Front. Agric. China 2011, 5(4): 666–670
DOI 10.1007/s11703-011-1130-2

RESEARCH ARTICLE

Potential of *Beauveria bassiana* for biological control *of Apriona germari*

Huiping LI (✉), Dazhuang HUANG, Zhigang WANG

College of Forestry, Agricultural University of Hebei, Baoding 071001, China; The Trees Germplasm Resources and Forest Protect Key Laboratory of Hebei Province, Baoding 071000, China

Abstract ***Apriona germari* is a destructive stem-boring pest. To date, the control of the pest is still based largely on the chemical insecticides. To meet the needs of people's high pesticide efficacy and environment safety, the alternative management strategy must be proposed. *Beauveria bassiana* is one of the most widely studied and used entomopathogenic fungi. The potential of *Beauveria bassiana* used to control *Apriona germari* was here evaluated. The result showed that the infective rate of *B. bassiana* to adults was 20%, with no pathogenicity to eggs. But it showed a high pathogenicity to larvae. The laboratory bioassays showed that the mortality, when concentration was 1×10^8 conidia/mL, was 96.47% ten days after inoculation. LT_{50} of *B. beauveria* to *Apriona germari* larvae was 4.53 d at the concentration of 1×10^8 conidia/mL; LC_{50} was 6.76×10^5 conidia/mL. The infecting experiments in field showed that, 20 days after control, the mortality was 68.4%. The present results suggested that *B. bassiana* has an excellent potential for biological control of *A. germari* larvae.**

Keywords *Apriona germari*, *Beauveria bassiana*, biological control, potential

Introduction

Apriona germari is a destructive stem-boring pest with difficulties in control for numerous reasons. In China, it has 1 generation every 1–3 years based on the timing of adult emergence and the climate conditions. Most adults emerge from June to August; however, the emergence is not synchronous and can well extend after August. After emergence, the adults feed on the phloem of twigs. And the females cut a small impression in the tree bark and lay eggs under the bark. After hatching, young larvae develop just under the bark but later live through the winter in the xylem. The fact is that it has a broad host and can attack or kill apparently healthy trees, which makes this species a serious pest. Due to the enormity of problems caused by this destructive pest, it poses an enormous threat to forestry, fruit industry, mulberry and sericiculture (Liu et al., 2002; Li et al., 2006).

Received May 25, 2011; accepted June 26, 2011
Correspondence: Huiping LI
E-mail: huipingli@sohu.com

Many scientists have made great efforts to control *A. germari*. To date, the control of the pests is still based largely on the chemical insecticides, to which the pests have developed strong resistance. Under the increasing concern over pesticide efficacy and safety to humans and the environment, the scientists have begun to seek an alternative management approaches.

Beauveria bassiana is one of the most widely distributed and extensively studied entomopathogenic fungi. It can infect more than 700 species of insects and mites belonging to 149 families and 15 orders, and the active agent in many products is currently in use and under development worldwide (Feng et al.,1994; Wu and Li, 1996; Wraight and Carruthers, 1999; Lacey et al., 2001; ST Leger and Screen, 2001; Bextine and Thorvilson, 2002; Felipe et al., 2004). Because of its attributes of easy mass culture, desiccation stability, compatibility with some chemicals, broad host range, and safety to vertebrates, it has been used successfully to control many important agricultural and forest insect pests (Zhang et al., 1992; Shimazu and Sato, 1995; Zhang and Inoue, 1998; Guo et al., 2001; Sun et al., 2001; Dubois, 2003; Xu et al., 2003; Ding et al., 2004; Dubois et al., 2004a, 2004b; Shimazu, 2004; He et al., 2005; Lin, 2005; Liu et al., 2007; Wang et al., 2007; Lu et al., 2008; Liu et al., 2009; He, 2010). However,

until now, there is no systemic study about the potential of *B. bassiana* against *A. germari*. The objectives of this study were to determine the virulence of *B. bassiana* to different stages of *A. germari*, and evaluate its potential use to control *A. germari*.

Materials and methods

Test insects

The *A. germari* adults were collected from the field. Eggs were collected by breeding *A. germari* adults in house to lay eggs. Larvae were hatched from the eggs in petri dish with a wetted filter. The first hatched larvae were soon inoculated to artificial groove of *Populus tomentosa* to breed for 20 d for bioassay.

Test isolates

Isolate Bi05 of *B. bassiana* used for the experiment was isolated from a larva's cadaver of *A. germari* from Dingzhou County, Baoding City, Hebei, China (Li et al., 2006).

Preparation of conidial suspension

B. bassiana was grown on PDA at 25°C. Conidia were harvested from 2-week-old surface cultures by scraping. Spore suspensions were made by placing harvested spores in 20 mL sterile distilled water in a small beaker containing 0.05% Tween-80. The beaker was agitated on a vibrant shaker for 10 min to produce a homogenous conidial suspension. The spore concentrations were then quantified with the neubauer hemocytometer and adjusted to 1×10^8 conidia/mL, then diluted to make concentrations of 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/mL. The control consisted of the same solution without fungi. Viability of the conidia was checked by a germination test prior to the experiment and assured to be $> 90\%$.

Evaluation of laboratory bioassays

Pathogenicity of B. bassiana to A. germari eggs

Two mL spore suspension of 1×10^8 conidia/mL was dropped into the egg niche on the day when the adult laid eggs. The branches were wrapped with the wet gauze on the end, and then cultured at room temperature. The amount of hatched eggs and larval survival rate were calculated 12 d after inoculation and 3 d after egg-hatching, respectively. The unhatched eggs were placed in moist petri dishes. The microscopic examination of hyphae and spores on the surface was used to confirm the dead due to *B. bassiana*. The sterile distilled water containing 0.05% Tween-80 was used as control. Each treatment had three replicates and each replicate had 25 eggs.

Pathogenicity of B. bassiana to A. germari adults

The branch brushed with conidial suspension of 1×10^8 conidia/mL was put into a breeding cage. The *A. germari* adults were bred in the cage until death. To keep fresh, the end of the branch was wrapped with the wet gauze. The branch brushed with sterile distilled water containing 0.05% Tween-80 was used as control. The dead adults were counted and then placed in moist petri dishes for observation of pathogen sporulation to determine whether the death was resulted from infection of pathogen. The mortality of insects was calculated by the percentage of infected dead adults. Each treatment had three replicates and each replicate had 25 insects.

Pathogenicity of B. bassiana to A. germari larvae

For each conidia concentration, 30 larvae were used. The larvae were dipped into the conidial suspension for approximately 30 s, drained of excess suspension by filter paper, and reared in the artificial groove. Controls were treated with sterile distilled water containing 0.05% Tween-80. Mortality was daily recorded for 10 d. Dead insects by *B. bassiana* were confirmed as described above. Cumulative mortality data were corrected for control mortality by Abbott's formula and subjected to a one-way analysis of variance. LT_{50} values were calculated by linear interpolation at the spore concentration of 1×10^8 conidia / mL. The LC_{50} was calculated using the Probit procedure of the SAS statistical package (Pu and Li, 1996).

Evaluation of field trial

Based on the laboratory bioassays results, the pathogenicity of isolate Bi05 of *B. bassiana* to *A. germari* larvae in the field was tested by the method described as below.

Sponge of 5 cm × 1 cm × 1 cm soaked with spore suspensions of 1×10^8 conidia/mL was stuffed in the bore holes of trunk. Controls were treated with sterile distilled water containing 0.05% Tween-80. The survivals were confirmed by the bore dust excreted from the excretory hole. The test was repeated 3 times, with 30 bore holes for each repetition. The field located in the living collection of Agricultural University of Hebei. The test was carried out in 2009 and surveys were made every five days.

Results

Pathogenicity of *B. bassiana* to eggs and adults of *A. germari*

The susceptible stage of most insects to *B. bassiana* was at larvae. Usually, eggs and pupae cannot be easily infected. To adults, the infection was different with different insects. Some insects were infected at most stages, and some only at one or two stages. The infectivities of *B. bassiana* Bi05 to eggs and adults were showed in Tables 1 and 2.

Table 1 Infectivity of *B. bassiana* to eggs of *A. germari*

Treatment	Inoculated number	Hatched number	Egg hatchability(%)	Dead larvae number 3 days after hatching	Infectivity
Inoculated	25	21	84	3	uninfected
Control	18	16	88.89	1	uninfected

Table 2 Infectivity of *B. bassiana* to adults of *A. germari*

Treatment	Inoculated number	Infected number	Infection rates(%)
Inoculated	25	5	20
Control	18	0	0

Table 3 Mortality of *B. bassiana* Bi05 to the larvae *A. germari* (10 d after inoculation)

Isolates	Cumulate mortality(%)	Correct mortality (%)
Bi05	96.7±0.01	96.47

Results showed that *B. bassiana* Bi05 has no influence on the egg hatching and larval survival rate. This is mainly related to the unusual structure of egg's cuticle.

Table 2 shows that the infective rate of *B. bassiana* Bi05 to adults of *A. germari* was 20%. Although the infective rate was low, we still concluded that *B. bassiana* could infect *A. germari* adults. This result provided us the possibility of screening highly virulent isolate to adult. In addition, we found in the test that the mycelial inside the infected adults grown first from femora, abdominal segment and antenna, indicating that the above-mentioned position was more sensitive to *B. bassiana* and the main part of infected naturally by *B. bassiana*.

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

Approximately 2 d after inoculation by *B. bassiana*, some responses of the treated insects were observed. In contrast to the control, the treated insects showed infested symptoms, often appearing to inappetency, weaken activity and sometimes being unresponsive to external stimuli. Later, some larvae died and rigidified. Dead larvae covered with white mycelium of *B. bassiana* were found 8 d after inoculation. No symptoms were observed on control larvae during the experimental period.

Mortalities of larvae inoculated with *B. bassiana* were shown in Table 3. The virulent of isolate Bi05 was 96.47% 10 d after infection, while the control had 6.6% cumulative larvae mortality, and no fungi were observed on the dead larvae.

The lethal speed was always noted by the use of the time taken to kill 50% larvae (LT_{50}). Table 4 shows that LT_{50} is 4.53 d when *A. germari* larvae are dipped in conidia suspension (1×10^8 conidia/mL) of *B. bassiana.*

LC_{50} value means the concentration taken to kill 50% larvae. It can be used not only to evaluate the virulence of isolates, but also to guide the applying dose in the actual use when controlling in the forestry. By dose-mortality analyses, the mortality of *A. germari* larvae differed significantly when inoculated with different concentration of isolates Bi05. The LC_{50} of *B. bassiana* Bi05 was shown in Table5.

The corrective mortality was only 11% when the concentration was 1×10^4 conidia/ml and amounted to 95% when 1×10^8 conidia/mL. By the Dose-Regression equation, the LC_{50} of Bi05 was 6.76×10^5 conidia/mL.

Evaluation of field trials

The results in the field test (Fig. 1) showed that there was a high mortality of *B. bassiana* on *A. germari* larvae. 10 d after control, the mortality rate of Bi05 was 20.1%. And till 20 d after control, the mortality increased to 68.4%.

Conclusion

The results of the laboratory bioassays and field trials indicated that *B. bassiana* Bi05 performed high virulence and had the excellent potential for biological control of *A.*

Table 4 LT_{50} (in days) of *B. bassiana* Bi05 to *A. germari* larvae at concentration of 1×10^8 conidia/mL

Isolates	LT_{50}±SE(d)	95% fiducial limits		Slope±SE
		Lower	Upper	
Bi05	4.53±0.631	3.87	5.21	2.13±0.210

Table 5 LC_{50} value of isolate Bi05 against larvae *A. germari* 10 d after treatment

	Concentration of Bi05(conidia/mL)				
	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8
Mortality of larvae (%)	0.19	0.33	0.52	0.86	0.95
Corrected mortality (%)	0.11	0.26	0.47	0.84	0.95
Dose regression equation	$Y = 0.514 + 0.777X(R^2 = 0.977)$, $F = 176.82 > F_{0.05}(1\ 3) = 10.13$				
LC_{50} (conidia/mL)	6.76×10^5				

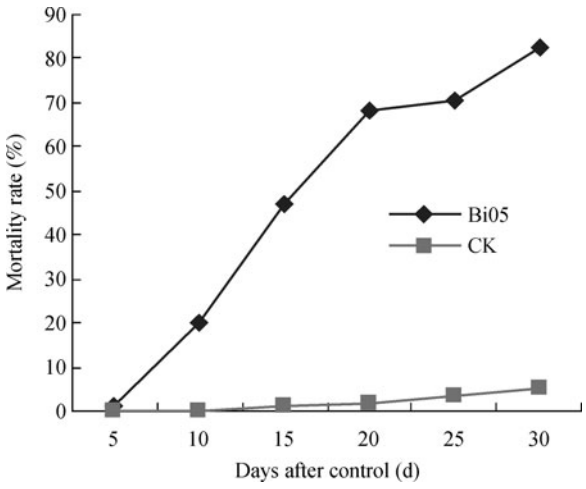

Figure 1 The control effect of *B. bassiana* on *A. germari* larvae in the field.

germari larvae. However, more studies about *B. bassiana* Bi05 are necessary before the practical application. The problems such as characteristics including the suitability for mass-production, inoculum stability, effects of formulation on conidium viability and efficacy, compatibility with commonly used pesticides and other natural enemies must be researched and resolved before the practical use of the biologic agent.

Acknowledgements

This research was supported by the Natural Science Foundation of Hebei Province, China (No. C2011204041).

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