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ORIGINAL PAPER

Performance of larval parasitoid, *Bracon brevicornis* on two *Spodoptera* hosts: implication in bio-control of *Spodoptera frugiperda*

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Abstract

Successful pest management using parasitoids requires careful evaluation of host-parasitoid interactions. Here, we report the performance of larval ecto-parasitoid wasp, *Bracon brevicornis* (Wesmael) on important agricultural pests, *Spodoptera litura* (Fabricius) and *S. frugiperda* (J.E. Smith). Biology of *B. brevicornis* was studied on different host instars under laboratory and cage setup. In no-choice assay, the parasitoid development was highest on fifth-instar *S. litura* larvae as the wasp laid ~ 253 eggs with 62% hatching, 76% pupae formation and 78% adult emergence. Similarly, these parameters were highest on fifth instar *S. frugiperda* larvae (293 eggs, 57% hatching, 80% pupae formation, 70% adult emergence). In two-choice assay, *B. brevicornis* preferred fourth or fifth over third instar larvae of both hosts. Successful parasitism depends on host paralysis and suppression of host immunity. *B. brevicornis* interaction downregulated cellular immunity of both hosts as shown by reduced hemocyte viability and spreading. The percent parasitism rate of *B. brevicornis* was unaltered in the presence of host plant, *Zea mays* in cage study. 76 and 84% parasitism was observed on fifth-instar larvae of *S. litura* and *S. frugiperda*, respectively. We evaluated the performance of *B. brevicornis* as a biocontrol agent on *S. frugiperda* in maize field. Our results show 54% average reduction in infestation after release of *B. brevicornis*. Taken together, we report the performance of *B. brevicornis* on important insect pests for the first time in laboratory and field conditions. Our findings indicate that *B. brevicornis* is a promising candidate for integrated pest management.

Keywords Biological control · *Spodoptera* · Maize · Immunity · *Bracon brevicornis*

Key messages

1. We report instar-dependent performance of *Bracon brevicornis* on two serious pests, *Spodoptera litura* and *S. frugiperda*.
2. Fifth instar larva of *Spodoptera* was most suitable for *B. brevicornis* development.
3. *B. brevicornis* induced permanent paralysis and down-regulated cellular immunity of *S. litura* and *S. frugiperda*.
4. Our field experiment confirmed *B. brevicornis* as an promising biocontrol agent for *S. frugiperda*.

Introduction

The Noctuid genus *Spodoptera* (Lepidoptera) encompasses 31 species, half of which are considered as serious pests worldwide, commonly referred to as 'armyworms' (Pogue

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2002). Among these, *Spodoptera litura* (Fabricius) and *Spodoptera frugiperda* (J.E. Smith) are important pest species that are highly polyphagous. *Spodoptera litura,* widely distributed throughout tropical and subtropical regions is a notorious pest of more than hundred plant species (Brown and Dewhurst 1975). For instance, in India, the economic impact of *S. litura* ranges between 30–98% of major crops like soybean and groundnut (Dhir et al. 1992; Natikar and Balikai 2015). In addition to high fecundity, short life cycle and polyphagy, resistance to chemical as well as biopesticides (Bt) is another concern (Cheng et al. 2017). The fall armyworm, *S. frugiperda* (FAW) is native to North America and it was first recorded in African continent in 2016 (Day et al. 2017; Goergen et al. 2016), then subsequently spread very quickly across Asia causing severe damage to cereal crops (Sharanabasappa et al. 2018). In India, it was first reported in 2018 on Maize and has now spread to more than 20 states (Shylesha et al. 2018; Suby et al. 2020). It is known to attack at least 80 different crop plants with preference towards cereals, especially maize. In African countries, the value of these losses is estimated to be ~ 2.48–6.19 billion USD (Prasanna et al. 2018). Effective control of FAW is an urgent need as the impact of FAW infestation on maize yield is alarming and favorable agro-ecological conditions can lead to large pest outbreaks causing lasting threat to important crops (Goergen et al. 2016). Natural enemies of insect pests exert top-down pressure and control insect populations (Weis and Abrahamson 1985). Despite reports on natural enemies, very few studies report larval parasitoids and their potential in controlling FAW (Shylesha et al. 2018; Sisay et al. 2018; Firake and Behere 2020; Gupta et al. 2020).

Insect parasitoids are fascinating model systems to study ecological interactions and are of high economic importance as biological control agents. As adults, parasitoids are free-living whereas in the larval phase, they need to feed on a single host to reach adulthood. For this, they develop either inside or on the host after inducing temporary or permanent paralysis by the adult parasitoid. The newly hatched parasitoid larvae feed on the paralyzed host and at the end of their feeding, the hosts are killed (Godfray 1994). Ecto-parasitoids inject maternal factors that cause permanent paralysis before oviposition making them effective natural enemies for pest management (Qian et al. 2013; Quicke 2015). *Bracon brevicornis* (Wesmael) (Hymenoptera: Braconidae) is a gregarious parasitoid that is known to attack stored grain and crop pests (Scholler et al. 2006). They are idiobionts and develop concealed or semi-concealed on larval hosts (Quicke 2015). Females are synovigenic as their eggs mature throughout their lifetime and exhibit host feeding behavior to acquire nutrients. Females also sometimes paralyze and host feed without depositing any eggs. *Bracon brevicornis* can accept a wide range of host species including *Spodoptera* but detailed study of their biology on *Spodoptera* spp. is lacking and is needed for proper utilization in management programs (Temerak 1984; Bakr et al. 2014; Kares et al. 2009).

The success of parasitoid depends on various factors related to their host viz. developmental stage, size, diet as well as environmental conditions (Pan et al. 2017; Khan et al. 2016). Studies comparing parasitoid performance and host size have shown positive correlation as these factors can directly influence parasitoid fitness (Charnov and Skinner 1985; Stoepler et al. 2011). Similarly, host plant quality and chemistry also influence parasitoid behavior, development and fitness (Gols et al. 2008; Sarfraz et al. 2009). Evaluation of these parameters is crucial to understand parasitoid biology, which in turn, is important for field application. Here, a comprehensive study on *B. brevicornis* performance on two lepidopteran hosts; *S. litura* and *S. frugiperda* is presented, with potential application for biological control in field conditions. Together, our results show that *B. brevicornis* is efficient in controlling FAW and could play an important role in integrated pest management programs of FAW.

Materials and methods

Insect rearing conditions

Laboratory culture of *S. litura* (NBAII-MP-NOC-02) and *S. frugiperda* (NBAIR-MP-NOC-03) were reared on castor bean plant (*Ricinus communis* L.) in a climatic chamber. *Bracon brevicornis* cocoon was collected on their natural host *Opisina aeronosella* from coconut field, Coimbatore, India during September, 2017. For their maintenance and mass production, the laboratory host rice moth *Corcyra cephalonica* (Stainton) was used (Srinivasan and Chandrikamohan 2017). The adult wasps were provided with 50% honey-water solution (v/v). 3-day-old mated female and male wasps (here onwards wasp couple) were used for our study. Rearing of insects and all laboratory experiments were conducted at 26 °C with a photoperiod of 12L:12D and 60% relative humidity.

Performance and preference of *B. brevicornis* on two *Spodoptera* hosts

B. brevicornis is known to parasitize older instars of *Spodoptera* larvae (Ghosh and Venkatesan 2019; Kares et al. 2009). Hence, third to fifth instar host larvae were chosen for this study. We performed no choice and two choice tests to assess the preference and performance of *B. brevicornis*. For no-choice assay, in a glass test tube (measuring 160 mm × 16 mm × 14 mm), single larva (of a specific instar) of *S. litura* or *S. frugiperda* was kept with one mated wasp couple of *B. brevicornis* for 24 h. Post 24 h, the wasp couple was removed and placed into another test tube with a fresh

larva of same instar for parasitization. The adult wasps were provided with 50% honey-water solution (v/v). This process was repeated until the death of the particular female wasp and replicated at least 5 times independently. The parasitized larvae were kept under same conditions and the total number of eggs laid (life-time fecundity/female wasp), percent larval hatching (number of larvae/total number of eggs laid × 100), percent pupae formation (number of pupae/total number of larvae × 100), adult emergence and number of females were recorded for comparative analysis. For calculating the fecundity, total number of eggs laid by the parasitoid was manually counted under a stereoscope. For two choice assay, female of *B. brevicornis* was released in a petri-plate and given a choice of: (a) third vs fourth, (b) third vs fifth and (c) fourth vs fifth instar larvae of *S. litura* and *S. frugiperda*, separately ($n = 25$). Single female wasp was released in the middle of the petri-plate and observed for a maximum duration of 6 h. This duration was chosen based on our preliminary experiment. The experiment was stopped after at least one larva was paralyzed.

Parasitism rates and fitness of *B. brevicornis* on infested maize

In our laboratory, both the host larvae were reared on *R. communis*. As *S. frugiperda* is a serious pest on maize, we recorded the parasitism, emergence and longevity of *B. brevicornis* on larvae feeding on maize. For this, plants (*Zea mays* DHM103) were grown in a pot (15 × 10 cm) with red soil and coco peat in green house (26 ± 5 °C) under natural light conditions. No insecticide was sprayed on plants during experiment. Five potted maize plants (~ 20 days old) randomly arranged in a cage (60 × 50 cm) were used for the study. A total number of 15 larvae of *S. litura* or *S. frugiperda* were used per cage where one plant had 3 larvae of a specific instar (3rd, 4th or 5th). Post 24 h of larval feeding, 5-wasp couples were released into the cage and allowed to interact for 24 h. 3-day old mated *B. brevicornis* was used for our study. The instars of *S. litura* and *S. frugiperda* were tested separately as independent experiments ($n = 5$). Percent parasitization was recorded considering the number of paralyzed larvae. The parasitized larvae from each treatment were carefully transferred into a test tube (160 mm × 16 mm × 14 mm) until adult emergence. The number of parasitoid eggs on each larva was counted under a stereoscope and the percent adult emergence was estimated by dividing the number of adults emerged over the total eggs laid on each larva, multiplied by 100. Adults emerged in each treatment were transferred into separate glass tubes with 50% honey-water solution and their longevity was calculated by recording the average length of their life-span in days.

Regulation of host immune-competence by *B. brevicornis*

Parasitoid venom is known to suppress host immune functions that help in better survival of their progeny (Teng et al. 2016; Ghosh and Venkatesan 2019). This includes reduction in hemocyte viability and spreading ability that are important to combat infections. Healthy laboratory reared castor fed fifth instar larva of *S. litura* and *S. frugiperda* was placed in a petri dish along with one mated female wasp for 24 h. Paralyzed larvae were removed and hemolymph samples were collected by cutting their abdominal prolegs with a pair of sterile scissors. Prior to hemolymph extraction, each larva was sterilized (70% ethanol) and rinsed with sterile water. 60 µl of hemolymph collected per larva was mixed with 200µL saline (PBS). 20 µl of this was placed on a teflon coated diagnostic slide (8 mm well) and fixed with 4% formaldehyde for 15 min to preserve the cell morphology, followed by two washes with PBT (PBS with 0.3% triton X). F-actin specific staining was done using alexafluor 488 (1:200, Thermo Fisher Scientific) for 2 h. The sample was mounted with DAPI vectashield (Vector laboratories). Images were recorded on Olympus FV3000 confocal microscope using 20X objectives. Hemocytes with filopodia, pseudopodia or flaring (all indicators of spreading) were counted for comparative analysis and the sample size was decided as per previous studies ($n = 5$) (Ghosh and Venkatesan 2019; Yang et al 2019). Hemocytes that retained intact shape and morphology were counted as viable while cells that showed blebbing or rupture were considered non-viable cells (Teng et al. 2016).

Field studies

To assess the potential of *B. brevicornis* in controlling *S. frugiperda*, a field experiment was conducted in FAW infested Maize plots, Chikkaballapur District, Karnataka (13° 28′ N, 77° 72′ E, 918 m above sea level), India, during August–September 2019. The experiment was performed in two Maize (cv. Rishi) plots of 2000 m^2, with a plant spacing of (75 × 20 cm) where plants were sown (66,000 plants/10,000 m^2) following ridges and furrow method in red sandy loam soil. Field inspection was done during early whorl stage to check for FAW infestation (20 days old) and the releases were made during true whorl stage of maize (25 days old) when FAW infestation reached its peak. The experiment had two plots (T1 = control plot; T2 = experimental plot where *B. brevicornis* was released). There were 20 replicates per plot (1000 m^2). To estimate the larval population of FAW, five plants were randomly selected per replicate (total 100 plants/plot) and tagged for inspection (details in Figure S1a). Releases were made (at the rate of 4000 adults/ha) at weekly intervals at ~ 9.00 AM

when temperature varied between (20–25 °C); humidity was 56–84% and wind speed was 14–24 km/h (Meteorological Centre, Bangalore, India) during all the three releases. 24 h old parasitoids were used for release in 1:1 ratio (male: female). They were transferred to a plastic container covered with black muslin cloth. In the field, parasitoids were released at 5 spots (Figure S1a). Four releases were made by removing the muslin cloth and tapping the container while walking at a slow pace along the row and one in the middle of the plot (Figure S1a). These two maize fields were separated by a buffer plot (100 m) occupied by weeds and trees which reduced the probability of parasitoid movement. Pre-release larval density was counted before 24 h of parasitoid release and the number of FAW larvae/plant was recorded by thorough inspection post 72 h of release. Number of releases was not decided a-priori, based on the reduction in larval number and practicalities of field work, a total of three releases were made. Percent parasitism in larval population was estimated by counting the visible number of paralyzed/parasitized larvae after every release without destroying the plants. For analysis, the larval number recorded pre and post-release from the same tagged plants from the plots were considered. After three releases, to monitor the parasitoid activity in the field, five sentinel pouches with *Corcyra cephalonica* larvae (10 larvae/pouch) were introduced in T1 and T2 plots. These were collected post 24 h to check parasitization.

Statistical analyses

Data from no-choice test tube assay were analyzed using one-way ANOVA considering instar (3rd, 4th and 5th) as main effect and fecundity, larval hatching, percent pupae formation, adult emergence and percent female emergence as dependent variables. To meet conditions of normality, data on fecundity and percent pupae formation were arcsine and square root transformed, respectively. When ANOVA indicated a significant result ($\alpha < 0.05$), all the relevant means were compared using Tukey's post-hoc significance test at a level of 5%. For choice assay, Pearson's chi-square test was done considering the parasitoid behavior as a binary response. The performance of *B. brevicornis* on different host instars in cage study was assessed using generalized linear model (GLM) with logit link function. The larval instar was treated as an independent factor and parasitism as the response variable in a binomial logistic regression model.

Data on total number of eggs laid per larva, percent emergence and fecundity from cage study were compared among the host instars (4th and 5th) using paired *t* test. Cell viability and cell spreading assay data were compared between control (un-paralyzed) and paralyzed larvae using paired *t* test. Field data on larval population were tested using independent *t* test to compare the mean number of larvae/plant between released and control plot. The performance of *B. brevicornis* between three releases was compared using percent parasitism data achieved from T2 plot with generalized linear mixed model (GLMM) having a binomial distribution and logit link function. Here release was considered as fixed factor, plant as random factor nested within the replicate taken as larval number and the parasitism as the outcome. The parasitism was calculated by considering the fate of each larva (as either alive or parasitized) counted on a plant before and after parasitoid release. This analysis was done using "lme4" package in R statistical program (R core Team 2016). We have cross-validated our model to check overdispersion by comparing the deviance over df residuals and it falls within 1.2–1.5. All other statistical analyses in this study were performed using SPSS Statistics version 25.0 (IBM). Assumptions of normality and homoscedasticity were tested before each test.

Results

Performance and preference of *B. brevicornis* on two *Spodoptera* hosts

The lifetime fecundity of *B. brevicornis* was affected by the larval instar of *S. litura*. The highest fecundity was recorded on fifth instar larvae (253 ± 17 eggs, Suppl. video S1) while there was no significant difference between the third and fourth instar, which ranged between 138 and 173 (Table 1). There were no significant differences in percent larval hatching among the tested host instars (47–62%). However, highest percent pupa formation was observed on fifth instar (76%) compared to third and fourth instar. Percent adult emergence and the number of female emergence varied significantly; an average of 26 (± 1) *B. brevicornis* females emerged from 5th instar, which was highest among the instars tested. The female wasps survived for 19–26 days and host instar had no impact on their longevity (Table 1).

The lifetime fecundity of *B. brevicornis* was highest on fifth instar *S. frugiperda* larvae as the female wasp deposited 293(± 80) eggs (Table 2). Egg hatching percentage was lowest on third instar. Percent hatching was not significantly different between fourth and fifth instars (Table 2). 80% of the parasitoid larvae on the fifth instar larva formed pupae and adult emergence was also recorded highest on this host developmental stage (70%). The number of females emerged increased with host age, fifth instar recorded the highest number of females and the life span of these females was also high. Females emerged from fifth-instar host larva had a life span of 24 days while females from fourth and third instar lived for 13 and 10 days, respectively. Although percent hatching was not different between fourth and fifth instar, pupal formation, adult emergence, number of females

Table 1 Biology of *Bracon brevicornis* on different instars of *Spodoptera litura*

Larval instar	Fecundity (mean ± SE)	% hatching (mean ± SE)	% pupae formation (mean ± SE)	% adult emergence (mean ± SE)	No of females (mean ± SE)	Female life span (days) (mean ± SE)
3rd	$138^{b} \pm 42$	47 ± 1.5	$50^{b} \pm 3$	$50^{b} \pm 1.3$	$13^{b} \pm 3$	19 ± 4
4th	$173^{b} \pm 32$	53 ± 5	$52^{b} \pm 5$	$52^{b} \pm 4$	$18^{b} \pm 3$	24 ± 5
5th	$253^{a} \pm 17$	62 ± 8	$76^{a} \pm 6$	$78^{a} \pm 9$	$26^{a} \pm 4$	26 ± 1
$F_{2, 27}$	8	3.2	24.7	25.5	9.4	3.5
P	< 0.001	$= 0.06$	< 0.0001	< 0.0001	< 0.0001	$= 0.06$

Data represent mean ± SE, different letters indicate statistical differences based on Tukey's post hoc test after one-way ANOVA ($n = 10$)

Table 2 Biology of *Bracon brevicornis* on different instars of *Spodoptera frugiperda*

Larval instar	Fecundity (mean ± SE)	% hatching (mean ± SE)	% pupae formation (mean ± SE)	% adult emergence (mean ± SE)	No of females (mean ± SE)	Female life span (days) (mean ± SE)
3rd	$108^{c} \pm 4.7$	$23^{b} \pm 1.5$	$25^{c} \pm 8$	$24^{c} \pm 4$	$11^{c} \pm 3$	$10^{b} \pm 0.8$
4th	$190^{b} \pm 43$	$47^{a} \pm 5.4$	$47^{b} \pm 5.3$	$45^{b} \pm 5.3$	$18^{b} \pm 5$	$13^{b} \pm 1.4$
5th	$293^{a} \pm 80$	$57^{a} \pm 8.5$	$80^{a} \pm 3.5$	$70^{a} \pm 6.2$	$33^{a} \pm 10$	$24^{a} \pm 5$
$F_{2, 12}$	5.48	9.8	41	28	13	8.1
P	$= 0.002$	$= 0.003$	< 0.0001	< 0.0001	$= 0.002$	$= 0.005$

Data represent mean ± SE, different letters indicate statistical differences based on Tukey's post hoc test after one-way ANOVA ($n = 5$)

and their life span was higher on fifth instar compared to fourth instar (Table 2). Third instar larvae recorded lowest on all these parameters among all the three developmental stages tested. Taken together, these results show that *B. brevicornis* development is dependent on host instar and fourth–fifth host larval stage is most suitable in the case of both *Spodoptera* hosts.

In two-choice experiment in a petri dish, the female wasp always preferred to parasitize the fourth or fifth instar larvae against third instar of both the tested hosts. When the choice was between fourth and fifth instar *S. litura*, *B. brevicornis* chose fourth over fifth instar larvae (Fig. 1a) ($P < 0.0001$). In case of *S. frugiperda*, there was no preference for the same treatment (Fig. 1b) ($P = 0.3$).

Parasitism rates and fitness of *B. brevicornis* on infested maize

When *S. litura* larvae on *Zea mays* were presented to *B. brevicornis* in a cage, mimicking natural conditions, their parasitism rate was 8 (± 5), 72 (± 5.2) and 76 (± 7.4) % against 3rd, 4th and 5th instar, respectively (Fig. 2a). *B. brevicornis* showed a higher parasitism rate on fourth- and fifth-instar *S. litura* larvae over 3rd instar similar to results obtained in earlier choice assay in the absence of the plant. The performance on *S. frugiperda* showed similar results, where 16 (± 4), 76 (± 4) and 84 (± 5.6) % parasitism was recorded on 3rd, 4th and 5th instar, respectively (Fig. 2b) ($P < 0.0001$). The average number of parasitoid eggs laid on fourth and fifth instar of *S. litura* larvae was 5.9 (± 1.3) and 5.5 (± 1.2), respectively. These parasitized larvae with eggs were incubated at laboratory conditions. From these eggs, 70% of adults could emerge from fourth instar while fifth instar supported 66% of adult emergence from *S. litura* (Fig. 3a, b). Although statistically there was no significant difference between fourth and fifth instar larvae of *S. litura* in terms of *B. brevicornis* oviposition and adult emergence, the adult longevity was higher from fifth instar host (Fig. 3c, $P = 0.0003$).

In case of *S. frugiperda*, the female parasitoid deposited 5.4 (± 0.8) on fourth and 6.6 (± 1.3) number of eggs on fifth instar larvae (Fig. 3d). In laboratory conditions, the adult emergence rate from fourth and fifth instar ranged from 57 to 62%. (Fig. 3e). Our results show that *B. brevicornis* can perform equally well on fourth and fifth instar of larvae of the hosts. However, the longevity of the emerged adults from fifth instar was significantly higher (Fig. 3f, $P = 0.004$).

Alteration in host immune-competence

Total hemocyte numbers were less in paralyzed larvae compared to control. In paralyzed larvae, the viability and spreading capacity of hemocytes were significantly reduced (Fig. 4a, c) in case of *S. litura*. Staining with

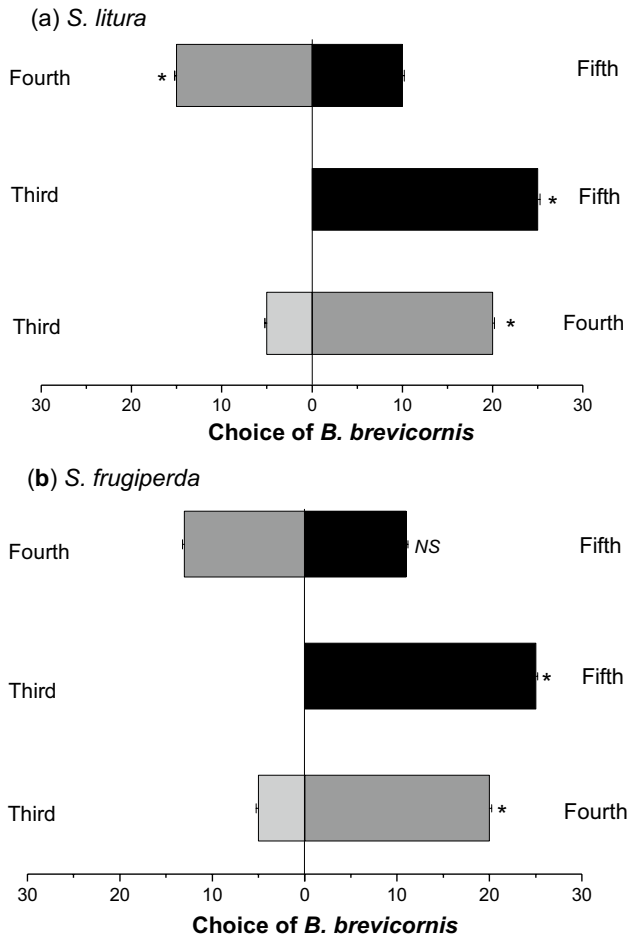

Fig. 1 Two-choice test of *B. brevicornis*. Choice of third vs fourth, third vs fifth and fourth vs fifth instar larvae of **a** *S. litura*, **b** *S. frugiperda* were offered to female parasitoids. Complete paralysis of the host was counted. Statistical significance is based on Pearson's chi-square test ($n = 25$)

phalloidin-DAPI showed these hemocytes had membrane blebbing and ruptured structure due to which they lost their spreading behavior (Fig. 4e, f). Parasitoid-induced immune suppression did not differ significantly from *S. litura* in case of *S. frugiperda* larvae. The viable hemocytes number was reduced after paralysis by *B. brevicornis* and these hemocytes rapidly lost their shape and spreading ability (Fig. 4b, g). Staining with phalloidin-DAPI showed similar morphology as in the case of *S. litura* (Fig. 4d, h). Also, all the replications under same treatment had remarkable consistency in their outcome. These results indicate that host regulation by *B. brevicornis* affects cellular immunity of host larvae and does not differ significantly between the two *Spodoptera* sp. tested.

From lab to field: *B. brevicornis* performance on FAW under field conditions

To test the potential of *B. brevicornis* in pest management, the wasps were released in a FAW infested maize field (Fig. S2, suppl. video S3). *Bracon brevicornis* were released three times at weekly intervals and the average number of larvae recorded per plant after each release was significantly low compared to control plot (T1) ($P < 0.0001$) (Fig. 5a; Figure S1b-c). Compared to pre-release, a total larval reduction of 65, 47 and 50% was observed in our treated plot after first, second and third release, respectively. Percent parasitism by *B. brevicornis* in treatment plot ranged between 10 and 22% after releases. There was no significant difference between different releases ($P = 0.34$) (Fig. 5b). After the releases, the remaining live larvae were noted to be largely in early developmental stages (1st, 2nd and 3rd instar), which is possibly be due to fresh egg laying by moths coming from the neighboring fields or larvae which escaped from parasitization and could complete their life cycle (Figure S2). To check the presence of parasitoids in treated and control plots, sentinel pouches containing *C. cephalonica* larvae were kept in both plots after our experiment. No parasitization was recorded in control plot (T1) while in the experimental plot (T2), 40% of *C. cephalonica* larvae were parasitized by *B. brevicornis*.

Discussion

Appropriate host selection is crucial for parasitoid progeny development (Mattiacci and Dicke 1995) and host developmental stage influences parasitoid attack rate, survival and sex ratio (Jervis et al. 2007; Kant et al. 2012; Li et al. 2006). Here, using *S. litura* and *S. frugiperda*, we examined the performance of *B. brevicornis* on different host developmental stages. It is interesting to note that three larval instars (3–5th) of both hosts were susceptible to *B. brevicornis* attack and the parasitoid could complete its lifecycle on 3–5th instar of these two hosts in the laboratory. However, the performance of the parasitoid in terms of average number of eggs laid, adult emergence and lifespan was best on 5th followed by 4th instar of the hosts, which indicate that late larval stages of these *Spodoptera* hosts are most suitable for *B. brevicornis* development. Our results are in accordance with Malesios and Prophetou-Athanasiadou (2014), where the biology of *B. brevicornis* on *Plodia interpunctalla* (Hübner) was shown to be strongly affected by host larval instar and preference of late instars was reported. Host suitability depends on factors like oviposition attempts, nutrient content and successful progeny development from them (Wiedenmann et al. 1992). Further, *B. brevicornis* is a gregarious parasitoid and their developing larvae acquire nutrients by constant feeding of host hemolymph (Suppl.

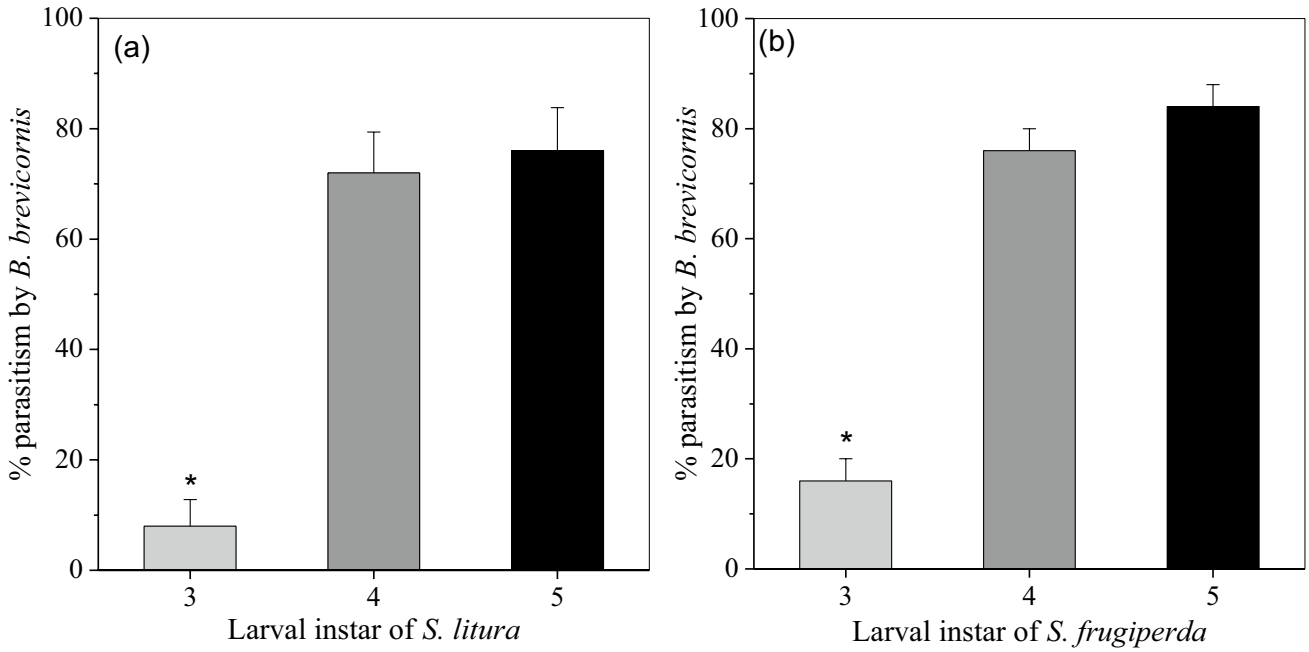

Fig. 2 Percent parasitism by *B. brevicornis* in the presence of host plant against **a** *S. litura* and **b** *S. frugiperda* in cage study. Data represent mean ± SE, statistical difference is based on generalize linear model ($n = 5$). 3: third, 4: fourth and 5: fifth instar larvae

video S2). Hence, the host must serve as an adequate nutrient pool to support more than one developing parasitoid larvae until they reach pupal stage. As fourth–fifth instar larvae contain higher amount of hemolymph (approx. 60 ul), they probably serve as better hosts. Although *B. brevicornis* could attack and parasitize all instars tested, the performance was best on late instar and choice test done clearly indicated that *B. brevicornis* prefers older instar larvae of both hosts tests. Whether *B. brevicornis* uses odors or other cues to distinguish larval instar remains to be studied.

Analysis by Brodeur and Vet (1995) shows that often endo-parasitoid oviposit in the early instars to avoid the well-developed immune system of the host. In the case of *S. litura*, previous study shows that there is an increase in immune response with ontogeny (Ghosh and Venkatesan 2019). However, the immune status did not influence the behavior of *B. brevicornis* as they could down-regulate cellular immunity of both hosts at fourth and fifth instar stages. Since late larval stages would serve as better nutrient resources and parasitoid-derived factors could successfully suppress host cellular immunity, *B. brevicornis* performance was higher on fourth and fifth instar and the female wasp showed a preference for late larval hosts in two-choice assay. Differences in the biochemical status, immune-competence, humoral immune responses and/or odor of host larvae could contribute towards parasitoid preference. Further, we tested the impact of host instar on parasitoid progeny. Since female offspring are more important for reproductive output (Godfray 1994) and for further biological applications, we assessed the number of females emerging from different host instars. Similar to earlier results, fourth or fifth-instar host larvae supported more female parasitoids. Taken together, our findings show that selection of late larval instar hosts increases the overall fitness of *B. brevicornis*.

Next, we evaluated if the parasitoid interaction is altered in the presence of host plant, *Zea mays*. Previous studies showed that host plant on which the herbivorous larvae develop can significantly alter parasitoid behavior (Li et al. 2014). The attraction of parasitoids to volatiles released by herbivore damaged plants is well documented. Such herbivore-induced plant volatiles (HIPV) play pivotal role in host location. For example, females of *Cotesia vestalis* (Haliday) are attracted only by HIPV from *Brassica rapa* plants infested with *Plutella xylostella* (Linnaeus) (Kugimiya et al. 2010). Such HIPV also help parasitoids to distinguish between larval stages, plant species and different levels of damage (Girling et al. 2011). These studies show that host plant can impact parasitoid host choice. In our study, *B. brevicornis* showed a similar preference for older instars in the presence and absence of host plant. In test tube assay, 3rd instar host could support parasitoid egg hatching and subsequent development, however, on host plants, *B. brevicornis* showed very low parasitism rate and did not oviposit or develop on 3rd instar larvae of both the hosts. This could be due to pre-oviposition interactions between host and parasitoid. We observed that, early instar larvae are comparatively more agile and can also hide well beneath the leaves, which could make the oviposition experience difficult

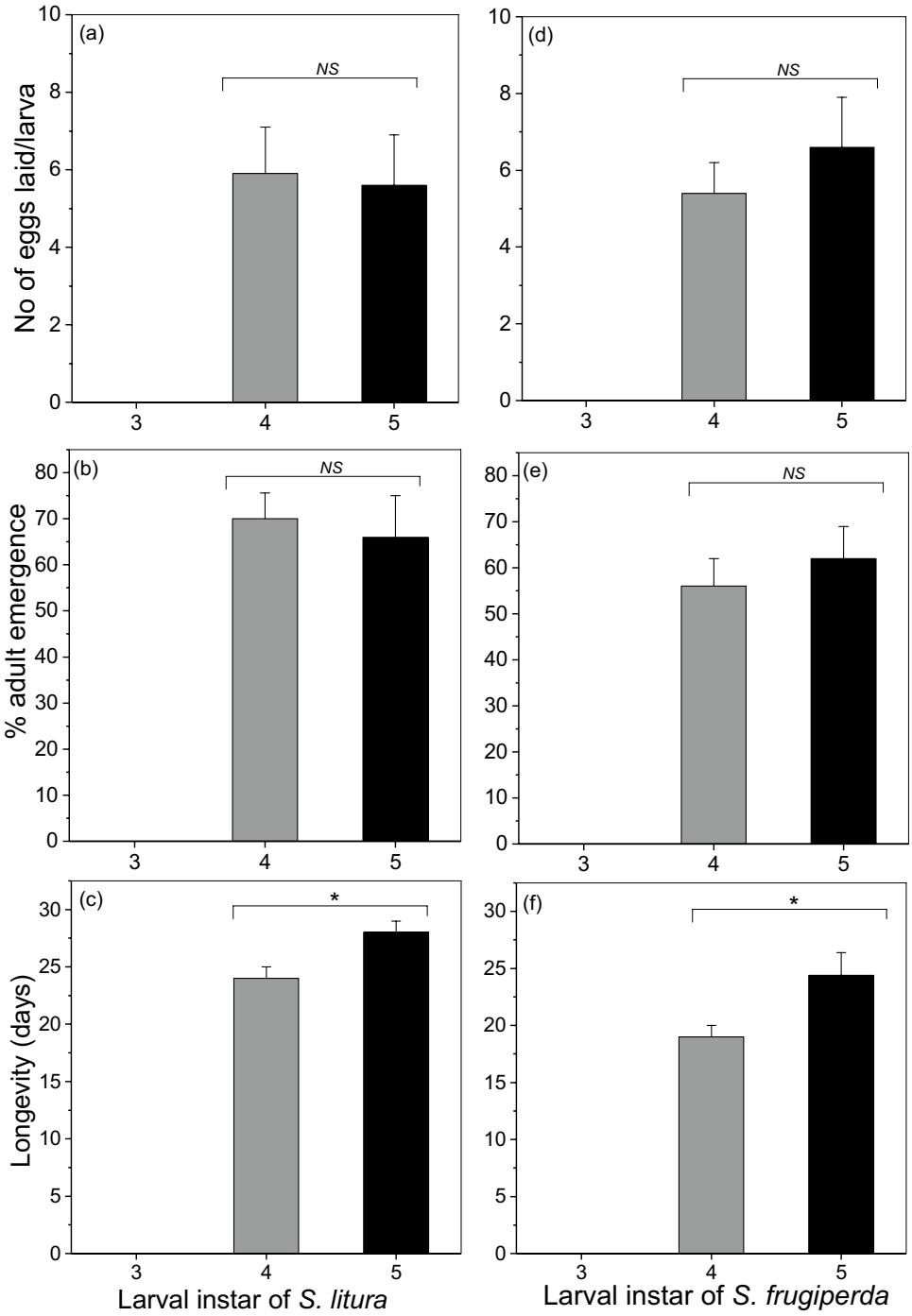

Fig. 3 Biology of *B. brevicornis* during cage study. No of eggs laid per larva in the presence of host plant, percent adult emergence and longevity of the adult emerged from parasitized larvae incubated at room temperature were recorded on **a, b, c** *S. litura* instars and **d, e, f** *S. frugiperda* larval instars, respectively. (Data represent mean ± SE, statistical differences are based on paired *t*- test ($n = 10$). 3: third, 4: fourth and 5: fifth instar larvae

for *B. brevicornis*. This is in line with previous study where parasitoid was reported to use larval cues to discriminate hosts and avoid hosts that counterattack by thrashing, biting, and regurgitating (Feltwell 1982). The role of plant volatiles induced by different larval instars on parasitoid choice warrants further investigation.

Encouraged by laboratory-based study, we evaluated the performance of *B. brevicornis* in a FAW infested maize field. Our results show that *B. brevicornis* can efficiently reduce the infestation and parasitize FAW larvae even under field conditions. Our first release showed a high reduction in larval population compared to second and third release due to the presence of high late instar larval population (4th and 5th instars). Since no other pest control was implemented in the experimental plot, factors such as fresh egg laying by moths from existing or neighboring field, presence of younger instar host larvae (during second and third release) and/or un-parasitized

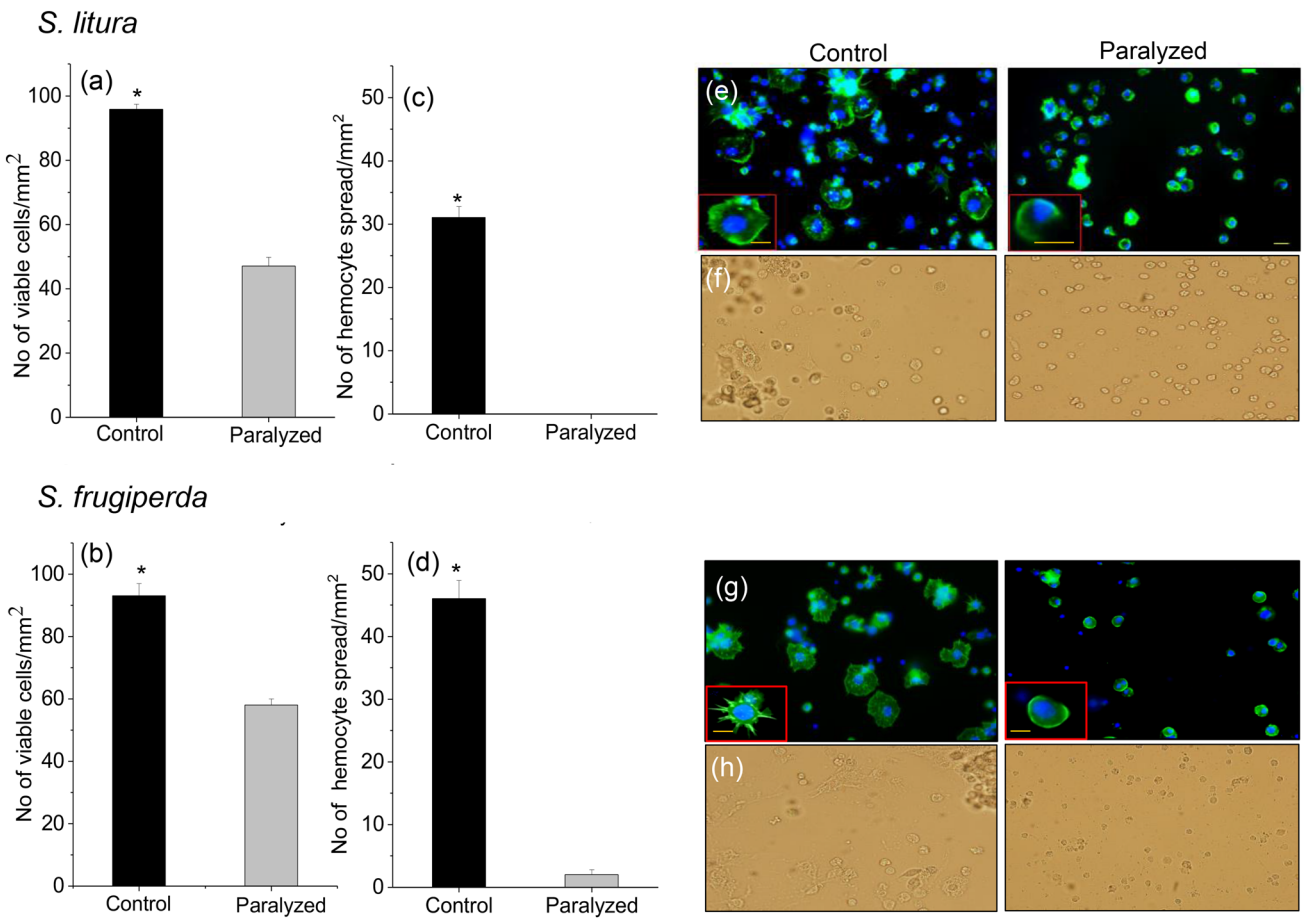

Fig. 4 Host regulation by *B. brevicornis*. Changes in hemocyte viability and spreading post paralysis of **a, b** *S. litura and* **c, d** *S. frugiperda*. Phalloidin-DAPI stained hemocytes in **e** control and paralyzed larvae of *S. litura* **f** control and paralyzed larvae of *S. frugiperda*. Alteration in hemocyte spreading activity in **g** control and paralyzed *S. Litura* **h** control and paralyzed *S. frugiperda.* Data represent mean ± SE, statistical differences are based on paired *t* test ($n = 5$), bars: 10 µm

larvae could be possible reasons for less percent reduction in larval population during other releases compared to the first. However, the significant difference between control and experimental plot after release indicates that *B. brevicornis* could be used in augmentative control of FAW. Interestingly, we found no movement of *B. brevicornis* between control and release plot which further strengthens our findings. Also, compared to the pre-release larval number, the average larvae per plant reduced significantly after three releases. The visible parasitism rate of in the field ranged from 10 to 22% in line with previous studies on *Bracon hebetor* and black-headed caterpillar where 8–27% parasitism rate was reported in coconut palm field (Rao et al. 2018). Since we counted only visible larvae, it is possible that our sampling method could be an underestimation of parasitism rate. Additionally, overlapping generations of FAW were found in the field that could lead to longer search time by the parasitoids. More releases could have also yielded better parasitism rate; however, in view of rapid crop growth, our experiment was terminated after three releases to avoid destructive sampling.

Taken together, *B. brevicornis* can be employed in combination with egg parasitoids like *Telenomus remus* (Kenis et al. 2019) and *Trichogramma* spp. to obtain greater success in pest management. Interestingly, *B. brevicornis* feeds on host hemolymph prior to egg laying. They also fed on third-instar larvae post paralysis, thus killing it. Hence, we anticipate that they might be able to control third-instar larvae also. These results make *B. brevicornis* a potential candidate for biological control program of these hosts in real field conditions. Furthermore, *B. brevicornis* is a generalist parasitoid and can produce a very high number of progeny on various lepidopteran pests like *Corcyra cephalonica*; mass production can easily be done by insectaries. In summary, the current study shows that *B. brevicornis* can be an effective biological control agent against *S. frugiperda* and *S. litura* larvae. Our findings provide valuable information about *B. brevicornis* and its

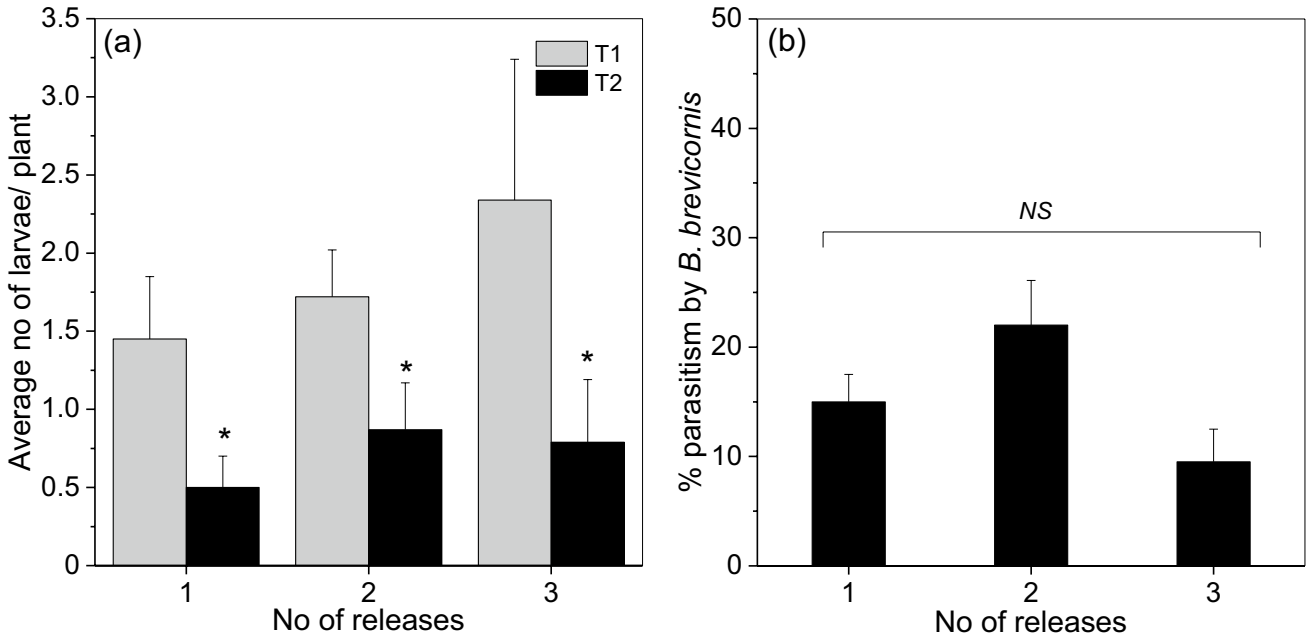

Fig. 5 The effectiveness of *B. brevicornis* in a FAW infested maize field. **a** Average number of larvae/plant after each release in T1 (Control plot) and T2 (experimental plot) **b** Percent parasitism by *B. brevicornis*. Data represent mean ± SE, statistical significance is based on *t* test and generalised linear mixed model ($n = 100$), respectively

role for the development of a biological control program and IPM strategies against the invasive pest *S. frugiperda*.

Author contributions

EG and RV designed and carried out experiments, performed field work, analyzed data and drafted the manuscript. RV conceptualized, supervised the project and experimental design, performed field work, acquired resources and drafted the manuscript. All authors read and approved the final manuscript.

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Declaration

Conflict of interest The authors declare that they have no conflict of interest.

References

Bakr RF, Gesraha MA, Guneidy NA, Farag NA, Ebeid AR, Elbehery HH, Abou-Ellail M (2014) Biological studies on *Bracon brevicornis* (Hymenoptera: Braconidae) reared on different host larvae. Egypt Acad J Biol Sci 7(2):89–95. https://doi.org/10.21608/eajbsa.2014.13138

Brodeur J, Vet LEM (1995) Relationships between parasitoid and host range and host defense: a comparative study of egg encapsulation in two related parasitoid species. Physiol Entomol 20:7–12. https://doi.org/10.1111/j.1365-3032.1995.tb00794.x

Brown ES, Dewhurst CF (1975) The genus *Spodoptera* (Lepidoptera, Noctuidae) in Africa and the Near East. Bull Entomol Res 65(2):221–262. https://doi.org/10.1017/S0007485300005939

Charnov EL, Skinner SW (1985) Complementary approaches to the understanding of parasitoid oviposition decisions. Environ Entomol 14:383–391. https://doi.org/10.1093/ee/14.4.383

Cheng T, Wu J, Wu Y, Chilukuri RV, Huang L, Yamamoto K, Feng L, Li W, Chen Z, Guo H, Liu J, Li S, Wang X, Peng L, Liu D, Guo Y, Fu B, Li Z, Liu C, Chen Y, Tomar A, Hilliou F, Montagné N, Jacquin-Joly E, d'Alençon E, Seth RK, Bhatnagar RK, Jouraku A, Shiotsuki T, Kadono-Okuda K, Promboon A, Smagghe G, Arunkumar KP, Kishino H, Goldsmith MR, Feng Q, Xia Q, Mita K (2017) Genomic adaptation to polyphagy and insecticides in a major East Asian noctuid pest. Nat Ecol Evol 1:1747–1756. https://doi.org/10.1038/s41559-017-0314-4

Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, Colmenarez Y, Corniani N, Early R, Godwin J, GomezJ MPG, Murphy ST, Oppong-Mensah B, Phiri N, Pratt C, Silvestri S, Witt A (2017) Fall Armyworm: impacts and implications for Africa. Outlooks Pest Manag 28:196–201. https://doi.org/10.1564/v28_oct_02

Dhir BCM, Mohapatra HK, Senapathi B (1992) Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian J Plant Prot 20:215–217

Feltwell J (1982) Large white butterfly: the biology, biochemistry and physiology of *P. brassicae* (L.). W. Junk, The Hague, p 273

Firake DM, Behere GT (2020) Natural mortality of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize agroecosystems of North-East India. Biol Control 148:104303–104310. https://doi.org/10.1016/j.biocontrol.2020.104303

Ghosh E, Venkatesan R (2019) Plant volatiles modulate immune responses of *Spodoptera litura*. J Chem Ecol 45(8):715–724. https://doi.org/10.1007/s10886-019-01091-3

Girling RD, Stewart-Jones A, Dherbecourt J, Staley JT, Wright DJ, Poppy GM (2011) Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. Proc Biol Sci 278:2646–2653. https://doi.org/10.1098/rspb.2010.2725

Godfray HCJ (1994) Parasitoids: behavior and evolutionary ecology. Princeton University Press, Princeton

Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS ONE 11:e0165632. https://doi.org/10.1371/journal.pone.0165632

Gols R, Bukovinszky T, van Dam NM, Dicke M, Bullock JM, Harvey JA (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. J Chem Ecol 34:132–143. https://doi.org/10.1007/s10886-008-9429-z

Gupta A, Soujanya PL, VAN Achterberg C, Sekhar JC (2020) *Coccygidium transcaspicum* (Kokujev) (Hymenoptera: Braconidae) parasitizing larvae of invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in India. Zootaxa. 4750(2). https://doi.org/10.11646/zootaxa.4750.2.13

Jervis MA, Ellers J, Harvey JA (2007) Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annu Rev Entomol 53:361–385. https://doi.org/10.1146/annurev.ento.53.103106.093433

Kant R, Minor MA, Trewick SA (2012) Fitness gain in a koinobiont parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) by parasitising hosts of different ages. J Asia Pac Entomol 15:83–87. https://doi.org/10.1016/j.aspen.2011.08.011

Kares EA, Ebaid GH, El-Sappagh IA (2009) Biological studies on the larval parasitoid species *Bracon brevicornis* Wesm. (Hymenoptera: Braconidae), reared on different insect hosts. Egypt J Biol Pest Control 19:165–168. https://doi.org/10.21608/eajbsa.2014.13138

Kenis M, Du Plessis H, Van den Berg J, Ba MN, Goergen G, Kwadjo KE, Baoua I, Tefera T, Buddie A, Cafà G, Offord L (2019) *Telenomus remus*, a candidate parasitoid for the biological control of *Spodoptera frugiperda* in Africa, is already present on the continent. Insects 10:92. https://doi.org/10.3390/insects10040092

Khan MAZ, Liang QF, Maria MSM, Liu TX (2016) Effect of temperature on functional response of *Aphidius gifuensis* (Hymenoptera: Braconidae) parasitizing *Myzus persicae* (Hemiptera: Aphididae). Florida Entomol 99:696–702. https://doi.org/10.1653/024.099.0419

Kugimiya S, Shimoda T, Tabata J, Takabayashi J (2010) Present or past herbivory: a screening of volatiles released from *Brassica rapa* under caterpillar attacks as attractants for the solitary parasitoid, *Cotesia vestalis*. J Chem Ecol 36:620–628. https://doi.org/10.1007/s10886-010-9802-6

Li J, Coudron TA, Pan W, Liu X, Lu Z, Zhang Q (2006) Host age preference of *Microplitis mediator* (Hymenoptera: Braconidae), an endoparasitoid of *Mythimna separata* (Lepidoptera: Noctuidae). Biol Control 39:257–261. https://doi.org/10.1016/j.biocontrol.2006.09.002

Li XW, Niu YQ, Liu TX (2014) The performance of *Oomyzus sokolowskii* (Hymenoptera: Eulophidae) parasitizing *Plutella xylostella* (Lepidoptera: Plutellidae) on different host plants. Appl Entomol Zool 49:67–75. https://doi.org/10.1007/s13355-013-0225-z

Malesios GA, Prophetou-Athanasiadou DA (2014) Development of the parasitoid *Bracon brevicornis* on different larval instars (L2–L5) of the Indian meal moth *Plodia interpunctella*. Entomol Hell 23:59–64. https://doi.org/10.12681/eh.11537

Mattiacci L, Dicke M (1995) Host searching behaviour of *Cotesia glomerata*, a larval parasitoid of *Pieris brassicae*: Infochemical use in the selection of the most profitable caterpillar instars. Entomol Exp Appl 76:39–50. https://doi.org/10.1007/BF01995321

Natikar PK, Balikai RA (2015) Screening of different varieties of soybean for their ovipositional preference to *Spodoptera litura* (Fabricius) under field conditions. J Exp Zool India 18(2):975–977

Pan MZ, Wang L, Zhang CY, Zhang LX, Liu TX (2017) The influence of feeding and host deprivation on egg load and reproduction of an aphid parasitoid, *Aphidius gifuensis* (Hymenoptera: Braconidae). Appl Entomol Zool 52:255–263. https://doi.org/10.1007/s13355-016-0474-8

Pogue MG (2002) A world revision of the genus *Spodoptera* Guenee (Lepidoptera: Noctuidae). Mem Am Entomol Soc 43:1–202

Prasanna BM, Huesing JE, Regina Eddy R, Peschke VM (2018) Fall Armyworm in Africa: a guide for integrated pest management. vii, México. CIMMYT, USAID

Qian C, Liu Y, Fang Q, Min-Li Y, Liu SS, Ye GY, Li YM (2013) Venom of the ectoparasitoid, *Nasonia vitripennis*, influences gene expression in *Musca domestica* hemocytes. Arch Insect Biochem Physiol 83(4):211–231. https://doi.org/10.1002/arch.21107

Quicke DLJ (2015) The braconid and ichneumonid parasitoid wasps: biology, systematics, evolution and ecology. Wiley, Oxford

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rao NBC, Nischala A, Ramanandam G, Maheswarappa HG (2018) Biological suppression of coconut black headed caterpillar *Opisina arenosella* outbreak in East Godavari district of Andhra Pradesh-eco friendly technology. Curr Sci 115(8):1588–1594. https://doi.org/10.18520/cs/v115i8/1588-1594

Sarfraz M, Dosdall LM, Keddie BA (2009) Host plant nutritional quality affects the performance of the parasitoid *Diadegma insulare*. Biol Control 51:34–41. https://doi.org/10.1016/j.biocontrol.2009.07.004

Scholler M, Flinn PW, Grieshop MJ, Zdarkova E (2006) Biological control of stored product pests. In: Heaps JW (ed) Insect management for food storage and processing, 2nd edn. AACC International, St. Paul, pp 67–87

Sharanabasappa KCM, Asokan R, MahadevaSwamy HM, Maruti MS, Pavithra HB, Hegde K, Navi S, Prabhu ST, Georgen G (2018) First report of the Fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Mana Hortic Ecosyst 24:23–29

Shylesha AN, Jalali SK, Gupta A, Varshney R, Venkatesan T, Shetty P, Ojha R, Ganiger PC, Navik O, Subaharan K, Bakthavatsalam N, Ballal CR (2018) Studies on new invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and its natural enemies. J Biol Control 32(3):1–7. https://doi.org/10.18311/jbc/2018/21707

Sisay B, Simiyu J, Malusi P, Likhayo P, Mendesil E, Elibariki N, Wakgiri M, Ayalew G, Tefera T (2018) First report of fall armyworm, *Spodoptera frugiperda* (Lepidoptera:Noctuidae), natural enemies from Africa. J Appl Entomol 142(8):800–804. https://doi.org/10.1111/jen.12534

Srinivasan T, Chandrikamohan (2017) Population growth potential of *Bracon brevicornis* Wesmael (Braconidae: Hymenoptera): A life table analysis. Acta Phytopathol Entomol Hung 52:123–129. https://doi.org/10.1556/038.52.2017.010

Stoepler TM, Lill JT, Murphy SM (2011) Cascading effects of host size and host plant species on parasitoid resource allocation. Ecol Entomol 36:724–735. https://doi.org/10.1111/j.1365-2311.2011.01322.x

Suby SB, Soujanya PL, Yadava P, Patil J, Subaharan K, Prasad GS, Babu KS, Jat SL, Yathish KR, Vadassery J, Kalia VK, Bakthavatsalam N, Shekhar JC, Rakshit S (2020) Invasion of fall armyworm (*Spodoptera frugiperda*) in India: nature, distribution, management and potential impact. Curr Sci 119(1):44–51. https://doi.org/10.18520/cs/v119/i1/44-51

Temerak SA (1984) Suitability of five lepidopteran host insects to the ectolarval parasitoid, *Bracon brevicornis* Wesmael. Z Angew Entomol 97:210–213. https://doi.org/10.1111/j.1439-0418.1984.tb03739.x

Teng ZW, Xu G, Gan SY, Chen X, Fang Q, Ye GY (2016) Effects of the endoparasitoid *Cotesia chilonis* (Hymenoptera: Braconidae) parasitism, venom, and calyx fluid on cellular and humoral immunity of its host *Chilo suppressalis* (Lepidoptera: Crambidae) larvae. J Insect Physiol 85:46–56. https://doi.org/10.1016/j.jinsphys.2015.11.014

Weis AE, Abrahamson WG (1985) Potential selective pressures by parasitoids on a plant-herbivore interaction. Ecology 66:1261–1269. https://doi.org/10.2307/1939179

Wiedenmann RN, Smith JW, Darnell PO (1992) Laboratory rearing and biology of the parasite *Cotesia flavipes* (Hymenoptera: Braconidae) using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as a host. Environ Entomol 21:1160–1167. https://doi.org/10.1093/ee/21.5.1160

Yang L, Wan B, Wang B-B, Liu M-M, Fang Q, Song Q-S, Ye G-Y (2019) The Pupal Ectoparasitoid *Pachycrepoideus vindemmiae* regulates cellular and humoral immunity of host *DROSOPHILA melanogaster*. Front Physiol 10:1282. https://doi.org/10.3389/fphys.2019.01282