Laboratory virulence and field efficacy of different *Heterorhabditis bacteriophora* strains against *Tetramoera schistaceana*

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Summary. Sugarcane is one of the most valuable economic crops worldwide. The yellow mealworm, *Tetramoera schistaceana* (Lepidoptera: Tortricidae: Olethreutinae), is a serious insect pest for sugarcane plants on Hainan Island where the pest often causes serious economic loss. However, there is a dearth of information concerning the control of this pest with entomopathogenic nematodes (EPN). In the present study, we compared laboratory virulence of four *Heterorhabditis bacteriophora* isolates (Hb SD, Hb I, Hb II and Hb III) toward *T. schistaceana*. Initial results showed that Hb SD isolates was more potent. Next Hb SD was further tested for field efficacy. In the first trial, survival of *T. schistaceana* following application of nematode infected cadavers was 61% lower compared with the untreated control. Similar results were obtained in a second trial (57% lower than the untreated control). Our results demonstrated that application of nematode infected host cadavers could cause a significant reduction in the pest population. Therefore, we conclude that Hb SD had an excellent ability for suppression of *T. schistaceana* in sugarcane.

Key words: biological control, entomopathogenic nematode, Hainan Island, sugarcane, yellow mealworm.

Entomopathogenic nematodes (EPN) are biological control agents capable of suppressing a variety of economically important insect pests (Klein, 1990). Sugarcane (*Saccharum sinensis* Roxb.) is one of the most important economic crops in tropical and subtropical regions. *Tetramoera schistaceana* Snellen (Lepidoptera: Tortricidae: Olethreutinae) is a serious pest of sugarcane and often causes losses of 25% economic value in the Chinese tropical area (Guo *et al.*, 2000). *Tetramoera schistaceana* is mainly distributed in China, Japan, Malaysia, Indonesia, Philippines and Vietnam (CAB, 1965; Williams, 1978). The pest is one of most serious pests of sugarcane; it bores into early shoots and causes death in young sugarcane seedlings (Williams, 1978). In the field, the sugarcane shoot borer pest damages sugarcane plants on Hainan Island all year round. A mixture of chemical pesticides, trichlorfon and dimehypo, is used to control *T. schistaceana* (Guo *et al.*, 2000). Chemical pesticides are harmful to humans and other non-target organisms as well as to the environment, so biological insecticides should be sought for application in the field.

Entomopathogenic nematodes have attracted attention in recent years, because they are biological control agents of some sugarcane pest species (Razak & Sivakumar, 2001; Sankaranarayanan *et al.*, 2003; Umamaheswari *et al.*, 2004). Entomopathogenic nematodes kill their invertebrate hosts with the aid of mutualistic bacteria, such as *Photorhabdus luminescens* (which is associated with *H. bacteriophora*). The nematode provides a safe harbour for intestinal symbionts in soil and delivers the symbiotic bacteria into the insect haemocoel. The symbiotic bacteria provide metabolites essential for nematode reproduction, and antibiotic preservation of the insect cadaver (Boemare, 2002; Bai *et al.*, 2013). Entomopathogenic nematodes are easily cultured *in vivo* for experimental use (Friedman, 1990; Adams & Nguyen, 2002; Shapiro-Ilan & Gaugler, 2002).

In the present study, four *Heterorhabditis bacteriophora* isolates were tested for control *T. schistaceana* in the laboratory and in field trials on Hainan Island. To our knowledge, this is the first detailed report on controlling *T. schistaceana* with *H. bacteriophora*.

MATERIAL AND METHODS

Nematodes. Four *H. bacteriophora* nematode isolates, Hb SD, Hb I, Hb II and Hb III, were collected from a field near Danzhou city, Hainan province, China. The yellow mealworm, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), was chosen as the target insect in the laboratory because it is one of laboratory hosts used in the laboratory for bioassays with EPN (Shapiro-Ilan *et al.*, 2010). The four nematode isolates were serially cultured for two passages through the hosts. Infective juveniles (IJ) were collected from White trap, modified from Kaya & Stock (1997). A genetically diverse foundation population was then created with about 5,000 IJ of each *H. bacteriophora* added to infect *T. molitor* larvae. Infective juveniles and nematode infected insect cadavers were prepared (Kaya & Stock, 1997).

Insects. The third instar larvae of *T. schistaceana* were used for the bioassay; the insects were fed on fresh young sugarcane seedlings at 25°C under laboratory conditions. They were originally collected from the sugarcane field in Haikou City, Hainan Province, China. Later, *T. molitor* were fed on wheat bran and a small amount of Chinese cabbage (Gao *et al.*, 2006) and used for the bioassay.

Laboratory bioassays. The bioassays were carried out at 25° unless otherwise indicated. One last instar *T. molitor* was infected with each of *H. bacteriophora* strain (ten IJ to one larva). Each Petri dish contained one insect and ten IJ (suspended with 200 µl of tap water). The mortality was recorded six days after exposure. A total of 36 insects were used for each treatment. Five treatments were applied. For the blank control, only tap water (without IJ) was added.

Virulence of Hb SD IJ against *T. schistaceana* was assessed. The bioassay methods were similar to the methods above, but different numbers of nematodes and insects were applied. Sugarcane leaves were cut into pieces (L: 2.0 cm; W: 0.5 cm), and then placed in a glass test tube with a strip of Whatman filter paper inside. The IJ were counted and added in the tube for testing the efficacy of the IJ toward *T. schistaceana*. All *T. molitor* were placed in Petri dishes with a Whatman filter pap disc, and then different number of IJ was added (0, 1, 3, 10, 33, 100 IJ to one insect).

Field trials. Both nematode infected *T. molitor* cadavers and the suspensions of Hb SD IJ were used for the field test. After collection and storage, more than 95% of nematodes survived. The SD IJ were applied for the field trials. The infected cadavers were dissected and the viability was counted. There were $7.71 \pm 0.89 \times 10^4$ Hb SD IJ per single insect cadaver.

Trial I. The field test was conducted in Chengxi Town, Haikou City (location: 20.05° N, 110.41° E) on June 25, 2013. Six replicate plots were used for the treatment and control. Nematodes were applied at 16:00 on a cloudy day with an ambient temperature ≤ 30°C, and soil temperature 26℃ (at 5 cm depth). The experimental plots were 4.0 m^2 (2 m × 2 m) with 5-m buffer space (without treatment) between the plots. The population density of sugarcane shoot borer larvae in the field plot was 15.33 ± 1.69 m^{-2} (on the day before the field trials). Samples were collected from at least five different places in the tested field. Nematode suspensions were made approximately 3 IJ ml^{-1} containing 0.05% Triton X – 100 (used as a surfactant) and then sprayed. The numbers of nematodes were sprayed at 1.02×10^4 IJ m^{-2}. The spray was repeated three times with 1 week intervals.

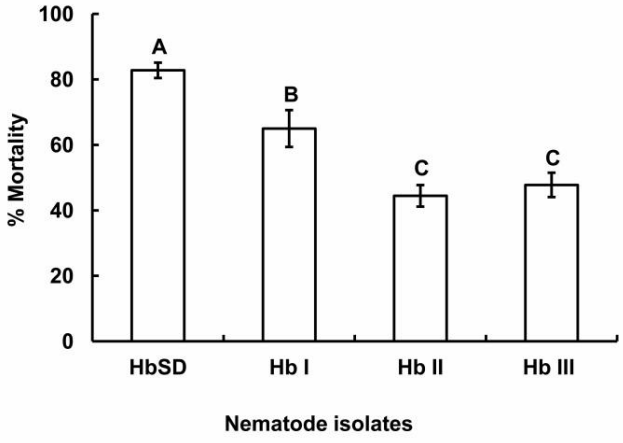

Fig. 1. Comparative virulence: mean percentage mortality of last instar *Tenebrio molitor* exposed to *Heterorhabditis bacteriophora* isolates. Four isolates were bioassayed under laboratory conditions. The insect larvae were experimentally infected with infective juveniles. Mortality was recorded at 6 days after exposure. Data are means ± SE of n = 6 biological replicates. Means denoted by the same letter do not differ significantly at $P < 0.05$ as determined by Duncan's multiple range test.

The location of repeated field test was same as the first test. Both of them were done simultaneously. The sample used was insect cadavers after infection by nematodes. A total of 12 cadavers were applied per 4 m^2, and the holes were 0.6 m distance from each other. The depth of the hole was about 5 cm, two cadavers were embedded in each hole, and six replicates were tested. Six plots without the cadavers were used as controls. After 15 and 30 days, the results of field trials were recorded.

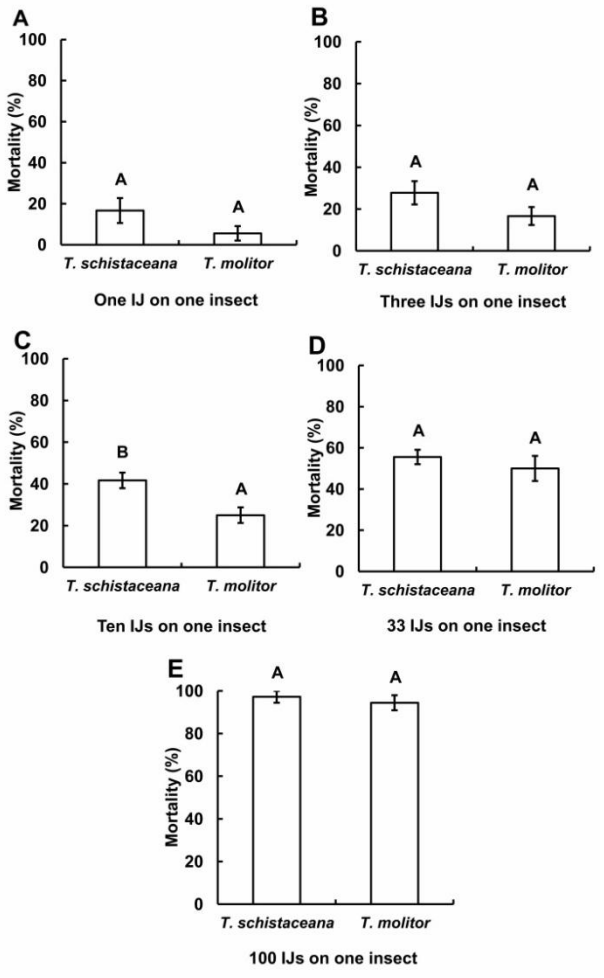

Fig. 2. Comparative virulence: mortality of third instar of *Tetramoera schistaceana* and last instar of *Tenebrio molitor* exposed to *Heterorhabditis bacteriophora* SD infective juveniles (IJ). The insect larvae were experimentally infected with the nematodes of 0, (A) 1, (B) 3, (C) 10, (D) 33 and (E) 100 IJ $(insect)^{-1}$, respectively. The mortalities were recorded at 6 days after exposure. Data are means ± SE of n = 6 biological replicates. Means denoted by the same letter do not differ significantly at $P < 0.05$ as determined by t-test.

Trial II. The field test was conducted in Baodao Xincun, Danzhou City, Hainan province, China (location: 19.52° N, 109.46° E) on September 28, 2013. The methods were similar to that of Trial I. The location of repeated field test was same as the test above. The two tests were done simultaneously. The methods of used for test were similar.

Statistical analysis. All data analyses were performed using SAS 9.1 (SAS Institute, Cary, North Carolina, USA). After bioassay, the analysis of results from multiple groups was compared used ANOVA and pair groups were compared used t-test (Ron, 2007).

RESULTS

Laboratory bioassay. Under laboratory conditions, virulence assays indicated that the Hb SD isolate resulted in significantly ($P < 0.05$) higher mortalities than Hb I, Hb II and Hb III isolates after *T. molitor* larvae were exposed to nematode suspensions (Fig. 1). Subsequently, the Hb SD isolate was chosen for further potency assays. After treatment with different concentrations of Hb SD suspensions, the mortalities of *T. schistaceana* and *T. molitor* varied (Fig. 2). Higher mortality was associated with higher doses of application. The nematode dose effect was significant for both insect pests. The LC_{50} values were 9.00 IJ $(insect)^{-1}$ for *T. schistaceana* (95% confidence limits = 3.57-22.71) and 16.80 $(insect)^{-1}$ for *T. molitor* (95% confidence limits = 6.60-42.74). These results indicate that there are no differences between the LC_{50} values because the confidence intervals overlap.

Field trials. Overall, after treatment with both Hb SD nematode infected *T. molitor* cadaver and nematode suspensions in the field trial I, the relative survival rates were lower than the untreated control ($P < 0.05$; Fig. 3). The mean number of surviving *T. schistaceana* was 5.50 ± 0.76 for nematode infected *T. molitor* cadaver group (Fig. 3A), and 10.17 ± 1.51 for nematode suspension group 15 days after treatment (Fig. 3B). Thirty days after treatment, the mean number of live *T. schistaceana* was 3.00 ± 0.37 for the nematode infected *T. molitor* cadaver group (Fig. 3C), and 8.00 ± 1.77 for the nematode suspension group (Fig. 3D). The relative survival rates of *T. schistaceana* nematode infected *T. molitor* cadaver group was 51% lower than untreated control 15 days after treatment (Fig. 3A) and 61% lower than control 30 days after treatment (Fig. 3C); *T. schistaceana* survival for nematode suspension treatments was 22% lower than control (Fig. 3B) and 30% lower than control (Fig. 3D) at 15 and 30 days, respectively. Thus, our results clearly indicated that after 30 days the survival of the pest populations was lower than that after 15 days.

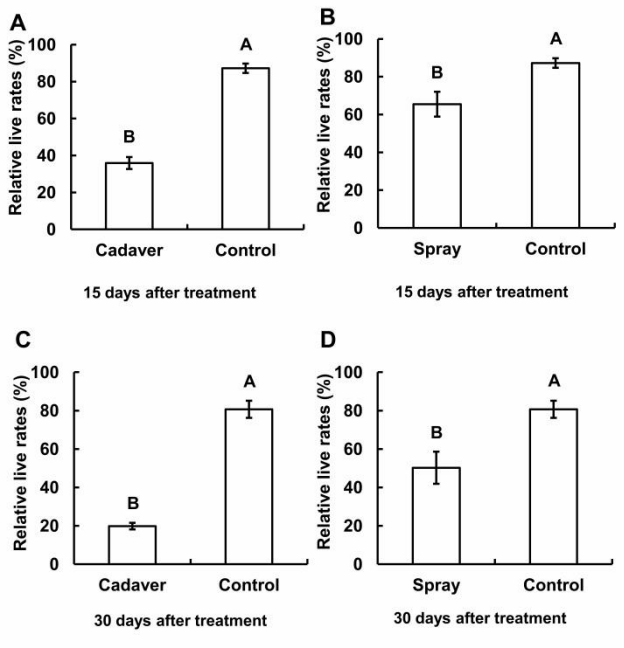

Fig. 3. Comparative potency following application of *Heterorhabditis bacteriophora* SD IJ in aqueous suspension and in infected *Tenebrio molitor* cadavers (Trial I): A and B: 15 days after exposure; C and D: 30 days after exposure. The Trial I was conducted on a large plot (which was divided into several small plots), located in Chengxi Town of Haikou City, Hainan Province, China. Data are means ± SE of n = 6 biological replicates. Means denoted by the same letter do not differ significantly at $P < 0.05$ as determined by t-test.

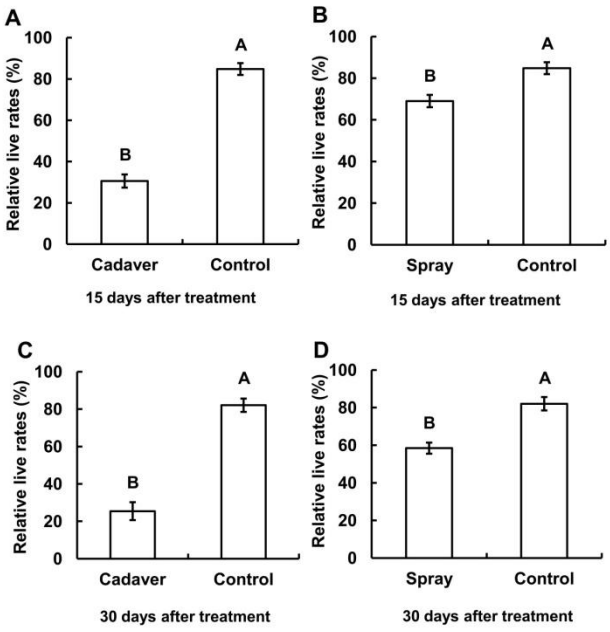

Fig. 4. Comparative potency following application of *Heterorhabditis bacteriophora* SD IJ in aqueous suspension and in infected *Tenebrio molitor* cadavers (Trial II): A and B: 15 days after exposure; C and D: 30 days after exposure. Trial II was conducted on a large plot (which divided into several medium plots), located in Baodao Xincun of Danzhou City, Hainan Province, China. Data are means ± SE of n = 6 biological replicates. Means denoted by the same letter do not differ significantly at $P < 0.05$ as determined by t-test.

Overall, the relative survival rates of both Hb SD nematode infected *T. molitor* cadaver and nematode suspensions in the field trial II were also lower than the control ($P < 0.05$; Fig. 4). The numbers of surviving *T. schistaceana* were 2.83 ± 0.31 for nematode infected *T. molitor* cadaver (Fig. 4A), and 6.50 ± 0.92 for nematode suspensions (Fig. 4B) 15 days after treatment. Thirty days after treatment, the mean numbers of *T. schistaceana* that survived were 2.50 ± 0.62 for nematode infected *T. molitor* cadavers (Fig. 4C), and 5.50 ± 0.76 for nematode suspensions (Fig. 4D). The relative *T. schistaceana* survival rates were 54% lower than control after 15 days (Fig. 4A) and 57% lower than control after 30 days (Fig. 4C) for infected *T. molitor* cadaver. The *T. schistaceana* survival rates for nematode suspension treatments were 16% lower than control (Fig. 4B) and 24% lower than control (Fig. 4D). Thus, our results for trial II also indicate that after applying Hb SD nematodes (both infected nematode cadavers and nematode suspensions), after 30 days the relative survival rates of the pest populations were lower than 15 days.

DISCUSSION

In the present study, laboratory experiments proved that Hb SD isolate was more virulent than Hb I, Hb II and Hb III isolates against larvae of the standard laboratory target insect *T. molitor*. In the field trial I, *T. schistaceana* mortality rates were much higher after treatment with Hb SD infected cadavers compared with the untreated control; and the mortality rate of *T. schistaceana* was also high after spraying with IJ compared with the untreated control. Similar results were obtained in the second field trial. The mortality by the spray method was 46%, and by cadaver application was 77% in the field trials. Our results confirmed that either Hb SD nematode suspensions or its infected *T. molitor* cadaver could significantly reduce populations of *T. schistaceana*.

Both nematode infected insect cadavers and suspensions have been tested for controlling different insect pest species. *Anagasta kuehniella* is highly sensitive to the higher doses with most species and/or strains of EPN (de Carvalho *et al*., 2013). Codling moth is susceptible to the nematode

isolates at a concentration of 50 IJ insect^{-1} (78-100% mortality) (de Waal *et al*., 2011). Others have shown that *H. bacteriophora* (German isolate) caused very high mortality of the sugarcane shoot borer, *Chilo infuscatellus* (Sankaranarayanan *et al*., 2003). From our laboratory results, it is evident that *T. schistaceana* larvae are very susceptible to Hb SD. Our field test results confirmed that *T. schistaceana* could be effectively controlled with Hb SD. To our knowledge, this is the first report of control of *T. schistaceana* by *H. bacteriophora*.

Temperatures, desiccation, UV irradiation, spray volume and nozzle type are important parameters since they are related to nematode infectivity (Brusselman *et al*., 2011, 2012). Based on our experimental record, a temperature range of 19-34°C was recorded during our field test period. There was no extreme weather occurring during the field test period. Such temperature conditions should be conducive to EPN survival and performance (searching and killing the host pests). In addition, the population of *T. schistaceana* may also be influenced by other predators, such as earwigs, spiders and ants, which were found in the field during the field trial period. These can attack *T. schistaceana* larvae, and could be considered as native natural enemies of *T. schistaceana* in the sugarcane fields.

It was reported that early instars of *Diaprepes abbreviatus* larvae are more susceptible to *H. bacteriophora* than later instars at the higher temperature (Shapiro-Ilan & McCoy, 2000). Here, at the same temperature, it appears that *H. bacteriophora* caused high mortality in last instar *T. molitor*, but could not cause any mortality in the third instar based on our results (unpublished observations). One possible reason is that the third instar larvae move much faster than the last instar in Petri dishes. Thus, the nematode IJ may be unable to attack the third instars. By contrast, the last instar of *T. molitor* was very susceptible to *H. bacteriophora*. It appeared that since the last instar *T. molitor* and both early instars and later instars of *D. abbreviates* moved so slowly, they may be vulnerable to attack by nematodes. The cause for differential virulence of Hb SD toward the last instar and the third instar of *T. molitor* remains unclear and requires further investigation.

Our results confirmed that *H. bacteriophora* SD infected cadaver can suppress populations of *T. schistaceana*. More extensive field trials are now needed and, in addition, Hb SD will be tested for efficacy against other sugarcane pests.

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Jianjun Yue, Cheng Bai and Haibo Long. Лабораторная инвазионность и полевая эффективность различных штаммов *Heterorhabditis bacteriophora* против *Tetramoera schistaceana*.

Резюме. Сахарный тростник относится к культурам с высокой удельной стоимостью урожая. Желтая моль сахарного тростника *Tetramoera schistaceana* (Lepidoptera: Tortricidae: Olethreutinae) – опасный вредитель сахарного тростника на плантациях о-ва Хайнань, вызывающая большие потери урожая. Отсутствуют данные о возможности контроля этого вредителя с помощью энтомопатогенных нематод. Оценивали инвазионность четырех штаммов *Heterorhabditis bacteriophora* (Hb SD, Hb I, Hb II and Hb III) для *T. schistaceana*. Лабораторные результаты показали, что штамм Hb SD наиболее эффективен. В первом полевом опыте применение штамма SD привело к снижению числа выживших *T. schistaceana* на 61% по сравнению с контролем. Во втором полевом эксперименте снижение составляло 57%. Предполагается возможность использования штамма *Heterorhabditis bacteriophora* SD для подавления *T. schistaceana* на сахарном тростнике.