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Potential use of *Beauveria bassiana* in combination with *Scleroderma guani* for improved control of *Apriona germari*

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Abstract: *Beauveria bassiana*, an important entomogenous fungus, and *Scleroderma guani*, a hymenopteran parasitoid, are the two main biological control agents for managing the spread of *Apriona germari*. The aim of the present study was to assess the potential value of combining the two agents for the purpose of controlling *A. germari*. First, the relative virulence of *B. bassiana* against both *A. germari* and *S. guani* was determined. The results showed that among the seven strains of *B. bassiana*, isolate BI05, which was isolated from another cerambycid, was far more virulent against *A. germari* (mortality 71.75%) than it was against *S. guani* (mortality 34.82%). The parasitic efficiency of *S. guani* against the larvae of *A. germari* was significantly affected by the size of the host and the host/parasitoid ratio. In this study, third instar *A. germari* larvae and a 1:3 host/parasitoid ratio of inoculation were used to evaluate the parasitization rate achieved by *S. guani* that were previously exposed to *B. bassiana* BI05. The mortality of *A. germari* treated with the previously inoculated *S. guani* was 73.30%. This was significantly greater than that of *S. guani* alone or *B. bassiana* alone (57.20% and 46.21%, respectively). The results indicated that the efficiency of both *S. guani* and *B. bassiana* are improved when used in combination, and this is promising for future control of *A. germari*.

Key words: *Beauveria bassiana*; *Scleroderma guani*; *Apriona germari*; Combination; Control

32 Introduction

33 *Apriona germari* Hope (Coleoptera: Cerambycidae) is commonly found in China,
34 Burma, India, Japan, Korea, Pakistan, and Vietnam. In these countries *A. germari*
35 infests a large number of broad-leaved trees, including maple, birch, elm, poplar,
36 horse chestnut, platanus, willow, and others (Li 2010; Hussain and Buhroo 2012;
37 Hussain 2015). The beetle completes most of its life cycle inside the host tree. Adult
38 beetles emerge from host trees between July and October and feed on twigs of *Morus*
39 *alba*, *Broussonetia papyrifera* and *Cudrania tricuspidata*. Females mate and lay eggs
40 10 to 15 days after emergence. Eggs are laid under the bark in oviposition slits
41 chewed out by the female. After 8 to 15 days, the eggs hatch and the young larvae
42 begin tunneling upward from the inner bark-sapwood interface for about 10 mm. They
43 then tunnel into the wood where their feeding slowly destroys the structural integrity
44 of host trees. Host trees are slowly killed over a 3- to 5-year period, although the time
45 period can be longer (Huang et al. 2015; Liu et al. 2002).

46 The use of chemicals for the control of arthropod pests can be problematic due to the
47 potential for both environmental contamination and resistance development. As a
48 result of these challenges, there is increasing interest in nonchemical alternatives.

49 Entomopathogenic fungi and natural enemies are two likely biological control agent
50 candidates for augmentation within the context of integrated pest management (IPM)
51 (Ekesi et al. 2007; Bukhari et al. 2011; Lacey et al. 2011; Niu et al. 2014;
52 Tamayo-Mejía et al. 2014; Yang et al. 2013).

53 *Beauveria bassiana* (Bals.) Vuill (Ascomycota: Hypocreales) is the most commonly

54 used fungus for control of insect pests and has served as the basis for a number of
55 commercially available mycoinsecticides (Hussein et al. 2012; Prasad and Syed
56 2010; Wraight et al. 2010). Many research studies have been conducted to determine
57 whether it is possible to control *A. germari* with *B. bassiana* (Li et al. 2011; Lu et al.
58 2013b). A serious concern that arose during the course of this body of research was
59 that the strains which performed well indoors did not achieve comparable results in
60 the field (Ding et al. 2000). This difference may be related to the virulence of the
61 strain, resistance, environmental conditions, or some combination of those factors
62 (Chen et al. 2012). Furthermore, because *A. germari* larvae live in the trunk of the
63 host tree, *B. bassiana* may not effectively contact the *A. germari* hosts and produce
64 mycosis. This has become a major limiting factor in *B. bassiana* field applications
65 (Liu et al. 2007).

66 *Scleroderma guani* Xiao et Wu (Hymenoptera: Bethyridae) is indigenous to China and
67 has been used widely for biological control of forest pests as it can parasitize the
68 larvae and pupae of more than 50 species of wood-boring insects belonging to 22
69 families in 3 orders (Li et al. 2015; Chen and Cheng 2000). This wasp has been
70 considered the most effective biological control agent of *Semanotus bifasciatus*
71 (Coleoptera: Cerambycidae), *Saperda populnea* (Coleoptera: Cerambycidae),
72 *A. germari* (Coleoptera: Cerambycidae), *Monochamus alternates* (Coleoptera:
73 Cerambycidae), *Acantholyda posticalis* (Hymenoptera: Pamphiliidae), *Tenebrio*
74 *molitor* (Coleoptera: Tenebrionidae), *Kytorrhinus immixtus* (Hymenoptera:
75 Bruchidae), and other pests (Wu et al. 2013). *Scleroderma guani* is an idiobiont (Lu et

al. 2013a) with a series of stereotypical host selection behaviors performed in a characteristic sequence (Hu et al. 2012). It uses olfactory, visual, or acoustic signals to locate concealed hosts and parasitize them (Torres et al. 2005; Wölfling and Rostás 2009).

In a study of *S. guani* behavior when searching for *M. alternatus*, Yao and Yang (2008) found females of *S. guani* search for hosts by crawling quickly around tree trunks. Once they find host frass, they follow it to the host tunnel where they locate host larvae under bark (Yao and Yang 2008). Females of *S. guani* prefer to parasitize mid-instars; late instars have strong defensive behaviors and are difficult to parasitize, whereas early instars are vulnerable to mortality during parasitism (Ma et al. 2010).

Technology on the use of artificial breeding and release in forests of *S. guani* against pest Lepidoptera has been developed (Zhou et al. 2005; Wang et al. 2015).

For a long time, the research on biological control mainly focused on the use of each agent separately. This is in part because there was incomplete knowledge of the interactions between biocontrol agents. In more recent years advances in our understanding of the ways that biocontrol agents interact are expanding, so that pests can be more effectively controlled using entomopathogenic fungi and parasitoids (Dean et al. 2012; Furlong and Pell 2005; Furlong and Pell 2000). In the Netherlands and former Czechoslovakia, the combined use of *Aschersonia aleyrodis* (Hymenoptera: Cordycipitaceae) and *Encarsia formosa* (Hymenoptera: Aphelinidae) against *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) achieved an 85% control effect (Ramakers and Samson 1984). However, the researchers who achieved these results

determined that the control rate for *E. formosa* alone was only 49% with a high host/parasitoid ratio, and that 3 applications of *A. aleyrodis* were needed to achieve an acceptable result (Ramakers and Samson 1984).

In China, *B. bassiana*, tachinid flies, and parasitic wasps were used in combination to control *Dendrolimus punctatus* (Li et al. 1996). The results of this research showed that the use of the three agents in combination merely achieved an additive effect rather than synergism or antagonism. Five entomogenetic fungi including *B. bassiana* failed to infect eggs of *D. punctatus*, and did not affect the parasitism by *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) (Li et al. 1996). By comparing the mortality of fourth instar *M. alternatus* using *B. bassiana* and *S. guani*, Liu et al. (2007) found that on the seventh day, the highest mortality achieved using only *B. bassiana* or *S. guani* individually was 26.3% and 55.0%, respectively; however, by inoculating *S. guani* with *B. bassiana*, mortality was 94.4 %. Thus, using the two biocontrol agents in combination may be better for control of *M. alternates* (Liu et al. 2007).

Apriona germari is known as a chronically damaging pest and its inaccessibility in woody hosts makes it difficult from a practical standpoint to implement control measures. *Scleroderma guani* can actively search for hosts, which overcomes the inability of *B. bassiana* to initiate contact with *A. germari* larvae inside trees. We hypothesized that *S. guani* inoculated with *B. bassiana* seek out *A. germari* larvae in their larval tunnels, and therefore have the ability to destroy the ecological defense of *A. germari* larvae. This in turn would lead to an epidemic of *B. bassiana*, which

forms the basis for a new approach to the biological control of *A. germari*. There is little research on the susceptibility of *S. guani* to *B. bassiana*. If there were negative effects on the *S. guani*, the simultaneous use of entomopathogenic fungi with parasitoids as biological control agents might be complicated or prevented. The aim of the present study was to evaluate the potential of the combined use of *B. bassiana* and *S. guani* to control *A. germari*. First, the relative virulence of *B. bassiana* isolates against both *A. germari* and the *S. guani* was determined. Next, the pathogenicity of *S. guani* inoculated with *B. bassiana* against *A. germari* was compared with that of *B. bassiana* and *S. guani* used separately against *A. germari* in order to compare the effectiveness of the combined approach in the control of *A. germari* to that of the existing separate approaches.

Materials and methods

Fungi

Seven isolates of *B. bassiana*, which are stored at -80°C at the Department of Forest Protection, College of Forestry, Agricultural University of Hebei, were used for the experiments. The details of the tested isolates are listed in Table 1. The isolates were cultured on potato dextrose agar (PDA) medium in 9cm petri dishes and kept at 25°C to allow fungal growth and conidial production. Conidia, harvested by scraping, were suspended in 0.05% Tween-80 and vortexed for 10 minutes to produce a homogenous suspension. The spore concentration was determined using a haemocytometer. Conidial viability prior to experiment was determined and assured to be > 90% using the technique of Inglis et al. (2012).

142 ***Apriona germari***

143 *Apriona germari* adults that were captured in the field were bred with twigs of *Morus*
144 *alba* L. in a cage to facilitate laying. Eggs were removed from the oviposition slits and
145 placed into a petri dish lined with sterile filter paper for hatching. The hatched larvae
146 were transferred to the artificial grooves in 30 to 40cm branches of *Populus tomentosa*
147 Carr. Both ends of the branches were wrapped with Parafilm to prevent water loss.
148 The artificial groove was made as follows. A U-shaped scar on the phloem was cut to
149 the depth of the xylem, with the bottom still linked to the branch. Under the cut bark,
150 a groove was made with a carving knife. The size of groove was determined based on
151 the size of insect body, meaning that each groove was a little larger than the insect
152 body. A larva was put into each groove and then covered with the cut bark and
153 Parafilm; the larvae then tunneled into the branches themselves. The instar was
154 determined by the width of head capsule according to Le et al. (2014).

155 ***Scleroderma guani***

156 *Scleroderma guani* adults were provided by the Propagation and Breeding Center of
157 Natural Enemies, Forest Protection Department, Zhangjiakou City. To obtained
158 *S. guani* adults, 5 mated *S. guani* females were confined with 2 third instar larvae of
159 *Saperda populnea* in a glass vial (5.5 cm height × 2.0 cm diameter) and kept in an
160 artificial climatic chamber at 26.0 °C, 16:8hr L:D photoperiod, and 58% RH for
161 collection of adults. Once the 2nd generation of *S. guani* adults emerged, mated
162 females were collected for the subsequent experiments.

163 **Pathogenicity of *B. bassiana* to *A. germari* larvae**

Thirty third instar *A. germari* larvae were tested against each isolate. Inoculations were performed by immersing the larvae in the conidial suspension of 1×10^8 conidia/mL for 30 seconds. Excess conidial suspension was removed by placing inoculated larvae onto filter paper. Thirty control larvae were immersed in distilled water plus 0.05% Tween-80 in the same manner as treated larvae. Treated and control larvae were reared in the artificial grooves of poplar to simulate the natural habitat of *A. germari* using the above method. The experiment was repeated 3 times for each isolate. Mortality was recorded daily for 8 days. The death of *A. germari* larvae was confirmed by a halt in production of frass. The death due to *B. bassiana* was determined by cutting into the branch 5 days after death to locate the dead larvae and inspect white hyphae on its surface.

Pathogenicity of *B. bassiana* to *S. guani*

The three fungal isolates with the highest mortality from the above bioassay were used for further study to determine virulence to adult *S. guani*. To inoculate healthy *S. guani* with *B. bassiana*, ten *S. guani* were placed on the surface of a full-grown colony of *B. bassiana* on a 9cm petri plate for 5 minutes. The amount of *B. bassiana* carried by *S. guani* was determined using the following method. Having crawled on the surface of a colony full of conidia for 5 minutes, 10 *S. guani* were washed with distilled water plus 0.05% Tween-80 and vortexed for 10 minutes to spread the conidial and produce a homogenous suspension. The spore concentration was determined using a haemocytometer, and this concentration was then divided by the total number of wasps washed per sample, in order to calculate the mean number

of spores per wasp. Using this method, individual *S. guani* were determined to carry approximately 1×10^6 conidia. The experiment was replicated 3 times. For each isolate, 30 *S. guani* individuals were used. The inoculated and control adults were placed in a U-shape tube for 36 hours, then transferred to another tube containing one freshly-hatched, rinsed with sterile water larva of *A. germari*. The tubes containing *S. guani* and *A. germari* were incubated in an artificial climate chamber (26°C, 16:8hr L:D photoperiod, and 58% RH), and the mortality was monitored daily. The experiment was repeated 3 times. In this experiment, total mortality was recorded because there was no outward growth of hyphae or spores on the surface of dead *S. guani*. So, the Abbott correction for control mortality was used to obtain corrected mortality.

Parasitism of *S. guani* on *A. germari* larvae

The *A. germari* larvae were reared in the previously described artificial grooves of *P. tomentosa*, with 5 grooves in each branch and one larva for each groove. They were then transferred to a glass container measuring 15 cm in diameter and 40 cm in height. Each container had 6 branches with 5 grooves per branch, for a total of 30 larvae per container. At the same time, mated female *S. guani* were placed into the glass container, and the container was covered with gauze cloth to prevent escape. The following inoculation method was used. The U-shaped tube with the designated number of healthy female *S. guani* was fixed to the poplar branch with the tube mouth inclined upward. The cotton cover was removed to allow the *S. guani* to search out the host autonomously.

The third, fourth, and fifth instar larvae of *A. germari* were used in the test. For each stage of instar larvae, 5 host/parasitoid inoculation ratios (1:1, 1:2, 1:3, 1:4, and 1:5) and one control without the *S. guani* were designed. One treatment of the test took place in one glass container and was repeated 3 times. The experiment was conducted under ambient laboratory conditions (26°C, 16:8hr L:D photoperiod, and 58% RH). Twenty-one days later, the poplar branches were dissected and total mortality of *A. germari* was recorded. Abbott correction for control mortality was used to obtain corrected mortality.

Parasitism by *S. guani* previously inoculated with *B. bassiana*

An isolate of *B. bassiana* that caused the highest mortality to *A. germari* while at the same time causing low mortality to *S. guani* was chosen for this experiment. The *S. guani* were inoculated with *B. bassiana* prior to the test using the inoculation method previously described. Each treatment involved 30 *A. germari* larvae. Controls consisted of *B. bassiana* alone, *S. guani* alone, and an untreated *A. germari* larvae. Because the previous test investigating 1:3 host:parasitoid ratio in third instar larvae showed adequate parasitism, this ratio was used for testing the combination of parasitoid and fungus. Using a similar setup as the above, six poplar branches carrying five third instar *A. germari* larvae in each branch were placed in each arena. In the first treatment, 90 *S. guani* inoculated with *B. bassiana* were added. In the second treatment, 90 *S. guani* alone were added. In the third treatment, 1mL of *B. bassiana* spore suspension of 3.9×10^6 conidia/mL was injected into the bore holes of the trunk.

For the untreated control, only distilled water was injected. Twenty-one days later, the branches were cut to inspect the mortality rates due to *B. bassiana* and *S. guani*, respectively. To determine if mortality was caused by *B. bassiana* or *S. guani*, larvae were observed for the presence of surface hyphae or *S. guani* eggs, respectively. The test was repeated 3 times.

Statistical analysis

Cumulative mortality data were corrected for mortality using Abbott's formula (Abbott 1925).

LT₅₀ values were determined using a computerized Probit analysis program. The data were examined using the analysis of variance (ANOVA) technique with a Statistical Package for the Social Science (SPSS, version 19, Chicago). Means were separated using the LSD multiple range test. Values of $P < 0.05$ were considered significant.

Results

Pathogenicity of *B. bassiana* to larvae of *A. germari*

Beauveria bassiana isolates were all found to be virulent to *A. germari* larvae (Table 2). Five to six days after treatment, white hyphae were observed on the cuticle; a few days later, this was followed by a dense conidiation. However, all tested isolates were significantly different in their level of virulence against *A. germari* ($F=375.420$, $df=20$, $P < 0.0001$). Isolate BI01 was the most virulent, achieving a mortality rate of 84.78%, followed by BS11 and BI05 with mortality rates of 71.74 % and 71.75%, respectively. For the control, cumulative larval mortality was 6% after 8 days, and no fungal hyphae were observed on the dead larvae.

LT₅₀ values differed significantly among the isolates when applied at a concentration

of 1×10^8 conidia·ml⁻¹ ($F=36.203$, $df=20$, $P<0.0001$) (Table 2). The LT_{50} values of *B. bassiana* BI01, BS11, and BI05 were 4.940 d, 5.939 d, and 5.442 d, respectively, and these values were significantly shorter than the values of the other isolates.

Pathogenicity of *B. bassiana* to *S. guani*

Scleroderma guani adults that were inoculated with *B. bassiana* were negatively affected (Table 3). Among the isolates tested, BI05 had a 34.82% mortality rate, which was significantly lower than those of BS11 and BI01 (46.20% and 76.91%, respectively) ($F=2184.635$, $df=11$, $P<0.0001$). However, even for the most virulent isolate, hyphae were observed only at the mouthparts and abdominal segments on a few dead wasps. Since no widespread sporulation appeared on their cuticles, *B. bassiana* thus presents almost no risk to *S. guani*. Moreover, the first death occurred 6 days after inoculation, which was enough time to allow *S. guani* to locate the host and carry the spores of *B. bassiana* to the tunnel.

Parasitism of *S. guani* on *A. germari*

In the experiment, we found that *S. guani* can find and locate the *A. germari* larvae and then get into the tunnel by biting a hole in the bark. And the size of *A. germari* and the inoculation ratio had a significant influence on the level of parasitism (Table 4). The parasitism rate of third instar larvae was 2.3% when the host/parasitoid ratio was 1:1, and this increased gradually as the ratio increased, until it finally leveled off at 1:4 which shared the same parasitism rate as 1:5 ($F=2244.446$, $df=14$, $P<0.0001$). There were no *S. guani* parasitized fourth instar larvae when the host/parasitoid ratio was 1:1 and 1:2. But as the ratio increased to 1:5, the rate of parasitism increased to

36.24% ($F=353.710$, $df=8$, $P<0.0001$). There was no parasitism in fifth instar larvae at any tested host/parasitoid ratio.

At high host/parasitoid ratios, *S. guani* will fight in group which accelerates the rate at which they affect the host. However, at a certain point parasitism rates decrease because of the limits of host availability. Large-sized, mature larva of *A. germari* can defend themselves against *S. guani*. Some *S. guani* were killed, either by being bitten to death or crushed.

Pathogenicity of *S. guani* previously inoculated with *B. bassiana* to *A. germari*

All *S. guani* inoculated with *B. bassiana* conidia find the host easily and attack it. In the time during which the conidia carried by *S. guani* was spread to surface of *A. germari* and caused it to fall ill, the *S. guani* made efforts to seek healthy *A. germari* to parasitize.

The efficacy of inoculated *S. guani* was significantly better than that of *S. guani* alone or *B. bassiana* alone. Twenty-one days after treatment *S. guani* inoculated with *B. bassiana* achieved a 73.3% mortality rate. That was significantly greater than the rate achieved by *S. guani* alone or *B. bassiana* alone (57.20% and 46.21%, respectively) ($F=90.001$, $df=8$, $P<0.0001$). And mortality was only 2.09% in the untreated control.

The results indicated that *S. guani* can successfully carry *B. bassiana* to the surface of *A. germari* and thus improves the efficiency of either *S. guani* or *B. bassiana* alone.

Throughout the entire study, only two *A. germari* larvae were found to be parasitized by *S. guani* and *B. bassiana* simultaneously. This indicates a possible competition between *S. guani* and *B. bassiana* in the *A. germari* host. In the treatment of *S. guani*

inoculated with *B. bassiana*, the number of eggs located on each *A. germari* larvae was less than that in the treatment of *S. guani* alone. In addition, most eggs in the treatment of *S. guani* inoculated with *B. bassiana* were unable to hatch. During the experiment, some *S. guani* adults were found to be dead not only when treated with *S. guani* inoculated with *B. bassiana*, but also when treated with *S. guani* alone. The mortality rate in the group treated with the inoculated *S. guani* was 49%, whereas it was only 7% in the group treated with *S. guani* alone.

Discussion

Scleroderma guani, an ideal natural enemy of stem borers, has been extensively used in the control of stem borers such as *M. alternates* (Xu et al. 2007), *Semanotus bifasciatus* (Wang 2007), *Saperda populnea* (Feng et al. 2005) and *A. glabripennis* (Liu et al. 2014). Many studies have been conducted on the feasibility of controlling *A. germari* using *B. bassiana* (Li et al. 2011). However, many isolates which perform well in the lab have not performed as well in the field. This may be due to the failure of *B. bassiana* to contact the *A. germari* hidden in the inner section of tree trunks. Data from the present study found that *S. guani* can be used to improve the biological efficacy of *B. bassiana* against *A. germari*. Of the isolates tested, *B. bassiana* strain BI05 showed the highest virulence to *A. germari* and the lowest virulence to *S. guani*. Furthermore, mortality was higher among *A. germari* specimens when *S. guani* that was inoculated with this isolate than when each natural enemy was used separately. The results showed that the combined use of parasitoids and entomopathogens against *A. germari* is promising and these kinds of combined strategies should be pursued.

Field studies testing these results and hypotheses should be conducted to confirm that *B. bassiana* poses a low risk to *S. guani* and to verify the practical efficiency of combined biological control using *S. guani* with *B. bassiana*. The optimum use model as well as the relationships among *S. guani*, *B. bassiana*, and *A. germari* also merit further research.

Using a time–response assay, we quantified the virulence of some *B. bassiana* isolates against *A. germari* and compared the calculated mortality and LT50 values for each isolate against *A. germari*. We found that the virulence of BI05 isolated from *A. glabripennis* was significantly higher than that of other isolates, followed by BI01 isolated from dead *A. germari* and BS11 isolated from soil. These results indicated that isolates from the original or similar hosts were substantially more virulent, and that soil appears to be a promising habitat for the screening of *B. bassiana* to be used as an biological agent. This observation supports with existing research (Imoulane and Elmezziane 2014; Li et al. 2006).

There was an inverse relationship between parasitic efficiency of *S. guani* in the larvae of *A. germari* and the size of the host larvae. Parasitism was also greater when a high *S. guani* host/parasitoid ratio was used. The pathogenic processes of *S. guani* against *A. germari* was different than that of *B. bassiana*, in that *S. guani* performs anesthesia, stings, and feeds to death using “circuitous tactics.” The pathogenic processes of *B. bassiana* include following steps: conidium adhering to the cuticle of *A. germari* and germinating; intrusion structure forming; penetrating the body wall into hemocoel; mycelium growing in the hemocoel, producing poison, overcoming

the immune system and killing the hosts. It was difficult for *S. guani* to conquer the older insects because of their larger size and thick cuticle. Some large hosts can crush *S. guani* to death during their reactions to attack by *S. guani*. For this reason, more *S. guani* were needed to conquer larger-sized hosts. In this study the fifth instar *A. germari* larvae failed to be parasitized, but fourth instar larvae can be partially parasitized; this finding was different from Xiao et al. (2003) who found that *S. sichuanensis* could not parasitize the fourth instar larvae at all. However, the difference may be attributable to the different natural enemy species and control targets that were used in the experiments.

One negative aspect of the present research is that *B. bassiana* appears to be somewhat virulent to *S. guani* and affects its rate of oviposition, parasitism, and egg hatching. However, the first death of *S. guani* occurred 7 days after inoculation, so we feel that there is enough time for *S. guani* to search for a host to carry *B. bassiana* to the larval tunnel. Our results showed a significantly higher pathogenic effect of *S. guani* combined with *B. bassiana* than either the *B. bassiana* treatment or the *S. guani* treatment alone. The likely mechanism is that *S. guani* spread the spores of *B. bassiana* on the cuticle of *A. germari* when fighting with the hosts. Plus, stinging and biting of *S. guani* may facilitate the infection of *B. bassiana* into the host. Although *B. bassiana* can infect *S. guani* to some extent, it is likely that the wasp recognizes infected hosts and oviposites in healthy host. In the experiment, most *A. germari* larvae cannot be parasitized simultaneously by *B. bassiana* and *S. guani*. All of these results indicated that there was some competition between the *B. bassiana* and *S.*

363 *guani* and that each had some ability to recognize and respond to threats caused by the
364 shared host and food source. However, the mechanisms related to the recognition and
365 response to competition remain poorly understood and merit further study.

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Table 1. Source and viability of *B. bassiana* for the experiments

Isolates	Host species or habitats	Locality	Collect time	Viability (%) ± SE
BS04	soil	Yi xian county Baoding	2005-06	90.39 ±3.21
BS05	soil	Baoding	2005-06	96.10 ±1.89
BS11	soil	Yixian county Baoding	2005-06	95.29 ±1.78
BI01	<i>A. germari</i> (Coleoptera: Cerambycidae)	Qinhuangdao	2010-09	95.23 ±2.91
BI08	<i>Hyphantria cunea</i> (Lepidoptera: Arctiidae)	Dingzhou county Baodin	2003-07	91.09 ±2.99
BI05	<i>Anoplophora glabripennis</i> (Coleoptera: Cerambycidae)	Baoding	2013-08	93.29±3.25
BI18	<i>A. glabripennis</i> (Coleoptera: Cerambycidae)	Xingtai	2014-05	95.81±4.01

Table 2. Mortalities and LT50 of *A. germari* larvae treated with various isolates of *B. bassiana*

Isolates tested	Mortality (corrected) /%							LT50 (days) (95% fiducial limits)
	2d	3d	4d	5d	6d	7d	8d	
BS04	0	0	0	10.42	14.89	25.53	32.61 d	10.524 (9.830-11.254) a*
BS05	2	0	8.33	14.58	21.28	23.40	30.43 d	10.80 (9.926-11.279) a
BS11	4	8.16	22.92	31.25	55.32	65.96	71.74 b	5.939 (5.582-6.342) b
BI01	8	12.24	33.33	56.25	76.60	80.85	84.78 a	4.940 (4.627-5.259) bc
BI05	4	12.24	43.75	50.00	57.45	70.21	71.75 b	5.442 (4.627-6.363) b
BI08	0	0	2.96	15.31	20.62	34.89	42.69 c	9.189 (8.213-9.987) a
BI18	0	0	0	10.99	23.41	38.92	42.99 c	8.969 (8.215-10.123) a
Control	0	0	2	4	4	6	6	

* Values followed by the same letter in the same column are not significantly different by LSD. (P=0.05)

Table 3. Mortalities of *S. guani* treated with various isolates of *B. bassiana*

Isolates tested	Mortality (corrected) /%							
	1	2	3	4	5	6	7	8
BS11	0	0	0	0	1.08	19.87	40.25	46.20 b*
BI01	0	0	0	3.48	13.48	33.96	55.80	76.91 a
BI05	0	0	0	0	0	3.99	26.91	34.82 c
Control	0	0	0	1.21	1.21	3.43	3.90	3.90 d

* Values followed by the same letter in the same column are not significantly different by LSD. (P=0.05)

Table 4. The parasitism rate of different inoculation ratios of *S. guani* on *A. germari* larvae (%)

	Mortality (corrected) /%				
	1:1	1:2	1:3	1:4	1:5
Third-instar larvae	2.3 a*	32.31 b	49.29 c	69.13 d	69.13 d
Fourth-instar larvae	0	0	10.21 a	19.93 b	36.24 c
Fifth-instar larvae	0	0	0	0	0

* Values followed by the same letter in the same row are not significantly different by LSD. (P=0.05)

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