubenzuron was applied after 48 h significant differences were obtained at concentrations of 0.1 and 0.5 ppm ($P < 0.05$ and $P < 0.001$, respectively) (Fig. 2). When novaluron was applied simultaneously with *B. bassiana* significant differences were obtained at concentrations of 0.5 ppm and 1 ppm ($P < 0.05$; $P < 0.001$, respectively) (Fig 2). When *B. bassiana* was applied first and then novaluron was applied after 48 h, a significantly different result was obtained at a concentration 1 ppm ($P < 0.001$) (Fig. 3).

Based on the formulae of Nishimatsu and Jackson (1998) if the calculated χ^2 was less than the tabular value of 3.84, the interaction between fungus and IGR was considered additive; if χ^2 was more than 3.84 and $P_{observed} < P_{expected}$ the interaction was considered antagonistic; and if χ^2 was more than 3.84 and $P_{observed} > P_{expected}$ the interaction was considered synergistic (where P_c and P_e are observed mortality and expected mortality, respectively).

Simultaneous application of *B. bassiana* and 0.1 ppm diflubenzuron, the application of diflubenzuron (0.1 ppm) first followed by *B. bassiana* after 48 h, and the application of *B. bassiana* first with diflubenzuron (0.1 ppm) after 48 h exhibited additive interaction (Table 2). Simultaneous application of *B. bassiana* and diflubenzuron (0.5 ppm) and the application of *B. bassiana* first then diflubenzuron (0.5 ppm) after 48 h, revealed additive interaction. The application of diflubenzuron (0.5 ppm) followed by *B. bassiana* after 48 h exhibited antagonism ($\chi^2 > 3.84$ and $Pc < Pe$) (Table 2).

Simultaneous application of *B. bassiana* and diflubenzuron (1 ppm) and the application of the fungus followed by diflubenzuron (1 ppm) after 48 h exhibited additive interaction because χ^2 was less then 3.84 ($\chi^2 < 3.84$). Application of diflubenzuron (1 ppm) followed by *B. bassiana* after 48 h exhibited antagonism ($\chi^2 > 3.84$ and $Pc < Pe$) (Table 2).

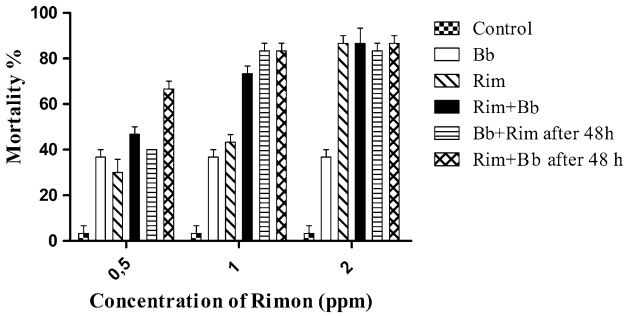

Fig. 3 Bioassay with different concentrations of novaluron ('*Rim*') and *B. bassiana* ('*Bb*' at the LC30); With combinations (novaluron alone, simultaneous application of *B. bassiana* and novaluron, application of novaluron first and *B. bassiana* after 48 h, application of *B. bassiana* first and novaluron after 48 h.)

Simultaneous application of *B. bassiana* and novaluron (0.5 ppm), application of novaluron (0.5 ppm) followed by *B. bassiana* after 48 h; and application of *B. bassiana* then novaluron (0.5 ppm) after 48 h all exhibited additive interactions ($\chi^2 < 3.84$) (Table 3).

Simultaneous application of *B. bassiana* and 1 ppm novaluron, and the application of novaluron (1 ppm) followed by *B. bassiana* after 48 h and application of *B. bassiana* first and novaluron (1 ppm) after 48 h exhibited additive interaction as χ^2 was less than 3.84 ($\chi^2 < 3.84$) (Table 3). Simultaneous application of *B. bassiana* and 2 ppm novaluron, application of novaluron (2 ppm) first then *B. bassiana* after 48 h, and application of *B. bassiana* first then novaluron (2 ppm) after 48 h, resulted in an additive interaction as χ^2 was less then 3.84 ($\chi^2 < 3.84$) (Table 3).

Discussion

In our studies an additive interaction was revealed in all instances where second-instar nymphs of migratory locusts were exposed to diflubenzuron (or novaluron) simultaneously with *B. bassiana* treatment and when *B. bassiana* was applied first and diflubenzuron or novaluron after 48 h. Additive interaction was also exhibited when novaluron was applied first and then *B. bassiana* after 48 h. Antagonism was demonstrated when nymphs were first fed with corn leaves treated with diflubenzuron (0.5 and 1 ppm) and after 48 h treated with the fungus.

Different interactions of *B. bassiana* and *M. anisopliae* with benzoylphenylureas have been observed by different authors. Hassan and Charnley (1989) provided the original observations of diflubenzuron synergizing the infectivity/pathogenicity of an entomopathogenic fungus and stimulated further research by others, to the present day. In their work, prior administration of the IGR synergized *M. anisopliae* in larval *Manduca sexta* with the effect becoming manifested when infection occurred after the next molt. Reuter et al. (1996) tested the effects of simultaneous administration of diflubenzuron and *B. bassiana* GHA onto grasshopper nymphs. Our reexamination of their data on the basis of the calculations of Nishimatsu and Jackson (1998) indicates either no interaction, additivity, or in one case, synergism, depending on the rates of fungus and IGR. The only synergistic combination was 2.5×10^{13} conidia ha^{-1} and 1.75 g diflubenzuron ha^{-1} (2.6×10^9 conidia ml^{-1} and 0.2 mg diflubenzuron ml^{-1}) and was manifested by a faster onset of mortality only evident 5 days after insects were treated. Higher and lower rates of either fungus or IGR had little effect. Subsequent field trials of diflubenzuron and *B. bassiana* (Foster et al. 1996) were marred by the absence of fungus only and IGR only treatments. Delgado et al. (1999) found that diflubenzuron had only an additive effect with *B. bassiana* when used against savanna grasshopper in field trials. Adult *Rhammatocerus schistocercoides*, separately and concurrently dosed topically with approx. 5,000 conidia of *M. acridum* and 8 ppm diflubenzuron, did not show any significant differences in either mortality rates or food consumption (De Faria 1999).

Mixed effects have been observed with other insects. Olson and Oetting (1999) showed that diflubenzuron interfered with the infectivity of *B. bassiana* for the aphid

Table 2 Interactions between *B. bassiana* and diflubenzuron in second-instar *L. migratoria*

Treatment	P_c* %	P_e** %	χ^2	Interaction
B. bassiana + diflubenzuron 0.1 ppm applied simultaneously	20	42	1.97	Additive
B. bassiana + diflubenzuron 0.1 ppm after 48 h	33	42	0.325	Additive
Diflubenzuron 0.1 ppm + *B. bassiana* after 48 h	16	42	2.756	Additive
B. bassiana + diflubenzuron 0.5 ppm applied simultaneously	83	65	1.424	Additive
B. bassiana + diflubenzuron 0.5 ppm after 48 h	77	65	0.633	Additive
Diflubenzuron 0.5 ppm + *B. bassiana* after 48 h	33	65	4.501	Antagonism
B. bassiana + diflubenzuron 1 ppm applied simultaneously	93	86	0.407	Additive
B. bassiana + diflubenzuron 1 ppm after 48 h	93	86	0.407	Additive
Diflubenzuron 1 ppm + *B. bassiana* after 48 h	57	86	6.985	Antagonism

* P_c observed mortality from combined treatments

** P_e expected mortality from combined treatments

Table 3 Interactions between *B. bassiana* and novaluron in second-instar nymphs of *L. migratoria*

Treatment	P_c* %	P_c** %	χ^2	Interaction
B. bassiana + novaluron 0.5 ppm applied simultaneously	43	51	0.262	Additive
B. bassiana + novaluron 0.5 ppm after 48 h	36	51	0.911	Additive
Novaluron 0.5 ppm + *B. bassiana* after 48 h	63	51	0.568	Additive
B. bassiana + novaluron 1 ppm applied simultaneously	70	60	0.433	Additive
B. bassiana + novaluron 1 ppm after 48 h	80	60	1.697	Additive
Novaluron 1 ppm + *B. bassiana* after 48 h	80	60	1.697	Additive
B. bassiana + novaluron 2 ppm applied simultaneously	83	89	0.312	Additive
B. bassiana + novaluron 2 ppm after 48 h	83	89	0.312	Additive
Novaluron 2 ppm + *B. bassiana* after 48 h	80	89	0.735	Additive

* P_c observed mortality from combined treatments

** P_e expected mortality from combined treatments

Myzus persicae (Sulzer), reducing efficacy up to 50 %. Ibrahim et al. (2011) observed no effect of diflubenzuron on the efficacy of a simultaneous application of diflubenzuron with a *B. bassiana* or a *M. anisopliae* for *Bemisia tabaci*, but saw a significantly greater degree of control in greenhouse tests, although several studies have suggested interesting explanations to these controversial results (e.g., Serebrov et al. 2003, 2006, Zibaee et al. 2009; Dubovskiy et al. 2012). In the case of synergy occurring with entomopathogenic fungi and OP insecticides, the insecticide inhibits nonspecific esterases and this mixture effectively might control insect pests (Serebrov et al. 2003). In general, insect infection by entomopathogenic fungi results in an increase of enzymatic activities of general esterases, P450 and glutathione *S*-transferase; i.e., treatment by *B. bassiana* causes tissue disruption and increase activity level of detoxifying enzymes. These enzymes probably affect the degradation and detoxification of different insecticides including IGRs. Vice versa, treatments with IGRs increase these activities and affect spore proliferation in insect's hemolymph.

The mechanism by which diflubenzuron or other CSIs could increase or decrease efficacy of a fungal pathogen is uncertain. The histological observations of Hassan and Charnley (1989) revealed that the *Manduca sexta* larval integument was weakened by diflubenzuron so that the fungus more readily penetrated through it into the hemocoel. Mietkiewski (unpublished, but cited in Bajan et al. 1995) observed that Dimilin 25WP stimulated the in vitro growth of *B. bassiana* strain 23 even at ten times the recommended field dose of the chemical. However, the observations of De Faria (1999) indicate that neither infectivity nor pathogenicity was increased in an adult grasshopper. Keller (1978) determined that diflubenzuron, as Dimilin 25WP, had deleterious effects on *B. tenella* (now *B. brongniartii*) and *Metarhizium anisopliae* at and above 250 ppm in an agar-incorporation assay, in terms of colony development.

On the other hand, the in vitro LC_{50} of teflubenzuron for *M. acridum* conidial germination was 3,596 ppm; for vegetative growth, it was 4,729 ppm (Magalhães et al. 2001). These data indicate teflubenzuron and, by inference, diflubenzuron and novaluron, has little or no effect on entomopathogenic fungi themselves.

As noted by Irigaray et al. (2003), however, it is very important to consider the formulations used for the agents in examining the effects of a chemical on the fungus in vitro. For example, the additives in the formulation of the diflubenzuron significantly affected fungal growth compared to the active ingredient on *B. bassiana* conidial germination (Anderson and Roberts 1983). Generally, the wettable powder and flowable formulations caused no inhibition and often increased colony counts, whereas the emulsifiable concentrate formulation frequently inhibited *B. bassiana* germination (Anderson et al. 1989). Conidia in a wettable powder are physically more accessible to the BPUs, than conidia in an oil-based emulsifiable formulation diluted in water, that is oil in water emulsion where the hydrophobic conidia remain in the oil phase (Jaronski, unpublished data), for application. These physical considerations must be taken into account. Olson and Oetting (1999) recommended that both the fungus and the diflubenzuron should not be mixed in the same tank, and when using diflubenzuron, it must dry on the foliage before *B. bassiana* can be applied.

Results of our experiments show that application of sub lethal concentrations of *B. bassiana* and IGRs (diflubenzuron and novaluron) may increase the rate of mortality, especially when the two are applied simultaneously. Synergism, whereby the effect of a combination of IGR and fungus is greater than either alone, an ideal outcome, was not observed. The additive interaction commonly observed in our experiments could still be useful, nevertheless, to achieve efficacious levels of grasshopper control at lower than usual rates of IGR, maintaining most of the environmental, health, and political benefits of a pure biological control agent, especially in an IPM context in which insect populations are attacked before they become economically damaging.

Elevated thermoregulation, called "behavioural fever," is a well-known defense against pathogens in Acrididae and members of several other orders (e.g., Ouedraogo et al. 2004), placing pathogens in stasis and reducing pathogenicity. The effects of benzoyl phenylureas on behavioral fever beyond their basic influence as a chemical toxicant are not known. Sublethal rates of diflubenzuron did not increase field efficacy of Beauveria against nymphal grasshopper populations beyond an potential additive effect, however (Jaronski unpublished data). It has been noted, however, that a metabolite of *M. robertsii*, Destruxin A, inhibited behavioral fever and enhanced pathogenicity of another *Metarhizium* that lacked this metabolite (Hunt and Charnley 2011). In our study we focused our attention on the basic interaction between novaluron and diflubenzuron, and the fungus, because of the chemicals' effect on chitin synthesis in the insect. Any effect of benzoyl phenylurea compounds on behavioral fever remains to the elucidated.

Acknowledgments We thank Galina Gindin and Marina Hazanovski for advice and all workers of Entomology Department of Gilat Research Center for their help. This research was supported (in part) under Grant No. CA23-022 funded by the US Israel Cooperative Development Research Program, Bureau for Economic Growth, Agriculture, and Trade, US Agency for International Development.

Conflict of interest None of the authors have any conflict of interest.

References

Anderson TE, Roberts DW (1983) Compatibility of *Beauveria bassiana* strain with formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. J Econ Entomol 76:1437–1441

Anderson TE, Hajek AE, Roberts DW, Preisler HK, Robertson JL (1989) Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides. J Econ Entomol 82:83–89

Bahiense TC, Bittencourt VREP (2004) Laboratory evaluation of the compatibility and the synergism between the entomopathogenic fungus *Beauveria bassiana* and deltamethrin to resistant strains of *Boophilus microplus*. Ann N Y Acad Sci 1026:319–322

Bajan C, Fedorko A, Kmitova K (1995) Reactions of entomopathogenic fungi to pesticides. Pol Ecol Stud 21(1):69–88

Bullen FT (1966) Locusts and grasshoppers as pests of crops and pasture—a preliminary economic approach. J Appl Ecol 3(1): 147–168

De Faria MR, de O Almeida D, Magalhaes BP (1999) Food consumption of *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) infected by the fungus *Metarhizium flavoviride* Gams & Rozsypal. An Soc Entomol Brasil 28(1):91–99

Delgado FX, Britton JH, Onsager JA, Swearingen W (1999) Field assessment of *Beauveria bassiana* (Balsamo) Vuillemin and potential synergism with diflubenzuron for control of savanna grasshopper complex (Orthoptera) in Mali. J Invertebr Pathol 73:34–39

Dubovskiy IM, Slyamova ND, Kryukova VY, Yaroslavtseva ON, Levchenko MV, Belgibaeva AB, Adilkhankyzy A, Glupov VV (2012) The activity of nonspecific esterases and glutathione-*S*-transferase in *Locusta migratoria* larvae infected with the fungus *Metarhizium anisopliae* (Ascomycota, Hypocreales). Entomol Rev 92:27–32

Foster RN, Reuter KC, Black L, Britton J (1996) Evaluation of the fungus *Beauveria bassiana* with selected insecticide stressors for control of unconfined rangeland grasshoppers. Arthropod Manag Tests 21:280

Harvey A (1990) Rearing and breeding of locusts in the laboratory. Natural Resources Institute, Chatham

Hassan AEM, Charnley AK (1989) Ultrastructural study of the penetration by *Metarhizium anisopliae* through dimilin-affected cuticle of *Manduca sexta*. J Invertebr Pathol 54:117–124

Horowitz AR, Ishaaya I (2004) Insect pest management—field and protected crops. Springer, Berlin

Hunt VL, Charnley AK (2011) The inhibitory effect of the fungal toxin, destruxin A, on behavioural fever in the desert locust. J Insect Physiol 57:1341–1346

Ibrahim L, Hamieh A, Ghanem H, Ibrahim SK (2011) Pathogenicity of entomopathogenic fungi from Lebanese soils against aphids, whitefly and non-target beneficial insects. Int J Agric Sci 3(3): 156–164

Irigaray FJSC, Marco-Mancebón V, Pérez-Moreno I (2003) The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the twospotted spider mite *Tetranychus urticae*. Biol Control 26:168–173

Ishaaya I (1990) Benzoylphenyl ureas and other selective control agents—mechanism and application. In: Casida JE (ed) Pesticides and alternatives. Elsevier, Amsterdam, pp 363–376

Ishaaya I, Casida JE (1974) Dietary TH 6040 alters cuticle composition and enzyme activity of house fly larval cuticle. Pesticide Biochem Physiol 4:484–490

Ishaaya I, Horowitz AR (1998) Insecticides with novel modes of action: an overview. In: Ishaaya I, Degheele D (eds) Insecticides with novel modes of action, mechanism and application. Springer, Berlin, pp 1–24

Jaronski ST, Goettel MS (1997) Development of *Beauveria bassiana* for control of grasshoppers and locusts. Mem Entomol Soc Can 171:225–237

Johnson DL, Goettel MS, Bradley C, Van der Paauw H, Maiga B (1992) Field trials with the entomopathogenic fungus *Beauveria bassiana* against grasshoppers in Mali, West Africa, July 1990. In: Lomer CJ, Prior C (eds) Biological control of locusts and grasshoppers. CABI, Wallingford, pp 296–310

Keller S (1978) Investigations on the influence of Dimilin (diflubenzuron) on growth and germination of conidia of some insect pathogenic fungi. Anz Schadlingskde Pflanzenschutz Umweltschutz 51:81–83

Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M (2001) Biological control of locusts and grasshoppers. Annu Rev Entomol 46:667–702

Magalhães BP, de Faria MR, Lecoq M, Schmidt FGV, Silva JBT, Frazão HS, Balança G, Foucart A (2001) The use of *Metarhizium anisopliae* var. *acridum* against the grasshopper *Rhammatocerus schistocercoides* in Brazil. J Orthoptera Res 10(2):199–202

Mohan MC, Reddy NP, Devi UK, Kongara R, Sharma HC (2007) Growth and insect assays of *Beauveria bassiana* with neem to test their compatibility and synergism. Biocontrol Sci Technol 17(10):1059–1069

Mondal K, Parween S (2000) Insect growth regulators and their potential in the management of stored-product insect pests. Integr Pest Manag Rev 5:255–295

Morales-Rodriguez A, Peck DC (2009) Synergies between biological and neonicotinoid insecticides for the curative control of the

white grubs *Amphimallon majale* and *Popillia japonica*. Biol Control 51(1):69–180

Nevo D (1996) The desert locust, *Schistocerca gregaria*, and its control in the land of Israel and the near east in antiquity, with some reflections on its appearance in Israel in modern times. Phytoparasitica 24:7–32

Nishimatsu T, Jackson JJ (1998) Interaction of insecticides, entomopathogenic nematodes, and larvae of the western corn rootworm. (Coleoptera: Chrysomelidae). J Econ Entomol 91(2):410–418

Olmert I, Kenneth RG (1974) Sensitivity of the entomopathogenic fungi *Beauveria bassiana*, *Verticillium lecanii* and *Verticillium* sp. to fungicides and insecticides. Environ Entomol 3:33–38

Olson DL, Oetting RD (1999) Compatibility of insect growth regulators and *Beauveria bassiana* (Balsamo) Vuillemin in controlling green peach aphids (Homoptera: Aphididae) on greenhouse chrysanthemums. J Entomol Sci 34:286–294

Ouedraogo RM, Goettel MS, Brodeur J (2004) Behavioral thermoregulation in the migratory locust: a therapy to overcome fungal infection. Oecologia 138:312–319

Prior C, Greathead DJ (1989) Biological control of locusts: the potential for the exploitation of pathogens. FAO Plant Prot Bull 37:37–48

Prior C, Streett DA (1997) Strategies for the use of entomopathogens in the control of desert locust and other Acridoid pests. Mem Entomol Soc Can 171:5–25

Purwar JP, Sachan GC (2006) Synergistic effect of entomogenous fungi on some insecticides against Bihar hairy caterpillar *Spilarctia oblique* (Lepidoptera: Arctiidae). Microbiol Res 161(1):38–42

Quintela ED, McCoy CW (1997) Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid. Environ Entomol 26:1173–1182

Reuter KC, Foster RN, Tompkins MT, Bradley C, Britton J, Underwood N, Black LR, Vigil E, Daye G, Radsick B, Fuller B, Brinkman M, Kahler E (1993) Development of the fungus *Beauveria bassiana* as a bio-insecticide for grasshoppers on rangeland. 1993 Annual Report, Grasshopper Integrated Pest Management Project. USDA/APHIS/PPQ, Boise, pp. 233–238

Reuter KC, Foster RN, Black L, Britton J (1996) Laboratory evaluation of *Beauveria bassiana* with an added chemical stressor. Arthropod Manag Tests 21:415–416

Russell CW, Ugine TA, Hajek AE (2010) Interactions between imidacloprid and *Metarhizium brunneum* on adult Asian longhorned beetles (*Anoplophora glabripennis* (Motschulsky)) (Coleoptera: Cerambycidae). J Invertebr Pathol 105(3):305–311

Serebrov VV, Kiselev AA, Glupov VV (2003) The study of some synergistic factors between entomopathogenic fungi and chemical insecticides. Mikol Fitopatol 37(1):76–82

Serebrov VV, Gerber ON, Malyarchuk AA, Martemyanov VV, Alekseev AA, Glupov VV (2006) Effect of entomopathogenic fungi on detoxification enzyme activity in greater wax moth *Galleria mellonella* L. (Lepidoptera, Pyralidae) and role of detoxification enzymes in development of insect resistance to entomopathogenic fungi. Biol Bull 33:581–586

Tingle CCD (1996) Sprayed barrier of diflubenzuron for control of the migratory locust *Locusta migratoria capito* (Sauss.) [Orthoptera:Acrididae] in Madagascar: short-term impact on relative abundance of terrestrial non-target invertebrates. Crop Prot 15:579–592

Zibaee A, Bandani AR, Tork M (2009) Effect of the entomopathogenic fungus, *Beauveria bassiana*, and its secondary metabolite on detoxifying enzyme activities and acetylcholinesterase (AChE) of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae). Biocontrol Sci Tech 19:485–498