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B M Sokame, J Obonyo, E M Sammy, Samira A. Mohamed, Sevgan Subramanian, et al.. Impact of the exotic fall armyworm on larval parasitoids associated with the lepidopteran maize stemborers in Kenya. *BioControl*, 2020, 66, pp.193-204. <10.1007/s10526-020-10059-2>. <hal-04293832>

HAL Id: hal-04293832

<https://hal.science/hal-04293832v1>

Submitted on 22 Nov 2023

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Impact of the exotic fall armyworm on larval parasitoids associated with the lepidopteran maize stemborers in Kenya

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Received: 11 August 2020 / Accepted: 12 October 2020

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Abstract Exotic invasive insect herbivores have the potential to interfere with existing herbivore-natural enemy interactions in new environments. Fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a new invasive pest in maize fields in Africa. Understanding the acceptability and suitability of FAW to existing maize stemborer-parasitoid interactions is the first step in elucidating the impact that this exotic insect pest can have on the existing natural enemies used in biological control of maize stemborers in Kenya. The most commonly used

larval parasitoids for biological control programs against maize stemborer communities in East Africa are *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) and two populations of the native *Cotesia sesamiae* (Cs-Inland and Cs-Coast) Cameron (Hymenoptera: Braconidae). All these parasitoid species attacked FAW larvae but none yielded offspring, although they induced high non-reproductive host mortality when compared to natural mortality. Furthermore, the parasitoids that inserted their ovipositor into FAW larvae exhibited no significant preference between FAW larvae and their respective stemborer hosts under dual-choice bioassays. In olfactometer bioassays, the parasitoids were more attracted to plants infested by FAW than uninfested plants and even showed a marked preference for the odours of plants infested by FAW over those of plants infested by their natural host counterparts. This study illustrates that exotic pests, such as FAW, can impact existing stemborer-parasitoid interactions associated with maize, even if they cannot be used as hosts by parasitoids associated with these stemborers. Although additional studies are needed, FAW might therefore have a negative impact on stemborer biological control existing before its invasion.

Handling Editor: Stefano Colazza

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Keywords *Busseola fusca* · *Chilo partellus* · *Sesamia calamistis* · *Sesamia nonagrioides* · Biological control · *Cotesia flavipes* · *Cotesia sesamiae* · *Cotesia typhae* · Non-reproductive host mortality

Introduction

Generally, exotic invasive insect herbivores have the potential to interfere significantly with existing herbivore-natural enemy interactions in invasion zones (Desurmont et al. 2014; Chabaane et al. 2015; Martorana et al. 2017). Exotic insect herbivores may interfere with the foraging behaviour of natural enemies as follows. Natural enemies such as parasitoids use the volatiles emitted by plants infested by their host insect herbivores in order to localize them easily from a distance (Vinson 1975). Interferences can occur if these volatiles attract the parasitoids towards unsuitable new exotic insect herbivores or if the plants infested by both exotic and native herbivores emit volatiles that are less attractive to parasitoids (Chabaane et al. 2015). After landing on infested plants, the parasitoids can attack (i.e., oviposit on) unsuitable exotic insect herbivores but be unable to develop in them, wasting time and energy, and this can negatively affect the fitness of the parasitoids (Hoogendoorn and Heimpel 2002; Chabaane et al. 2015). These interferences can have detrimental consequences on a pre-existing biological control process (Hoogendoorn and Heimpel 2002).

Few studies have explored the impact of exotic invasive insect herbivores on existing herbivores-natural enemy interactions (Chabaane et al. 2015; Martorana et al. 2017). Most previous studies have focused on the use of exotic natural enemies of insect herbivores in classical biological control programs, for example, and the consequences of introducing these exotic natural enemies on native parasitoid-host interactions (Chabaane et al. 2015). Similar to a study by Chabaane et al. (2015), we hypothesized that exotic insect herbivores impact existing herbivore-natural enemy interactions, even if they cannot be used as hosts by natural enemies already present in the new environment.

In Sub-Saharan Africa, there is a new destructive pest of maize *Zea mays* L. (Hruska and Gould 1997), the fall armyworm (FAW) *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), which originated from the Americas. It started to invade Africa in 2016 (Goergen et al. 2016) and has spread throughout maize fields all over sub-Saharan Africa, causing up to 11.5 and 30% yield losses in Ethiopia and Kenya, respectively (Kassie et al. 2020; De Groot et al. 2020). Before the introduction of FAW in Kenya, an

effective biological control program for the maize stemborer communities with larval parasitoids was implemented (Midingoyi et al. 2016). The most commonly used larval parasitoids are *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), used in a classical biological control programs against the invasive *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) (Overholt et al. 1994), and two populations of the native *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), *Cs-Coast* and *Cs-Inland*, controlling *Sesamia calamistis* Hampson and *Busseola fusca* Fuller (Lepidoptera: Noctuidae), respectively (Kfir et al. 2002). In addition, *Cotesia typhae* Fernández-Triana (Hymenoptera: Braconidae), a recently described species, was found to be associated with *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae) in Kenya (Kaiser et al. 2017).

The invasion of FAW is likely to interfere with pre-existing trophic interactions between maize, stemborers, and the stemborers' natural enemies in Africa as demonstrated by Desurmont et al. (2014) and Chabaane et al. (2015). In maize fields, FAW second instars are commonly found with stemborer species in the whorl of the plants in communities of mixed species (Sokame 2020). It is also frequent to find older FAW larvae in the bored holes and tunnels made by stemborers, as well as FAW larvae feeding on corn with stemborer larvae (Sokame 2020). This suggests that FAW and the native African stemborers are sharing the same ecological niche. Such associations of FAW with existing trophic interactions could potentially impact the biological control efforts of both FAW and maize stemborers. However, little is known about the interactions of FAW with lepidopteran stemborer communities and their natural enemies in maize fields. Such information could help understand how parasitoids associated with maize stemborers might cope with the invader FAW in Sub-Saharan Africa. We focused our study on the consequences of the introduction of FAW on the foraging behaviour and reproductive output of *C. flavipes*, two *C. sesamiae* populations, and *C. typhae*. Specifically, we addressed the following questions: (1) Are FAW larvae attacked and suitable to *C. flavipes*, two *C. sesamiae* populations (*Cs-Coast* and *Cs-Inland*), and *C. typhae*? For the most effective parasitoid species attacking FAW: (2) Do they prefer to attack FAW larvae as compared to their natural host? (3) Are they attracted to volatiles from maize plants infested by

FAW larvae? (4) Do they prefer volatiles emitted by plants infested by their natural host over volatiles from FAW-infested plants?

We investigated the first question in no-choice conditions by observing attacks of FAW larvae by each parasitoid species in Petri dishes and then by following the development of attacked FAW larvae. The second question was investigated using observations of attacks on two herbivores (two-choice conditions) by stemborer parasitoids in Petri dish bioassays. The third and fourth questions were addressed by using behavioural Y-tube olfactometer tests.

Material and methods

Fall armyworm and stemborer colonies

A FAW colony was initiated from specimens collected in the field in 2018. The colony was maintained at 25 ± 2 °C, 50–70% RH and, L:D 12:12, at the animal rearing and containment unit (ARCU) in the international centre of insect physiology and ecology (ICIPE), Nairobi, Kenya, for at least two generations before being used for the bioassay. Adult moths were placed in Perspex® cages (40 × 40 × 45 cm) with netting materials fitted at the rear and a sleeve at the front. They were fed with cotton soaked with 1:9 honey:water solution. Moths were provided with potted maize plants for oviposition which were removed after one night. Then, the leaves with eggs were transferred to plastic boxes (15 × 7 × 5 cm) lined with a paper towel to absorb excess moisture until egg hatching. The emerged larvae were reared on fresh detached maize leaves and kept in plastic boxes until pupation. The leaves were changed every two days during the rearing process. Pupae were incubated under similar rearing conditions until adult emergence. *Chilo partellus* larvae were reared at ICIPE on artificial diets according to Ochieng et al. (1985), whereas *B. fusca*, *S. calamistis* and *S. nonagrioides* larvae were reared on an artificial diet according to Onyango and Ochieng'-Odero (1994). Field-collected larvae were introduced to the colonies twice a year to maintain genetic diversity.

Parasitoid colonies

Cotesia flavipes, *C. sesamia* (*Cs*-Inland and *Cs*-Coast populations) as well as *C. typhae* individuals were obtained from laboratory-reared colonies established at ARCU, ICIPE. The *C. flavipes* colony was initiated with parasitoids from *C. partellus* collected in maize fields in 2005 in Mombasa, Kenya. *Cotesia sesamiae* *Cs*-Inland and *Cs*-Costal populations were initiated from field-collected samples of *B. fusca*-infested maize from Kitale, Kenya in 2006 and *S. calamistis*-infested maize from Mombasa, Kenya in 2007, respectively. Colonies of *C. typhae* were obtained from *Typha domingensis* Persoon infested by *S. nonagrioides* larvae in Kobodo, Kenya in 2013. Field-collected parasitoids were introduced to the colonies twice a year to maintain genetic diversity.

Adult parasitoids were fed with a 20% honey:water solution and placed under artificial light for 8 h to mate (Overholt et al. 1994). One-day-old mated females that were naïve (i.e., without oviposition experience) were used. Similar to Overholt et al. (1994), rearing conditions were 25 ± 2 °C, 50–80% RH and a L:D 12:12 photoperiod.

Acceptability and suitability tests

The experiments were conducted at 25 ± 1 °C, $50 \pm 6\%$ RH and a L:D 12:12 photoperiod. A single FAW larva was placed in an inverted Petri dish (height = 1.5 cm; diameter = 3 cm), together with a wasp, as previously reported by Obonyo et al. (2010). A naïve, one-day-old mated female parasitoid was randomly selected and introduced into an inverted Petri dish on a flat surface and allowed to climb to the top (i.e. the bottom of the inverted Petri dish). Once the parasitoid had reached the top, the Petri dish was placed over a FAW larva. The wasp and FAW larva were observed until the parasitoid stung the larva, for a maximum of 5 min. The number of dead parasitoids was recorded in case the host's defense behaviour led to its death. Second (L2), third (L3), and fourth (L4) instars were tested with each parasitoid species. These instars were chosen because they generally constitute the most suitable host for larval parasitoids (Godfray 1994).

Each wasp and larva was used once. For each instar tested, a total of 60 FAW larvae were exposed to each parasitoid species in three replicates of 20 larvae tested

individually. After each replicate, each Petri dish was cleaned with distilled water and air-dried. For each FAW larva, the searching (approaching and contact) and stinging (probing) times of each parasitoid species were recorded.

After parasitoid exposure, the FAW larvae were maintained individually in glass vials, and provided with fresh maize leaves. They were monitored daily until cocoon formation, pupation, death, or until the host or parasitoid reached adulthood. The control consisted of individually kept FAW larvae ($N = 60$) that were not exposed to parasitoids in glass vials in three replicates of 20 larvae under the same rearing conditions to assess natural mortality of the host. In nature, adult *C. flavipes* and *C. sesamiae* are generally active throughout the day but mostly around midday, when light intensity and temperatures are high (Obonyo et al. 2010). Therefore, all experiments were conducted between 10:00 am and 2:00 pm, in arenas kept at 25 ± 2 °C under a white 60 W light bulb (Obonyo et al. 2010).

Larval dissection

To confirm egg deposition by the parasitoids and to evaluate possible encapsulation of the eggs by FAW larvae, each larva tested was exposed to a gravid female of each parasitoid species using the hang stinging method (Overholt et al. 1994). The method consisted of exposing the host larvae individually to parasitoids by holding them with soft forceps inside the cage. The oviposition could be detected by carefully watching the encounter between the parasitoid and its host. Parasitized larvae were placed individually into glass vials on fresh maize leaves maintained under laboratory conditions for 12 to 24 h post-parasitism before dissection, as previously reported by Buchori et al. (2009). *Chilo partellus* larvae parasitized by *C. flavipes* and *S. calamistis* parasitized by each population of *C. sesamiae* were used as positive controls for observation on egg deposition (Sallam et al. 1999), while *B. fusca* larvae parasitized by either *C. flavipes* or *Cs-Coast* were used as positive controls for determining encapsulated egg observations (Gitau et al. 2006). Thereafter, parasitized hosts were dissected, suspended in a drop of Ringer's saline on a concave glass slide under a binocular microscope (Olympus UK Ltd, London, UK) in order to count the number of normal and

encapsulated eggs (which was determined by observing a capsule around the parasitoid egg) per host larva. For each examination, five replicates of six parasitized larvae per instar and parasitoid species were used.

Two-choice bioassays

Based on the outcome of the FAW larvae acceptability experiments (see results), the most effective parasitoid species to attack FAW larvae, namely *C. flavipes* and *C. sesamiae* *Cs-Inland*, were used in both two-choice bioassays and Y-tube olfactometer tests. This experiment was conducted using the same procedure described above, except that the parasitoid was offered two larvae instead of one (FAW vs. *C. partellus* for *C. flavipes* and FAW vs. *S. calamistis* for *Cs-Inland*). The behaviour of each female wasp was observed until it either stung the preferred larva or for a maximum of 5 min if the wasp did not sting any of the larvae. Second and third instar hosts were tested with each parasitoid species. For each instar, three replicates of 20 choices were performed. After each replicate, each Petri dish was cleaned in distilled water and air-dried. The conditions of the experiment remained the same as described previously.

Olfactometer behavioural bioassay

In Y-tube olfactometer choice experiments, maize plants of hybrid H513 (Simlaw, Kenya Seed Company, Nairobi, Kenya) grown in plastic pots (12 cm in height \times 13 cm in diameter) were used when they were between four and six weeks old. Maize plants were infested with five third instars of either FAW or a given stemborer species. Third instars were chosen to ensure a high degree of feeding damage within 24 h before their use. Each assay lasted for a maximum of 10 min. The following combinations were used for each female parasitoid: uninfested maize vs. empty chamber, uninfested maize vs. FAW infested maize. Additionally, for *C. flavipes* the treatments were uninfested maize vs. *C. partellus* infested maize and FAW infested maize vs. *C. partellus* infested maize, while for *C. sesamiae* *Cs-Inland* they were uninfested maize vs. *S. calamistis* infested maize and FAW infested maize vs. *S. calamistis* infested maize.

For each parasitoid, gravid females were released individually into the base of a Y-tube and allowed to choose either one of the two arms. The Y-tube had the

following dimensions: stem length of 18 cm, 34 cm for each arm and 4 cm in diameter. For each test, a choice was recorded when the insect passed 5 cm from the intersection into one arm and remained motionless there for more than 20 s. Those that made no choice were also recorded. After every five insects, odour source connections to the chambers were reversed to minimize any locational bias, and the chambers were cleaned thoroughly with tap water. In each combination, the number of gravid female parasitoids tested ranged from 30 to 36. For each choice tested, the percentage of parasitoids that made a distinct choice was calculated.

Data analysis

The percentage of accepted hosts was expressed as the number of larvae stung divided by the total number of larvae exposed, while successful parasitism was calculated as the number of parasitoid cocoon masses divided by the sum of host pupae and parasitoid cocoon masses. Larval and pupal non-reproductive mortality (i.e. larval or pupal mortality induced by parasitism not resulting in development of parasitoid offspring (Abram et al. 2019)), as well as larval and pupal mortalities in the control were computed using Abbott formula (Abbott 1925).

The proportion of hosts stung and the proportion of parasitoids killed by the host were analyzed using a generalized linear model (GLM) with binomial error distribution (with a logit link function), due to the binary nature of these parameters (accepting host vs. failure to accept, killed vs. not killed). Means were separated with Tukey's multiple comparison tests, performed in the R package 'lsmeans'. The searching and oviposition times of parasitoids and the actual non-reproduction host mortality were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, respectively, and analysed using Kruskal–Wallis non-parametric procedure with the dunn.test R package. The number of eggs laid per female parasitoid and the number of eggs encapsulated were compared among instars by ANOVA, after being tested for normality and homogeneity of variance using Shapiro–Wilk and Bartlett tests, respectively. Means were separated with the Student–Newman–Keuls (SNK) method at 5% significance level. Results of the olfactometer assays and the response in the dual bioassay were analyzed by χ^2 at

5% significance level. All analyses were performed with R software version 3.5.1 (R Core Team 2018).

Results

Acceptability tests

Upon release into the experimental arena, some parasitoids were killed by FAW larvae. *Cotesia flavipes* and *Cs-Coast* mortality significantly increased with host age (*C. flavipes*: 0.00, 0.00 and 5.00 ± 2.83 for L2, L3 and L4 respectively, $\chi^2 = 6.69$, $df = 2$, $P = 0.03$; *Cs-Coast*: 3.33 ± 2.33 , 10.00 ± 3.90 and 23.33 ± 5.50 for L2, L3 and L4 respectively, $\chi^2 = 11.93$, $df = 2$, $P = 0.002$). No significant difference was recorded for *Cs-Inland* mortality among instars (0.00, 3.33 ± 2.33 and 6.67 ± 3.24 for L2, L3 and L4 respectively, $\chi^2 = 5.68$, $df = 2$, $P > 0.05$). Within parasitoid species, *Cs-Coast* was significantly killed by L3 ($\chi^2 = 9.87$, $df = 2$, $P = 0.007$) and L4 ($\chi^2 = 11.27$, $df = 2$, $P = 0.003$). No mortality was observed for *C. typhae*, since this parasitoid species never attempted to attack the larvae.

For parasitoid species that attacked FAW larvae, the attack occurred on 16.67 to 71.67% of the total FAW larvae exposed and the probing time ranged from 4.24 to 19.97 s (Table 1). For *C. flavipes* and *Cs-Inland*, the percentage of FAW larvae attacked did not vary among instars (Table 1). By contrast, for *Cs-Coast*, the percentage of FAW larvae attacked varied with host age, being significantly higher for L2 than the other instars. Moreover, the time taken by female parasitoids to search the host and to insert their ovipositor tended to increase with FAW larval age (Table 1). Among parasitoid species, the percentage of L3 and L4 hosts attacked by *C. flavipes* and *C. sesamiae* *Cs-Inland* were significantly higher than those by *Cs-Coast* (Table 1). Moreover, *Cs-Coast* took more time to search for L3 instar of the host as compared to the other species. The probing time of each instar also differed among parasitoid species (Table 1).

Suitability tests

None of the FAW instars yielded cocoons, irrespective of parasitoid species (Table 2). For all FAW instars, larval non-reproductive mortality was significantly

Table 1 Percentage (\pm SE, $n = 3$) of stung larvae of fall armyworm (FAW) and searching and ovipositor insertion times (\pm SE, $n = 3$) by parasitoids

Parameters	FAW stages	<i>Cotesia flavipes</i>	<i>Cs-Inland</i>	<i>Cs-Coast</i>	<i>Cotesia typhae</i>	χ^2	df	P
% of larvae attacked	L2	71.67 \pm 5.85aA	71.66 \pm 5.86aA	65.00 \pm 6.20aB	— ^a	0.82	2	0.66
	L3	70.00 \pm 5.96 bA	68.33 \pm 6.05bA	28.33 \pm 5.86Aa	—	27.55	2	< 0.0001
	L4	66.67 \pm 6.13 bA	63.33 \pm 6.27 bA	16.67 \pm 4.85aA	—	40.13	2	< 0.0001
χ^2		0.365	0.96	33.28				
df		2	2	2				
P		0.83	0.61	< 0.0001				
Searching time (in seconds)	L2	47.74 \pm 8.03aA	40.76 \pm 7.61aA	33.57 \pm 5.52aA	—	1.76	2	0.41
	L3	34.48 \pm 6.26aA	54. 83 \pm 10.19bB	77.40 \pm 23.36cAB	—	16.12	2	0.0003
	L4	54.78 \pm 9.06aB	59.23 \pm 10.82aB	119.64 \pm 22.2aB	—	0.84	2	0.65
χ^2		5.65	5.53	16.01				
df		2	2	2				
P		0.05	0.04	0.0003				
Ovipositor insertion time (in seconds)	L2	4.30 \pm 0.37aA	4.24 \pm 0.22aA	6.00 \pm 1.10bA	—	15.08	2	0.0005
	L3	4.63 \pm 0.20aA	5.18 \pm 0.39aA	11.90 \pm 2.40bB	—	13.8	2	0.001
	L4	6.86 \pm 0.95aB	19.97 \pm 2.65bB	16.47 \pm 3.36bB	—	40.98	2	< 0.0001
χ^2		17.33	50.06	4.38				
df		2	2	2				
P		0.0001	< 0.0001	0.04				

The searching time corresponded to the total time spent by each parasitoid from release to host stung

^a0% of attacked larvae has been observed. Non-significant different means ($p > 0.05$) are shown by identical letters within each row (i.e. parasitoid species comparisons) in lower-case and within each column in upper-case (i.e. larval instars)

higher by *C. flavipes* (23 to 36%) and *Cs-Inland* (22 to 37%) than by *Cs-Coast* (8 to 29%) (Table 2). Non-reproductive mortality of FAW larvae tended to decrease with larval age, but the differences were not always significant. Except for *Cs-Coast* at the L3 and L4 stages, FAW larval mortality was significantly higher for all instars attacked by parasitoids compared to their respective control (Table 2). For pupal mortality, no significant difference was recorded either among parasitoid species or within host larval stages (Table 2). Comparing pupal non-reproductive mortality for each parasitoid vs. its control at each host instar, larvae attacked by the parasitoids resulted in significant pupal non-reproductive mortalities in all host larval stages tested, except for the L4 stage with *Cs-Coast* and *Cs-Inland* (Table 2).

Larval dissection

No normal or encapsulated eggs were found in any of the FAW larval stages stung by the parasitoid species (Table 3). By comparison, the positive control hosts yielded both normal and encapsulated eggs (Table 3).

Two-choice bioassays

In two-choice experiments, *C. flavipes* and *Cs-Inland* equally accepted FAW and *C. partellus* ($\chi^2 = 3.37$, $df = 1$, $P = 0.06$; $\chi^2 = 0.28$, $df = 1$, $P = 0.59$, respectively). Furthermore, there was no significant difference in the preference between FAW and *S. calamistis* larvae either for *C. flavipes* ($\chi^2 = 0.92$, $df = 1$, $P = 0.33$) or for *Cs-Inland* ($\chi^2 = 0.01$, $df = 1$, $P = 0.89$) (Fig. 1).

Table 2 Successful parasitism (i.e. cocoon mass production after ovipositor insertion by *Cotesia flavipes*, *C. sesamiae* Cs-Inland or *C. sesamiae* Cs-Coast) and non-reproductive mortality in control (= FAW larvae not exposed to parasitoids) andthose induced after ovipositor insertion by parasitoids of fall armyworm larvae at different development stages (% , \pm SE, $n = 3$)

Parameters	FAW stages	<i>Cotesia flavipes</i>	Cs-Inland	Cs-Coast	Control	χ^2	df	P
Successful parasitism (%)	L2	— ^a	—	—				
	L3	—	—	—				
	L4	—	—	—				
FAW larval non-reproductive mortality (%)	L2	36.98 \pm 0.96bB	37.96 \pm 2.54bB	29.05 \pm 3.81bA	16.66 \pm 2.40aA	7.37	3	0.04
	L3	23.00 \pm 3.33bA	34.12 \pm 0.79cB	16.98 \pm 1.65aA	15.00 \pm 2.88aA	8.22	3	0.04
	L4	25.09 \pm 2.87bA	22.59 \pm 2.59bA	8.33 \pm 2.65aA	13.33 \pm 1.66aA	8.25	3	0.04
χ^2		5.80	6.21	4.39	0.32			
df		2	2	2	2			
P		0.04	0.04	0.11	0.85			
FAW pupal non-reproductive mortality (%)	L2	11.49 \pm 1.51bA	14.39 \pm 1.89bA	14.05 \pm 0.84bA	6.03 \pm 0.32aA	7.55	3	0.04
	L3	15.05 \pm 2.14bA	11.20 \pm 0.72bA	20.55 \pm 2.42cA	5.89 \pm 0.20aA	9.17	3	0.03
	L4	10.06 \pm 0.58aA	8.33 \pm 2.16aA	8.33 \pm 4.16aA	3.92 \pm 1.96aA	0.34	3	0.83
χ^2		3.77	2.98	2.87	0.68			
df		2	2	2	2			
P		0.15	0.22	0.24	0.71			

^aNo successful parasitism (i.e. cocoon mass production) has been observed. Non-significant different means ($p > 0.05$) are shown by identical letters within each row (i.e. parasitoid species comparisons) in lower-case and within each column in upper-case (i.e. larval instars)

Olfactometer behavioural bioassay

In the Y-tube olfactometer assays, 83–93% of both *C. flavipes* and *Cs-Inland* females orientated significantly towards volatiles emitted by uninfested maize plants when compared to blanks (Fig. 2, $\chi^2 = 10.8$, $df = 1$, $P = 0.001$ for *C. flavipes* and $\chi^2 = 8.53$, $df = 1$, $P = 0.003$ for *Cs-Inland*). The parasitoids showed a significant preference for plants infested by either FAW or stemborer larvae over uninfested plants (for *C. flavipes*: comparison uninfested vs. *C. partellus* infested plants: $\chi^2 = 4.8$, $df = 1$, $P = 0.02$; comparison uninfested vs. FAW infested plants: $\chi^2 = 8.53$, $df = 1$, $P = 0.003$) (for *Cs-Inland*: comparison uninfested vs. *S. calamistis* infested plants: $\chi^2 = 10.8$, $df = 1$, $P = 0.001$; comparison uninfested vs. FAW infested plants: $\chi^2 = 4.8$, $df = 1$, $P = 0.02$). However, *C. flavipes* did not discriminate between the odours from plants infested by *C. partellus* and plants infested by FAW ($\chi^2 = 0.53$, $df = 1$, $P = 0.46$), while *Cs-Inland* showed marked preference towards the odours of plants infested by FAW over plants infested by *S. calamistis* ($\chi^2 = 8.53$, $df = 1$, $P = 0.003$) (Fig. 2).

Discussion

In the present study, the parasitoids of maize stem-borers, *C. flavipes* and *C. sesamiae* (*Cs-Inland* and *Cs-Coast* populations), exhibit searching behaviours (approaching, touching, probing and defense movement) on FAW larvae and were then able to attack the larvae. For most of them, attacks on non-host or new host larvae have already been reported (Obonyo et al. 2008). However, in our study, escape and defense behaviours between the parasitoid and FAW larvae occurred mostly at the older instars, resulting in the death of some female parasitoids. Parasitoid death following the aggressive reaction of the host in response to the ovipositor insertion process has already been observed in *Cotesia* spp. by Takasu and Overholt (1997) and in other parasitoids (e.g. Gordh 1976). The time spent by the parasitoids searching the FAW larvae increased with the FAW larval stage. This could be due to the decreasing suitability and increased aggressiveness of FAW with age. Chau and Mackauer (2000), working with the aphid parasitoid *Monoctonus paulensis* Ashmead (Hymenoptera:

Table 3 Mean number of eggs (\pm SE, $n = 5$) laid by a given female parasitoid per ovipositor insertion and the number of eggs (\pm SE, $n = 5$) encapsulated in *Busseola fusca* larvae

Larval stages	Number of eggs per larva			Number of eggs encapsulated per larva	
	<i>Chilo partellus</i>	<i>Sesamia calamistis</i> <i>Cotesia flavipes</i>	<i>Spodoptera frugiperda</i>	<i>Busseola fusca</i>	<i>Spodoptera frugiperda</i>
L2	16.34 \pm 1.73a		— ^a	9.16 \pm 1.82a	— ^b
L3	25.48 \pm 3.34a		—	8.21 \pm 1.51a	—
L4	37.61 \pm 3.94b		—	11.92 \pm 3.65a	—
<i>F</i>	11.48			0.58	
<i>df</i>	2, 12			2, 12	
<i>P</i>	0.001			0.57	
<i>Cotesia sesamiae</i> (Inland)					
L2		17.14 \pm 2.20a	—		
L3		19.10 \pm 2.08a	—		
L4		27.81 \pm 2.07b	—		
<i>F</i>		7.16			
<i>df</i>		2, 12			
<i>P</i>		0.008			
<i>Cotesia sesamiae</i> (Coast)					
L2		16.34 \pm 2.38a	—	7.21 \pm 1.16a	—
L3		27.25 \pm 2.22b	—	9.23 \pm 1.19a	—
L4		31.94 \pm 2.73b	—	7.90 \pm 1.08a	—
<i>F</i>		10.59		0.79	
<i>df</i>		2, 12		2, 12	
<i>P</i>		0.002		0.47	

Neither normal eggs (—^a) nor eggs encapsulated (—^b) have been observed. Non-significant different means ($p > 0.05$) are shown by identical letters within columns (i.e. larval stages comparisons)

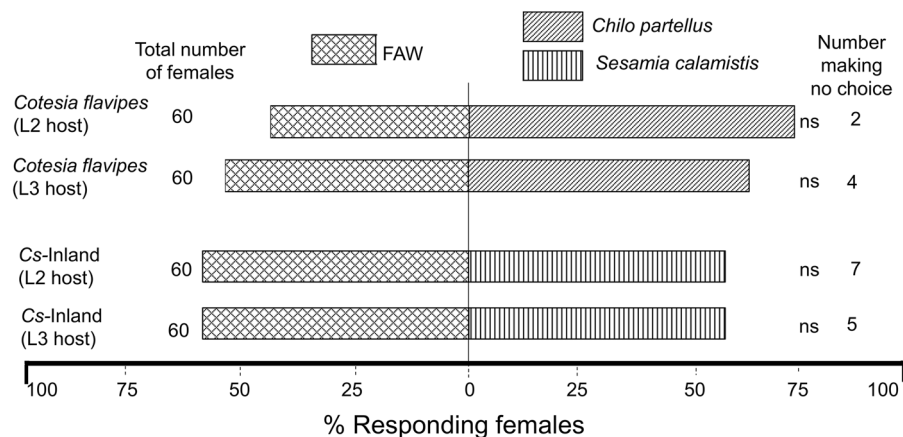


Fig. 1 Responses of *Cotesia flavipes* and one population of *C. sesamiae* (Cs-Inland) to fall armyworm (FAW) larvae and two species of maize stemborer larvae (dual choice situation) in Petri dish arenas. On each bar, the number of parasitoids choosing a host was given, as well as the number of parasitoids making no

choice. The number of females making a choice was set to 100% to calculate the percentage of responding females. ns, non-significant; $P > 0.05$ according to χ^2 test at 5% significance level

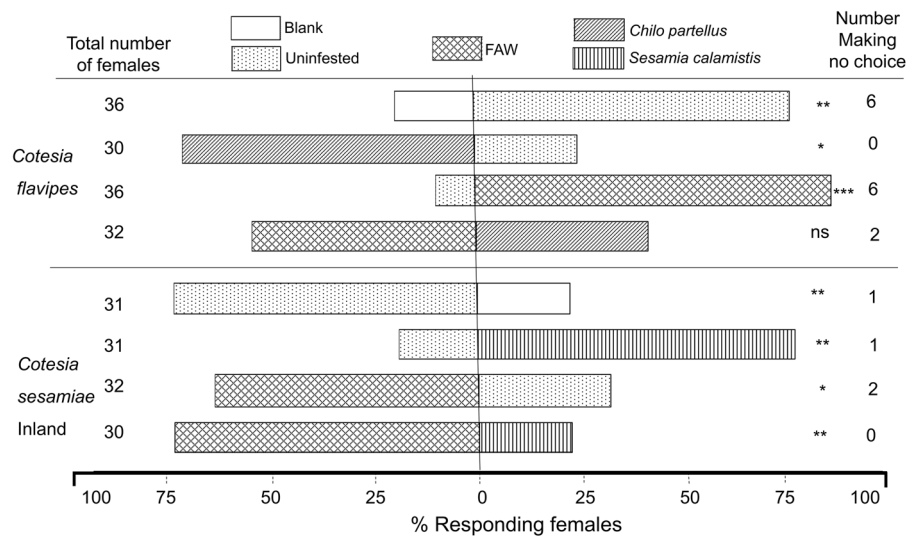


Fig. 2 Responses of *Cotesia flavipes* and one population of *C. sesamiae* (Cs-Inland) to two different odor sources (blank, uninfested plants and plants infested by fall armyworm (FAW) and two species of maize stemborer larvae) in a Y-tube olfactometer. On each bar, the number of parasitoids choosing

the odors was given as well as the number of parasitoids making no choice. The number of parasitoids making a choice was set to 100% to calculate the percentage of responding parasitoids. ns = non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$ according to χ^2 test at 5% significance level

Braconidae), showed that the total time needed by a female to capture, and parasitize an aphid varied with host instars, with fourth instars requiring nearly twice as much time as for the more suitable first, second and third instars. The same authors reported that longer ovipositor insertion times increased the likelihood of the parasitoids' death.

Cotesia flavipes and Cs-Inland attacked all FAW instars tested while Cs-Coast mostly preferred the second instar. On the other hand, *C. typhae* did not attack any instar of the host. This might be due to the higher host specificity of this parasitoid, which has been previously documented (Kaiser et al. 2017; Bichang'a et al. 2018). Host habitat specificity could also play a key role in the behaviour of the four parasitoids in terms of their attraction to and attacks on FAW larvae. *Cotesia flavipes* and *C. sesamiae* (both populations) share the same maize ecosystem, while *C. typhae* is predominantly found on wild *Typha* and *Cyperus* habitats, where its preferred host *S. nonagrioides* is found in Kenya (Kaiser et al. 2017).

Surprisingly, there was no significant difference in larval choice for ovipositor insertions under dual-choice conditions between FAW and either *C. partellus* larvae for *C. flavipes* or *S. calamistis* larvae for Cs-Inland. Since older FAW larvae are frequently found in the bored holes and tunnels left by stemborers and

even on corn with stemborer larvae in maize fields (Sokame B.M., pers. obs.), whether these two *Cotesia* parasitoids will evolve to successfully develop in FAW larvae is unknown. Other African native *Cotesia* species, such as *Cotesia icipe* Fernandez-Triana & Fiafoe (Hymenoptera: Braconidae), have been reported to successfully parasitize FAW larvae (Sisay et al. 2018).

The Y-tube experiments confirmed the preference of Cs-Inland for maize plant infested by FAW over those infested by *S. calamistis*, while no preference was observed for *C. flavipes* between maize infested by FAW and *C. partellus*. The results for Cs-Inland could be due to the fact that plants infested by FAW might be more important than those of plants infested by *S. calamistis*, since defoliation and plant damage by FAW larvae is higher than that caused by *S. calamistis* (Sokame et al. 2020) and this could have affected odours emitted by these plants. This is aligned with the finding by Gouinguéné et al. (2003) who reported a positive correlation of herbivore-induced plant volatile emissions and the amount of damage inflicted by *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae).

No successful parasitism of FAW larvae was recorded, indicating that FAW larvae are not suitable to all parasitoid species tested. However, both

larval and pupal non-reproductive mortality caused by the parasitoid species that attacked FAW larvae were significantly higher than natural mortality in the control treatment. The non-reproductive mortalities might be due to either mutilation or pseudo-parasitism, since neither normal eggs nor encapsulated eggs were observed in FAW larva attacked by parasitoids (Abram et al. 2019). Mutilation comes from mechanical damage to hosts resulting from ovipositor insertion during the stinging process, while pseudo-parasitism occurs when parasitoids inject chemical (e.g. venom) or biological (e.g. symbiont, viruses) substances that alter host physiology but do not lay eggs (De Pedro et al. 2018; Hegazi and Khafagi 2000; Quednau 1970).

Those non-reproductive mortalities induced by native parasitoids on FAW may significantly reduce FAW populations in the field, mostly in regions where stemborer species and their associated *Cotesia* species cohabitate. For example, a model developed by Kaser et al. (2018) on the biological control effects of non-reproductive host mortality caused by insect parasitoids predicts that native parasitoids may have a large biological control impact on the population dynamics of the invasive agricultural pest, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in areas where suitable native hosts coexist. Nevertheless, since plants infested by FAW larvae are attractive to *C. flavipes* and *Cs*-Inland and these parasitoids equally stung FAW as compared to their respective natural and native hosts in dual choice, we can also expect FAW to have a negative impact on stemborer biological control already established before its invasion. It has been reported that exotic insect herbivores unsuitable to native parasitoids have the potential to disrupt existing native parasitoid-host interactions via two mechanisms: interference with the foraging behaviour of the parasitoids and costs associated with unsuccessful attacks on the exotic non-host (Chabaane et al. 2015; Martorana et al. 2017). Parasitoids might waste time and energy in unproductive foraging behaviours when they do not complete development in the new host but respond to volatile cues from the plant-herbivore complex and oviposit in the unsuitable host. This may directly affect the reproductive output of the parasitoids under field conditions (Hoogendoorn and Heimpel 2002), leading to reduction in parasitism rates on its native hosts. In this respect, FAW may represent a sink or an evolutionary trap for stemborer-

associated parasitoids. Indirectly, this might be an advantage for stemborer species which might exhibit significant outbreaks due to a lower demographic pressure from natural enemies (Abram et al. 2014).

In conclusion, the present study showed that FAW larvae are not suitable for *Cotesia* spp. associated with lepidopteran stemborers in Kenya. However, *C. flavipes*, *Cs*-Inland and *Cs*-Costal are able to induce non-reproductive mortality possibly through mutilation and pseudo-parasitism. In the areas where stemborers coexist with FAW, *Cotesia* spp. associated with stemborers could also contribute to the regulation of FAW populations through host non-reproductive mortality. However, on the other hand, FAW can have a negative impact on stemborer biological control already established before its invasion. Further studies are needed to confirm under field conditions the contributions of non-reproductive mortality by native *Cotesia* spp. on FAW populations and its impact on stemborer biological control.

Acknowledgements The authors wish to thank the German Academic Exchange Service (DAAD) for funding the PhD fellowship under the Grant Number 91636630, and the University of Nairobi and ICIPE Capacity Building Program (ARPPIS) for hosting the PhD student. This research was funded by the 'Institut de Recherche pour le Développement' (IRD)-France through the IRD Collaborative Research project (Grant Number B4405B) and the integrated pest management strategy to counter the threat of invasive fall armyworm to food security in eastern Africa (FAW-IPM) (Grant No. DCI-FOOD/2017/) financed through the European Union. We also acknowledge the financial support for this research by the following organizations and agencies: the UK's Department for International Development (DFID), the Swedish International Development Cooperation Agency (SIDA), the Swiss Agency for Development and Cooperation (SDC), and the Kenyan Government. Thanks are also due to the stemborer rearing unit at the ARCU-ICIPE, especially to Josphat Akhobe and John Buluma, for rearing and supplying insect larvae and artificial diets. Thanks also to Fritz Schulthess for his critical review of the manuscript and to Malcolm Eden for his English correction.

Author contributions PAC and BMS conceived the ideas and designed methodology; BMS, JO and EMS collected the data; BMS analysed the data; PAC, BMS, SAM, SS, DCK, and GJ led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability Data will be made available after the manuscript accepted.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval This research complied with all local and national standards for ethical conduct in research. This research did not involve vertebrates or humans, and thus, no IRB approvals were needed.

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