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# Cereal stem borer species complex and establishment status of highland larval parasitoid, *Cotesia sesamiae* (Cameron) in coastal Taita Hills, Kenya

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## Abstract
Sorghum and maize are important crops whose production is constrained by stem borers such as *Busseola fusca.* In Kenya, two distinct populations of *Cotesia sesamiae,* a parasitoid of *B. fusca* exist. The virulent highland biotype was released in the coastal region where the avirulent biotype existed. However, post release surveys were not done to assess for establishment. This survey was undertaken in release areas where 100 maize plants were inspected for infestation and ten plants dissected to recover stem borers in each farm. Results revealed that *B. fusca* was the most dominant pest (85.5%). Overall infestation was 19.17±2.48% and varied significantly among stem borer species ($\chi^2_2$ =16.86, *p*=0.00022) Overall parasitism was 10.78±4.34% and did not show significant variation from parasitoid pre-release rates (*V*=3, *p*>0.05). *Cotesia sesamiae* was recovered from *C. partellus* only suggesting that it was the avirulent biotype. The highland *C. sesamiae* did not establish in coastal Kenya.

**Keywords:** *Cotesia sesamiae, virulent, avirulent, biotype, Busseola fusca, redistribution*

## Introduction
*Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), is one of the important field pests of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L) Moench) in Sub-Saharan Africa (SSA) [1, 2]. Before the introduction of maize from Meso America in the 16th century [3] and the extensive cultivation of sorghum, *B. fusca* subsisted on non-cereal wild host plants [1]. Following the introduction and extensive cultivation of these crops, *B. fusca* shifted to cultivated habitat [4] where yield losses associated with its infestation was estimated at 10-14% [5]. In Kenya, these losses are mostly incurred in regions considered the ‘bread basket’ of the country [6, 7] and characterized as high potential zones (highland tropics, moist transitional and moist mid-altitude) [8]. Distribution of *B. fusca* is favoured by low temperatures, an attribute found in the afore-mentioned high potential zones [9-15].

*Busseola fusca* infestations cause considerably higher yield losses among small scale farmers who cannot afford chemicals but rely mainly on naturally available enemies. Various natural enemies including forty parasitoids, five predators and eight pathogens have been reported to attack *B. fusca* in East Africa [16]. Of these, the most common is the braconid, larval endoparasitoid, *Cotesia sesamiae* (Cameron) [17, 18]. In addition to *B. fusca*, *C. sesamiae* can attack *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* (Strand) and *Sesamia calamistis* Hampson [19]. Several strains of *C. sesamiae* varying in their insect host ranges have been found in Africa [20]. In Kenya, studies have shown that two *C. sesamiae* biotypes with variation in developmental success in *B. fusca* exist. The virulent strain (found in highlands) is able to develop successfully while the avirulent strain (found at the coast) is encapsulated by *B. fusca* [12, 21-23]. Evolution of biotypes was the result of trade-offs brought by local adaptation to host community structure [24] and is of vital importance in pest management.

*Cotesia sesamiae* distinct populations have been utilized in the biological control redistribution programme during which natural enemies were exchanged between African regions and different localities within a country to manage indigenous stem borer pests [25]. In Kenya, the virulent highland *C. sesamiae* population was collected and released at three localities in Taita Hills in 2006 (*icipe*, unpublished data). However, no post release surveys have been undertaken to ascertain the establishment and spread of the parasitoid from release sites, a gap that was addressed through this study.

## 2 Methodology

### 2.1 Description of the study area

This study was undertaken in Taita Hills at sites where *C. sesamiae* had been released. Survey farms were marked around Josa, Wesu and Prison's farm. Taita Hills is in the coastal region and stretches across mid to high elevations. The areas within the mid elevations lie between 1000-1800masl, with temperatures ranging within 17-32°C and receive between 500-1000mm of rainfall annually. These areas are generally moist. The areas within high elevations lie above 1800masl with temperatures ranging within 7-24C° and receive above 1000mm of rainfall annually. These areas are generally wet [8]. Taita hills have bimodal rainfall pattern with long rains received between March to May/June and short rains falling from September/ October to December.

### 2.2 Sampling protocol

Maize farms surrounding *C. sesamiae* release points were inspected for stem borer infestation. In each farm, a total of 100 maize plants were inspected during which 10 infested maize stems were destructively sampled and dissected. Immature stem borer stages were collected, identified and categorized (as small {$1^{st}$ and $2^{nd}$ instars}, medium, {$3^{rd}$ and $4^{th}$ instars} and large {$5^{th}$ and $6^{th}$ instars}). Identified larvae were placed individually in glass vials containing artificial diet [26] and transported to the laboratory at *icipe* where they were reared at ambient temperatures of 24-25°C and a relative humidity of 55-65%, with a 12:12 light: dark photoperiod. Samples were inspected daily for parasitoid cocoons, pupal development and adult moth emergence. Pupae were transferred into plastic jars lined with wet paper towels. Humidity in the jars was maintained by moistening the soft paper towels once every 2 days using a few drops of distilled water. Larval parasitoids and adult stem borer moths were identified and recorded. *Cotesia flavipes* and *C. sesamiae* were morphologically separated using the shape of male genitalia [27].

### 2.3 Statistical analyses

Percentage infestation was computed by expressing the number of infested plants as a percentage of the total number of plants inspected. Larval densities were obtained by expressing the total number of stem borer larvae collected from dissected plants as a proportion of number of infested plants. To estimate percentage parasitism, suitable larval stages that were parasitized were expressed as a proportion of the total number of stem borer larvae collected. Percentage infestation and parasitism were tested for normality using Shapiro-Wilk normality test after which data that failed ($p<0.05$) were transformed. Data that failed to normalize after transformation were subjected to Kruskal-Wallis rank sum test and significantly different means separated using Nemenyi post-hoc test ($p<0.05$). Wilcoxon rank sum test was used to compare rates of infestation and parasitism computed during this study with parasitoid pre-release rates.

## 3 Results

### 3.1 Stem borer species composition and diversity

Three species of stem borers; *B. fusca, C. partellus* and *S. calamistis* were found occurring in Taita Hills. The most dominant was *B. fusca* which occurred at altitudes ranging within 1362-1736masl. *Busseola fusca* constituted 85.5% of the total stem borers collected. *C. partellus* which accounted for 9.7% of stem borers collected was found at 1099-1453masl. The least abundant stem borer species was S. *calamistis* which constituted 4.8% and was found at altitudes ranging within 1362-1712masl (Table 1). Mean larval density of the three stem borer species varied significantly ($\chi^2_2$ =17.64; $p<0.05$) (Table 1). *Busseola fusca* exhibited a significantly higher larval density (2.5±0.5) in comparison to *C. partellus* (0.3±0.1) and *S. calamistis* (0.1±0.05). Mean larval density of *C. partellus* and *S. calamistis* did not show significant difference.

**Table 1:** Percentage composition, mean larval density and altitude at which stem borer species were recovered in Taita Hills

| Stem borer species | % composition | Larval density ( $\bar{X}$ ±SE) | Altitude (masl) |
|---|---|---|---|
| *Chilo partellus* | 9.7 | $0.3\pm0.1^a$ | 1099-1453 |
| *Sesamia calamistis* | 4.8 | $0.1\pm0.1^a$ | 1362-1712 |
| *Busseola fusca* | 85.5 | $2.5\pm0.5^b$ | 1362-1736 |
| $\chi^2$ value | | 17.64 | |
| *df* | | 2 | |
| *p* value | | 0.00015 | |

Larval density $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different ($p>0.05$)

### 3.2 Parasitoid and hyperparasitoid species composition and diversity

Parasitoid species recovered from Taita Hills were *Dolichogenidea polaszeki* Walker, *C. sesamiae, C. flavipes* and the hyperparasitoid *Aphanogmus fijiensis* (Ferriére). The most dominant parasitoid was *C. sesamiae* which constituted 35.0% of the parasitoids collected and was only recovered from *C. partellus*. The exotic *C. flavipes* was recovered from *C. partellus*, while *D. polaszeki* was recovered from *B. fusca* larvae only. No parasitoid was recovered from *S. calamistis* despite the availability of the appropriate larval stages in the field (Table 2). Parasitism among the parasitoid species was not significantly different ( $\chi^2_3$ =2.56; $p>0.05$). The solitary larval parasitoid *D. polaszeki* parasitized the highest number of stem borers though the *C. sesamiae* and *C. flavipes* numbers were higher owing to their gregarious nature (Table 2).

**Table 2:** Parasitoid species recovered, their percentage composition, stem borer species and life stage attacked and their respective rates of parasitism in Taita Hills

| Parasitoid species | % composition | Guild | Stem borer species parasitized | Parasitism ($\bar{X}$ ±SE) |
|---|---|---|---|---|
| *D. polaszeki* | 5.1 | Larval | *B. fusca* | 3.1±1.8[a] |
| *C. sesamiae* | 35.0 | Larval | *C. partellus* | 0.4±0.4[a] |
| *C. flavipes* | 29.1 | Larval | *C. partellus* | 0.8±0.8[a] |
| *A. fijiensis* | 30.8 | Hyperparasitoid | *C. partellus* | 0.7±0.7[a] |
| $\chi^2$ value | | | | 2.56 |
| *df* | | | | 3 |
| *p* value | | | | 0.47 |

Percentage parasitism by parasitoid species mean $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different (*p*>0.05)

### 3.3 Stem borer infestation and parasitism levels

Mean stem borer infestation levels in Taita Hills were 19.2±2.5%. Infestation was significantly different among the three stem borer species ($\chi^2_2$=16.86; $p$<0.05). Mean infestation by *B. fusca* was 15.2±3.0% which was significantly higher than infestation by *C. partellus* (3.3±1.6%) and *S. calamistis* (0.7±0.4%). Mean infestation levels by *C. partellus* and *S. calamistis* were not significantly different (Fig. 1). The current record of *B. fusca* infestation was significantly lower than figures recorded during a *C. sesamiae* pre-release survey in 2002/2003 (*V*=0; *p*<0.05). Significantly lower percentage infestation by *C. partellus* and *S. calamistis* (*V*=0; *p*<0.05) was also observed in comparison to pre-release results (Fig. 2).

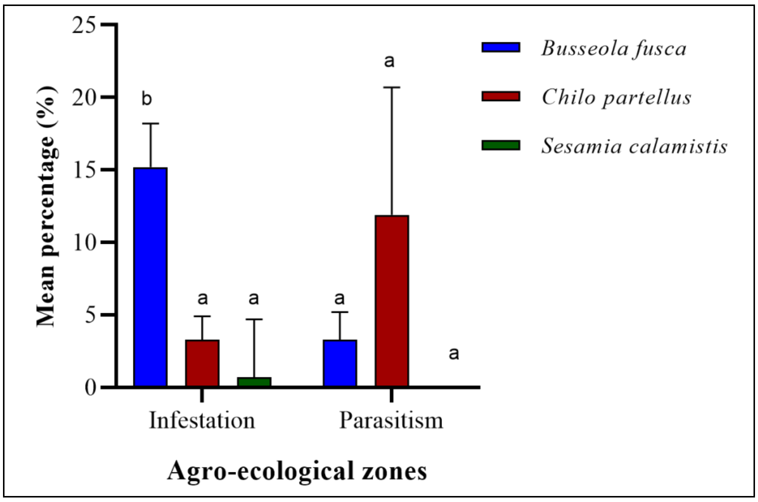


**Fig 1:** Percentage infestation and parasitism of stem borer species recovered in Taita Hills

Overall levels of parasitism were 10.8±4.3%. There was no significant difference in parasitism rates among the stem borer species ($\chi^2_2$=2.95; $p$>0.05). Though *C. partellus* exhibited a higher parasitism rate (11.9±8.8%), it was not significantly different from *B. fusca* (3.3±1.9%) and *S. calamistis* which did not yield any parasitoids (Fig 1). There was no significant difference in *C. partellus* parasitism rate recorded during this study in comparison with pre-release records (*V*=3; *p*>0.05). The same trend was demonstrated by *B. fusca* (*V*=6; *p*>0.05) (Fig. 2).

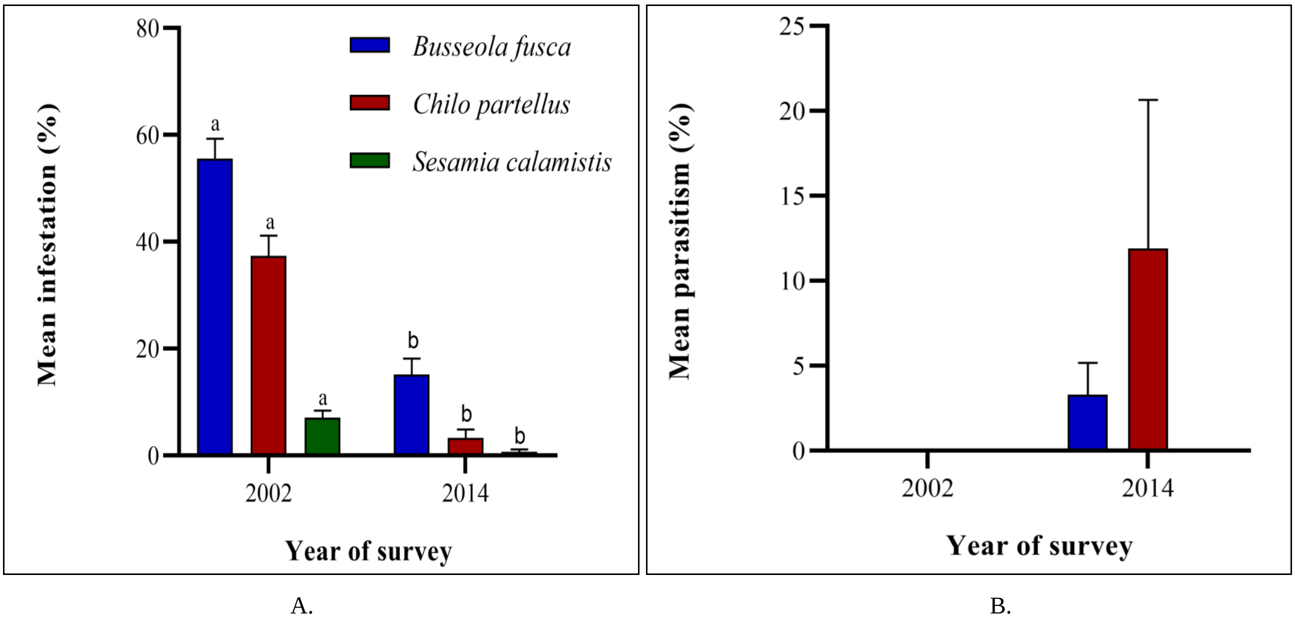


**Fig 2:** (a) Comparison of *C. partellus*, *S. calamistis* and *B. fusca* infestation and (b) parasitism levels before and after *C. sesamiae* release in Taita Hills. Percentage infestation with same letters above the bars are not significantly different ($p>0.05$)

## 4. Discussion

Three stem borer species were found in Taita Hills; namely *B. fusca*, *C. partellus* and *S. calamistis*, results that corroborated findings by [2]. Among the above three pest species, *B. fusca* is reportedly the most important pest in highlands of East and South Africa [1, 28]. This pest has been found occurring above 1000masl [2, 9, 14, 29-32], areas considered high altitude. Considering that Taita Hills lies in mid to high altitudinal range, the stem borer community was dominated by *B. fusca*, upholding findings by previous researchers. Earlier reports from mid to high altitudinal zones documented *B. fusca* as the predominant species followed by *S. calamistis*. However, results obtained from this survey indicated that *C. partellus* was the second most dominant stem borer species. In addition to the growing proportions of *C. partellus*, it was found to occurring at altitudes ranging between 1099 and 1453masl. Previously known to occur in lowland tropical and dry mid altitude zones (below 600masl) [1, 2], these results validate findings that the exotic *C. partellus* distribution is expanding into higher altitude areas [15]. With it's superior competitive abilities, is it possible that it is displacing *S. calamistis* as an important pest in mid-altitude agro-ecological zones? This proposition is further supported by the observed average larval densities and rates of infestation of *C. partellus* which were also higher than *S. calamistis*. Predictive modelling studies done in Taita Hills forecast a future increase in proportion of *C. partellus* which may eventually become an economically important pest in the area [33].

The objective of the redistribution programme was to suppress *B. fusca* population in Taita Hills. However, parasitism rates by individual parasitoid species remained low. The same trend was demonstrated by parasitism figures obtained on individual stem borer species. Various researchers also reported low parasitism by indigenous parasitoids [34-36]. This study showed that parasitism level have not changed since the release of *C. sesamiae* in Taita Hills in 2006.

Contrary to the redistribution programme's expectation, *C. sesamiae* was not recovered from *B. fusca* which was the principal target stem borer species, neither was it recovered from *S. calamistis* which spatially overlaps in distribution with *B. fusca*. Interestingly, *C. sesamiae* was recovered from *C. partellus*. If *C. sesamiae* had been recovered from *B. fusca* during this survey, this could have warranted further tests (gel electrophoresis) to distinguish if it was the virulent highland or the avirulent coastal biotype. Since it was recovered from *C. partellus*, it was most probably the avirulent, coastal biotype as it was unable to parasitize *B. fusca* despite the host's availability as depicted by the stem borer composition results. Further to this, *C. sesamiae* recovery from *C. partellus* confirmed that indigenous parasitoids expanded their host range to include the exotic stem borer [37, 38]. The other parasitoid, *C. flavipes*, was only recovered from its old association host, *C. partellus.*

## 5. Conclusion

The highland *C. sesamiae* did not establish in Taita Hills. This is explained by the low stem borer parasitism rates coupled with high *B. fusca* infestation rates. Parasitoid establishment and spread is dependent on suitable climatic parameters and host availability. Considering that climatic matching was done between the source of *C. sesamiae* and the recipient ecosystem, failure to establish cannot be attributed to difference in climatic parameters nor to non-availability of suitable hosts. Non-establishment in this area, may be attributed to the single release as CBC proponents agree that parasitoid establishment is enhanced by boosting natural enemy population through multiple releases [39]. In order to increase chances of *C. sesamaie* establishment, multiple releases should be done over various maize growing seasons and possibly years so as to boost the natural enemy population in a new environment.

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