BIOLOGICAL CONTROL

Screening of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) Isolates Against Nymphs of *Bemisia tabaci* (Genn.) Biotype B (Hemiptera: Aleyrodidae) with Description of a New Bioassay Method

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Avaliação de Isolados de *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) Sobre Ninfas do Biótipo B de *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) com Descrição de Nova Metodologia de Bioensaio

RESUMO – Estabeleceu-se uma metodologia de bioensaio com o emprego de folhas de melão como substrato para ovos e ninfas do biótipo B de *Bemisia tabaci* (Genn.). A formação de raízes no pecíolo foliar imerso em água de torneira garantiu turgor e coloração normais de folhas por um período de 20 a 25 dias, possibilitando a realização de testes com fungos entomopatogênicos. Avaliou-se a virulência de 50 isolados de *Beauveria bassiana* (Balsamo) Vuillemin visando ao desenvolvimento de um bioinseticida para o controle de ninfas de *B. tabaci* em lavouras de melão. Folhas de melão foram infestadas por 18 casais de adultos do biótipo B durante 26 horas. Ninfas de 1º estádio foram selecionadas através de observação em microscópio estereoscópico cerca de 4-5 dias após a remoção dos adultos. A aplicação dos isolados fúngicos foi feita com o emprego de uma torre de pulverização. O número médio (± EP) de propágulos infectivos depositados sobre a superfície foliar foi de $1,2x10^4 \pm 9,12x10^2$ conídios por cm^2. Para a testemunha aplicou-se uma solução de Tween 80 a 0,1%. Foram realizadas 3-4 repetições por tratamento. A incubação foi realizada em incubadora regulada para 27±1ºC, 70±5% UR e fotofase de 12 horas. Avaliações do número de ninfas mortas foram realizadas aos sete e 14 dias após a pulverização. A mortalidade média na testemunha aos 14 dias foi de 1,2%, com 94,4% dos indivíduos atingindo o estágio adulto. A mortalidade média de ninfas nas avaliações realizadas sete dias após a pulverização atingiu um valor máximo de 25,7%. A mortalidade média decorridos 14 dias da pulverização variou de 6,1% a 92,3%.

PALAVRAS-CHAVE: Insecta, mosca branca, controle biológico, fungo entomopatogênico.

ABSTRACT – A bioassay method that uses melon leaves as substrate for eggs and nymphs of the biotype B of *Bemisia tabaci* (Genn.) was established. Root formation of petioles immersed in tap water guaranteed the turgidity and normal coloration of leaves for a period of 20 to 25 days, enabling the execution of tests with entomopathogenic fungi. The virulence of 50 isolates of *Beauveria bassiana* (Balsamo) Vuillemin was assessed, as an initial action of a research project that aims the development of a bioinsecticide for controlling *Bemisia tabaci* nymphs in melon crops. Melon leaves were infested with 18 couples of biotype B adults for 26 hours. First-instar nymphs were selected through microscopic observation approximately 4-5 days after adults removal. Application of fungal isolates was performed with a spray tower. Average number (± SEM) of infective propagules deposited on leaf surface was $1.2x10^4 \pm 9.12x10^2$ conidia per cm^2. For the control it was applied a Tween 80 solution at 0.1%. Three or four replicates were performed per treatment. Incubation was in chamber regulated to 27±1ºC, 70±5% RH and 12 hours photophase. Assessments of dead nymphs were performed at seven and 14 days following spraying. Average mortality in the control treatment was 1.2% at day 14, and 94.4% of the nymphs became adults. Average nymphal mortality at day seven post-spray reached a maximum value of 25.7%. Average mortality at day 14 post-spray varied from 6.1% to 92.3%.

KEY WORDS: Insecta, silverleaf whitefly, biological control, entomopathogenic fungus.

Since its introduction in Brazil in the beginning of the last decade (Lourenção & Nagai 1994), the silverleaf whitefly [biotype B of *Bemisia tabaci* (Genn.)] has been reported on a variety of crops. Consistent and expressive damages have been observed in tomato and melon, especially in the Northeastern region. Chemical control of this pest has been the only alternative measure available for growers. The possibility of resistant pest populations to appear as a consequence of intensive use of chemicals has stimulated studies on integrated pest management strategies, in which biological control is considered a relevant tool.

Predators, parasitoids and fungi have been commercially used in many countries for biological control of aleyrodids, emphasis being on *Trialeurodes vaporariorum* (Westwood), the greenhouse whitefly (Cranshaw *et al.* 1996, Heinz 1996, Lenteren *et al.* 1997). In non-protected crops the potential of parasitoids and, above all, entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith has been assessed for biotype B of *B. tabaci* control (Wraight *et al.* 1996, Akey & Henneberry 1998, Wraight *et al.* 1998).

In Brazil, there are reports of the occurrence of fungi on *Bemisia* spp. (Lacey *et al.* 1996, Lourenção *et al.* 1999, D.R. Sósa-Gomez, pers. communication). In some cases, the epizootics observed were able to cause drastic reductions of pest populations, but the slow dissemination of the fungus usually is not sufficient to avoid the economic threshold from being reached. Also, an epizootic is strongly influenced by environmental conditions and cultural practices, making its occurrence uncertain. Therefore, the better approach for the microbial control of whiteflies seems to be the use of bioinsecticides. Studies aiming at determining the potential of Brazilian fungal isolates toward *Bemisia* spp. were not carried out so far. The recent introduction of biotype B in the country, associated to the inaccessibility of a practical and accurate bioassay method could explain, at least partially, the complete lack of applied research on this area.

The availability of a bioassay that allows assessment of different fungal isolates is the first step in order to develop a biopesticide. In this paper a bioassay method that uses melon leaves kept in tap water as substrate for the silverleaf whitefly is described. Furthermore, data on the virulence of 50 isolates against nymphs of this pest are presented.

Material and Methods

Insects. Adults of *B. tabaci* were obtained in a greenhouse colony maintained at Embrapa Recursos Genéticos e Biotecnologia (Cenargen), Brasília-DF. Identity of biotype B insects was confirmed through techniques of molecular biology (Lima *et al.* in press).

Fungal Isolates. Although natural infections of aleyrodids by *B. bassiana* are uncommon, this pathogen was selected for this study, among other factors, for the ease of mass production. Diphasic fermentation may result in a 4-fold spore yield when compared to the fungus *P. fumosoroseus* (Wraight *et al.* 1998). All 50 isolates studied were *B. bassiana* isolated from insects belonging to orders Coleoptera (18), Hemiptera / suborder Heteroptera (21), Hemiptera / suborder Homoptera (9), Hymenoptera (1) and Lepidoptera (1), according to Table 1. Isolates were collected in Brazil (45), Argentina (3), France (1) and USA (1), between 1973 and 1994, and preserved in liquid nitrogen at the culture collection maintained at Cenargen. Conidia were harvested approx. 18 days after seeded on complete medium. Germination tests were carried out and results used for adjusting the concentration to $4.0x10^6$ viable conidia / ml.

Bioassay. Melon leaves, genotypes Gaúcho and AF-682, were removed from plants 40-55 days post-planting and put in glass vials (7 x 3.5 cm), with the petiole immersed in tap water. Leaves were individually kept inside plastic cups (14 x 9 cm) with two screened windows in the lateral of the cups, in chamber regulated to 27±1ºC, 70±5% RH and 12h photophase. Each leaf was infested with 18 adult couples of *B. tabaci* biotype B for 26h. Approximately 4-5 days after removal of adults, the majority of insects were at the 1st nymphal instar , and were selected through observations under a dissecting microscope and then marked with a spot nearby their position. This methodology for standardization of insect age is used at University of Vermont (M. Brownbridge, pers. communication). A Potter Spray Tower (Burkard Manufacturing, Hertfordshire, England) working at 15 PSI, was used for spraying 2 ml of conidial suspension on the underside of each leaf. Three or four replicates were performed per treatment. The number of conidia deposited per unit of area was calculated by spraying the fungal suspension and collecting it in a glass Petri dish with internal diameter of 7.4 cm containing 9 ml of 0.1% Tween 80. Total number of conidia sprayed within the Petri dish area was determined using a Neubauer chamber. At the set conditions, an average (±SEM) of $1.2x10^4$±$9.12x10^2$ conidia were applied per cm^2. In the control treatment, a 0.1% Tween 80 solution was sprayed.

Mortality Assessment. Incubation conditions were the same mentioned above for root formation. Measurements taken within the plastic cup containing plants with a digital thermo-hygrometer (Templec, China) showed values that followed parameters set for the chamber. Assessments were performed at seven and 14 days post-spraying, when the number of adults and number of live and dead nymphs were recorded. Data observed at days 7 and 14 post-spraying failed the normality test (Kolmogorov-Smirnov), indicating a non normal frequency distribution. Therefore, it was used a Kruskal-Wallis non-parametric analysis of variance followed by a non-parametric multiple comparison test (Dunn´s Method), in which all isolates were compared to the control treatment using the software SigmaStat (Kwo *et al.* 1992). For day 14, isolates statistically different from the control treatment were compared among themselves, using CG 224 as the reference isolate.

Results and Discussion

A bioassay method that enables the use of melon leaves as substrate for eggs and nymphs of the biotype B of *B. tabaci*

Table 1. Isolates of *Beauveria bassiana* assessed against *Bemisia tabaci*, biotype B.

Order	Code	Host	Family	Date	Origin
Coleoptera	CG 007	*Leptinotarsa decemlineata*	Chrysomelidae	14/09/78	USA
	CG 011	*Sternechus subsignatus*	Curculionidae	-	Brazil
	CG 015	*Lebia concinna*	Carabidae	-	Brazil
	CG 017	*Hypothenemus hampei*	Scolytidae	24/04/84	Brazil
	CG 079	*Maecolaspis monrosi*	Chrysomelidae	08/04/86	Argentina
	CG 082	*Diabrotica speciosa*	Chrysomelidae	28/03/86	Argentina
	CG 138	*Cosmopolites sordidus*	Curculionidae	-	Brazil
	CG 152	-	Chrysomelidae	12/03/84	Brazil
	CG 207	*Chalcodermus aeneus*	Curculionidae	06/01/82	Brazil
	CG 208	*Chalcodermus aeneus*	Curculionidae	06/01/82	Brazil
	CG 212	-	Cerambydae	29/04/82	Brazil
	CG 213	*Crimissa* sp.	Chrysomelidae	01/01/92	Brazil
	CG 215	-	Tenebrionidae	01/01/92	Brazil
	CG 218	*Cerotoma arcuata*	Chrysomelidae	01/01/92	Brazil
	CG 220	*Cerotoma arcuata*	Chrysomelidae	01/01/92	Brazil
	CG 223	*Chalcodermus aeneus*	Curculionidae	01/01/92	Brazil
	CG 224	*Cerotoma arcuata*	Chrysomelidae	01/01/92	Brazil
	CG 227	*Crimissa* sp.	Chrysomelidae	01/01/92	Brazil
Hemiptera, suborder Heteroptera	CG 010	*Nezara viridula*	Pentatomidae	-	Brazil
	CG 013	*Nezara viridula*	Pentatomidae	-	Brazil
	CG 014	*Podisus* sp.	Pentatomidae	-	Brazil
	CG 020	*Nezara viridula*	Pentatomidae	01/01/82	Brazil
	CG 021	-	Pentatomidae	01/01/73	France
	CG 022	*Nezara viridula*	Pentatomidae	01/01/83	Brazil
	CG 023	*Piezodorus guildinii*	Pentatomidae	01/01/86	Brazil
	CG 024	*Euschistus heros*	Pentatomidae	01/01/86	Brazil
	CG 074	*Tibraca limbativentris*	Pentatomidae	20/12/82	Brazil
	CG 077	-	Pentatomidae	26/01/82	Brazil
	CG 078	*Nezara viridula*	Pentatomidae	01/06/86	Argentina
	CG 080	-	Pentatomidae	16/03/83	Brazil
	CG 081	*Nezara viridula*	Pentatomidae	02/04/84	Brazil
	CG 083	*Nezara viridula*	Pentatomidae	11/07/86	Brazil
	CG 084	*Nezara viridula*	Pentatomidae	15/03/85	Brazil
	CG 145	*Leptopharsa heveae*	Tingidae	01/01/89	Brazil
	CG 261	*Edessa meditabunda*	Pentatomidae	04/09/92	Brazil
	CG 306	*Thyanta perditor*	Pentatomidae	26/09/90	Brazil
	CG 470	*Podisus* sp.	Pentatomidae	28/04/84	Brazil
	CG 474	*Podisus sp.*	Pentatomidae	01/01/84	Brazil
	CG 488	*Oebalus poecilus*	Pentatomidae	26/03/86	Brazil
Hemiptera, suborder Homoptera	CG 001	*Deois flavopicta*	Cercopidae	23/08/88	Brazil
	CG 004	*Deois flavopicta*	Cercopidae	23/08/88	Brazil
	CG 018	*Deois schach*	Cercopidae	01/01/81	Brazil
	CG 069	*Deois flavopicta*	Cercopidae	25/04/88	Brazil
	CG 136	-	Cercopidae	-	Brazil
	CG 149	*Deois flavopicta*	Cercopidae	04/05/82	Brazil
	CG 154	*Deois flavopicta*	Cercopidae	01/12/83	Brazil
	CG 234	-	Cicadellidae	16/03/83	Brazil
	CG 432	-	Membracidae	27/06/94	Brazil
Hymenoptera	CG 067	-	-	01/04/88	Brazil
Lepidoptera	CG 151	*Spodoptera frugiperda*	Noctuidae	06/01/83	Brazil

was established. Root formation of leaf petioles immersed in tap water guarantees the turgidity and normal coloration for a period of 20 to 25 days, wich is long enough for the bioassay purpose. Average mortality of nymphs at seven and 14 days in the control was 0 and 1.2%, respectively, with 94.4% of individuals reaching the adult stage at day 14, indicating a satisfactory condition of the substrate. Until recently, bioassays for assessment of fungi against aleyrodids were based on complex and laborious or excessively artificial techniques. In some cases, experiments were carried out using whole plants. In the method employed by Wraight *et al.* (1998), a portion of non-rooted *Hibiscus* leaves lost the turgid state and presented clorotic spots after 4-5 days, and mortality in the control treatment ranged from two to 26.9%. In another method, nymphs detached from leaves were kept on glass slides at 100% RH for at least 12h after spraying (Landa *et al.* 1994). A bioassay technique based on root formation of cabbage leaves in specific solutions was recently published by Lacey *et al.* (1999). A simpler method, based on root formation of bean leaves maintained in Oasis®cubes embedded in water, was developed at the University of Vermont (M. Brownbridge - pers. communication). However, when tested for Brazilian bean varieties it did not work properly (M. Faria - unpublished) and, therefore, nymphs could not be reared for many days.

In the present study, nymphs treated with *B. bassiana* dried and/or presented reddish coloration upon death, as a likely consequence of oosporein production (Eyal *et al.* 1994, Wraight *et al.* 1998). It was observed a significant variability among tested fungal isolates concerning virulence against 1st instar nymphs of the biotype B. Seven days after-spraying, mortality rates (Fig. 1) were lower than at 14 days (Fig. 2). This fact was expected, since entomopathogenic fungi have a slow mode of action. At the day 7, nine out of 50 isolates caused mortality on nymphs statistically different from the control treatment ($P<0.001$). At the day 14, the number of isolates statistically different from the control treatment reached 16 ($P<0.001$). When the top 15 most virulent isolates were compared to the 16th one (CG 224) at the day 14, the nearly 90% nymphal mortality caused by CG 136 and CG 149 isolates showed statistically different ($P<0.001$).

Isolates originally obtained from insects belonging to the same taxon as the whitefly (Order Hemiptera, suborder Homoptera) were more virulent then the others. At day 7 post-spraying, four out of nine isolates that were better than the control treatment belonged to suborder Homoptera. At day 14, eight out of 16 isolates were from insects belonging to suborder Homoptera, including the five most virulent ones. In other words, 88.9% of tested isolates from suborder Homoptera ranked among the top 16. For Coleoptera, Heteroptera, Hymenoptera and Lepidoptera, the rates were 38.9, 4.8, 0 and 0%, respectively. Based on the observed values, surveys of *B. bassiana* isolates collected from homopterans seems to be a suitable approach for silverleaf whitefly microbial control programs.

The lack of a standardized bioassay method, including substrate for the host, developmental stage of the insect, concentration and inoculation technique of the fungus, incubation conditions and the way data are presented, impede results obtained in most publications to be compared with results presented in this paper. In bioassays with nymphs of the biotype B of *B. tabaci*, data are expressed as LC_{50} values (Wraight *et al.* 1998) or as an index based on the developmental stage of the fungus on the host (Landa *et al.* 1994) and, less common, as percentage mortality. For example, when conidia or blastospores of two *P. fumosoroseus* isolates were applied onto eggs and 2nd instar nymphs of the biotype B using rooted cabbage leaves as substrate, mortality rates ranged from eight to 20% for eggs, and from 27 to 68% for nymphs (Lacey *et al.* 1999). In the referred paper, spraying was performed with a sprayer tower delivering $3.8x10^3$ conidia / cm^2, which is 1/3 of the dose used in the present study.

In preliminary bioassays, CG 136 and CG 149 were proved more virulent than some fungal isolates that are the active ingredient of bioinsecticides recomended for whitefly control (M. Faria, S. Vicentini & M.R.V. Oliveira - unpublished). Studies aiming at assessing the efficiency of *B. bassiana* on other developmental stages of the B biotype, as well as studies to determine the compatibility of selected fungal isolates with chemicals and suitability of different bioinsecticide formulations, are being carried out.

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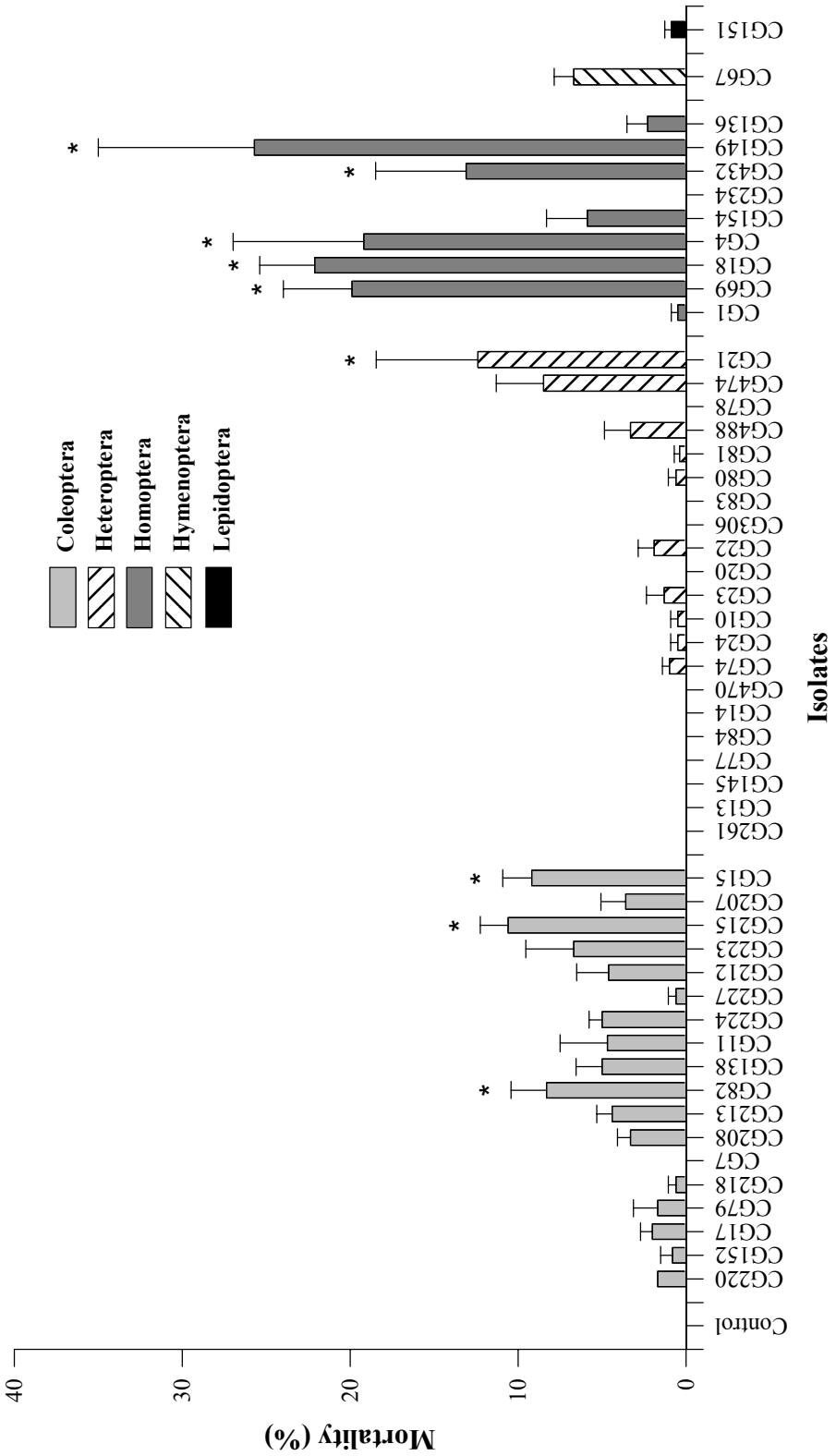

Figure 1. Mortality of *Bemisia tabaci* nymphs infected with different isolates of *Beauveria bassiana* seven days after spraying. Treatments with * above the bar (average ±SEM) are significantly different from control ($P < 0.05$; Dunn's Method).

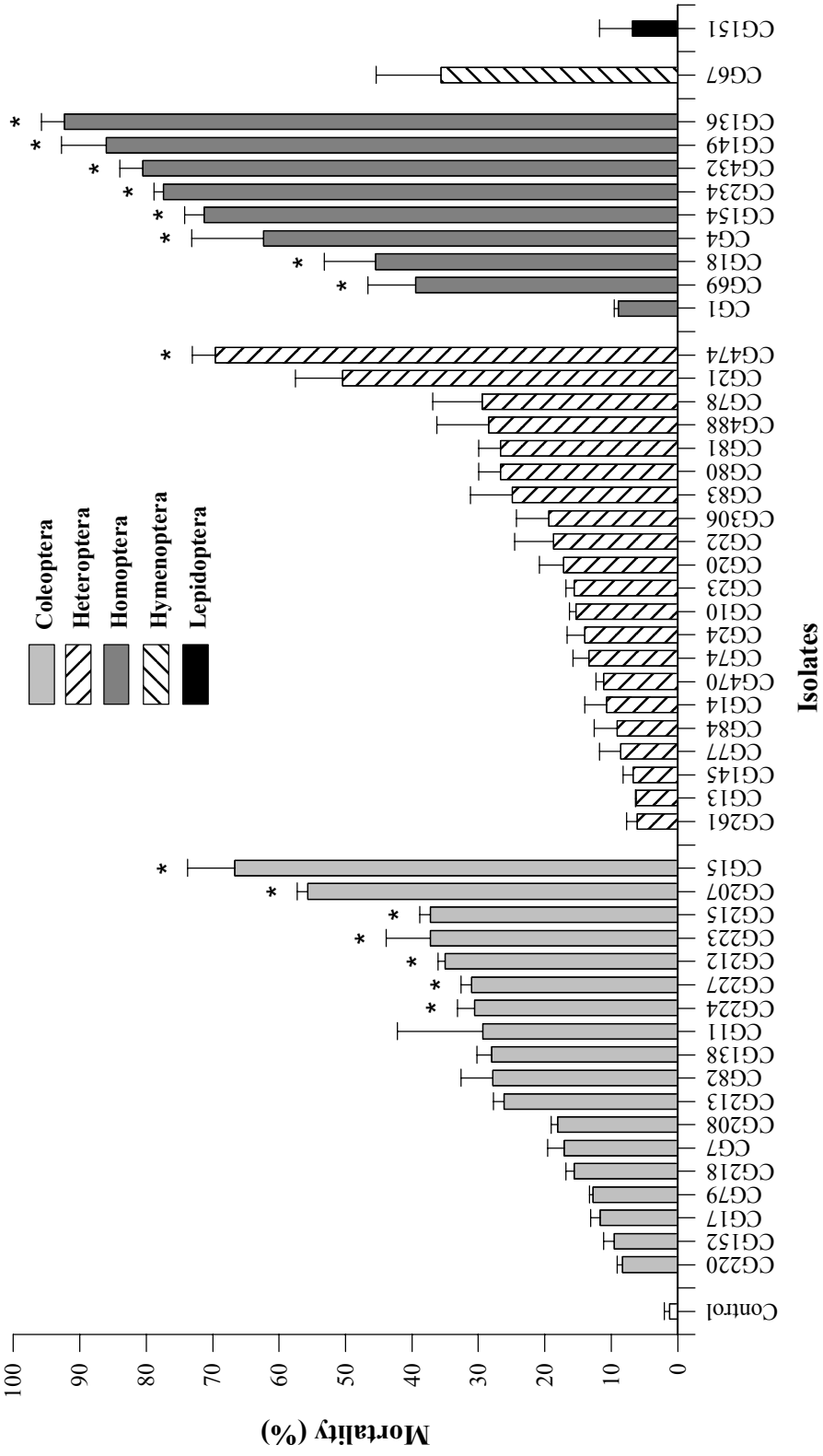

Figure 2. Mortality of *Bemisia tabaci* nymphs infected with different isolates of *Beauveria bassiana* 14 days after spraying. Treatments with * above the bar (average ±SEM) are significantly different from control ($P < 0.05$; Dunn's Method).

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