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Prospecting for fine scale establishment of exotic stem borer pupal parasitoid (X*anthopimpla stemmator* Thunberg) in Kenya

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Abstract

Lepidopteran stem borers are an important constraint to cereal production in Sub-Saharan Africa. The exotic *Chilo partellus* (Swinhoe) is one of the most economically important stem borer pest causing extensive losses on cereal crops in Kenya. This pest has also displaced indigenous species of stem borers while expanding its range in warm, mid and high altitude areas. In order to exert control on various developmental stages of this pest, both *Cotesia flavipes* Cameron (larval endoparasitoid) and *Xanthopimpla stemmator* Thunberg (pupal endoparasitoid) were imported and released in 2002 in the Eastern region of Kenya. This study was conducted to assess the establishment status, spread and impact of *X. stemmator* on *C. partellus* following its release in Kenya. Stem borer sampling was done on farms where the biocontrol agents had been released and on transects radiating outwards from them every 15km to assess spread. A total of 100 maize plants were inspected for stem borer infestation and destructive sampling done on 10 maize stems per farm to collect immature stem borer stages. Emerging parasitoids and adult moths were identified, counted and recorded. Results of this study showed that *C. partellus* was the most dominant stem borer species (constituted 71.2%) followed by *Sesemia calamistis* and *Busseola fusca* (26.0 and 2.8% respectively). A decrease in overall stem borer infestation (22.47±7.42%) with no significant difference across distances from parasitoid release points (F=0.4; df=3, 51; p>0.05) was also recorded. Seven parasitoid species were recovered, the most abundant being *C. flavipes* and this coupled with a significant increase in parasitism (25.27±3.27%) (V=1213, p<0.05) from pre-release levels. The parasitoid of interest, *X. stemmator* was not recovered. This suggests a failure to establish though there is need to sample alternative hosts before this is declared.

Key words: Classical biological control, lepidoptera, pupal parasitoid, post release survey, *Xanthopimpla stemmator*, Eastern Kenya

Introduction

Lepidopteran stem borers constitute important biotic factors that constrain maize and sorghum production in Sub Saharan Africa (SSA) (Brownbridge, 1991; Odindo, 1991; Schulthess *et al.*, 1997; 2007). However, losses associated with stem borer pest infestation varies among regions in SSA depending of stem borer community composition. In East Africa, the economically important lepidopteran stem borers are *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Family: Noctuidae) and *Chilo orichalcociliellus* Strand and *Chilo partellus* (Swinhoe) (Family: Crambidae) (Nye, 1960, Bonhof *et al.*, 1997, Overholt *et al.*, 2001). All the aforementioned pest species are indigenous to African continent except for *C. partellus* (Nye, 1960; Bleszynski, 1970; Van Hamburg, 1979) which was accidentally introduced from Asia in 1930s (Tams, 1932). Since its introduction, *C. partellus* has become one of the most economically important pests with losses associated with its infestation varying between 73 and 100% in maize, and 88 and 100% in sorghum (Seshu Reddy, 1983; 1988; Ampofo, 1986; Seshu Reddy & Walker, 1990).

Due to economic importance of *C. partellus*, different management strategies including chemical, cultural, habitat management and host plant resistance have been utilized to reduce its populations (Seshu Reddy, 1985; Bonhof, 2000; Kfir *et al.*, 2002). Focus shifted towards biological control in order to find ecologically sound, technically and economically feasible techniques (De Bach, 1974; Sanda & Sunusi, 2014). A wide range of indigenous parasitoids including *Cotesia sesamiae* (Cameron), *Dolichogenidea polaszeki* Walker, *Chelonus curvimaculatus* (Cameron), (larval parasitoids), *Pediobius furvus* (Gahan), and *Dentichasmias buseolae* (Heinrich) (gregarious pupal parasitoids) and *Psilochalsis soudanensis* (Steffan) (solitary pupal parasitoid) expanded their range to include this exotic species (Kfir, 1992; Zhou *et al.*, 2003). However, the effect of this native parasitoid assemblage has been recorded at less than 5% and is considered negligible (Mohyuddin &

Greathead, 1970; Oloo & Ogeda, 1990; Bonhof *et al.,* 1997; Zhou *et al*., 2003). *icipe*'s biological control programme thus spearheaded the importation and eventual release of the exotic, koinobiont, larval endoparasitoid *C. flavipes* Cameron from *C. partellus'* native range in 1993 (Overholt *et al*., 1994a).

To further suppress *C. partellus* population and build on stem borer natural enemy complex in Kenya, a solitary, idiobiont, pupal endoparasitoid, *Xanthopimpla stemmator* Thunberg (Hymenoptera: Ichneumonidae) was imported from South Africa in 2001. *Xanthopimpla stemmator* which is Asian in origin, is known to parasitize pupae of various lepidopteran stem borers. Prior to release in 2002, various pre-release studies were carried out regarding host suitability (Gitau *et al*., 2007), interspecific competition with native parasitoid species (Muli *et al*., 2006) and its performance in the field (Muturi *et al*., 2005). After these studies, releases were done in the Eastern region of Kenya, at two locations, Machakos and Kitui. Despite its potential, no post release assessments have been carried out to confirm its establishment. This study was thus undertaken to document fine scale establishment status and spread of *X. stemmator* since its release in Kenya in 2002.

Methodology

Description of study area

This study was carried out in the Eastern region of Kenya where pupal parasitoid, *X. stemmator*, was released in 2002. *Xanthopimpla stemmator* was released on various farms in Machakos and Kitui counties (Figure 1). The Eastern region is located in dry mid-altitude agro-ecological zone, characterized by temperatures ranging from 14 to 33°C. The area lies at an altitude of 700-1,400masl and receives annual rainfall varying between 300 and 550mm (Corbett, 1998).

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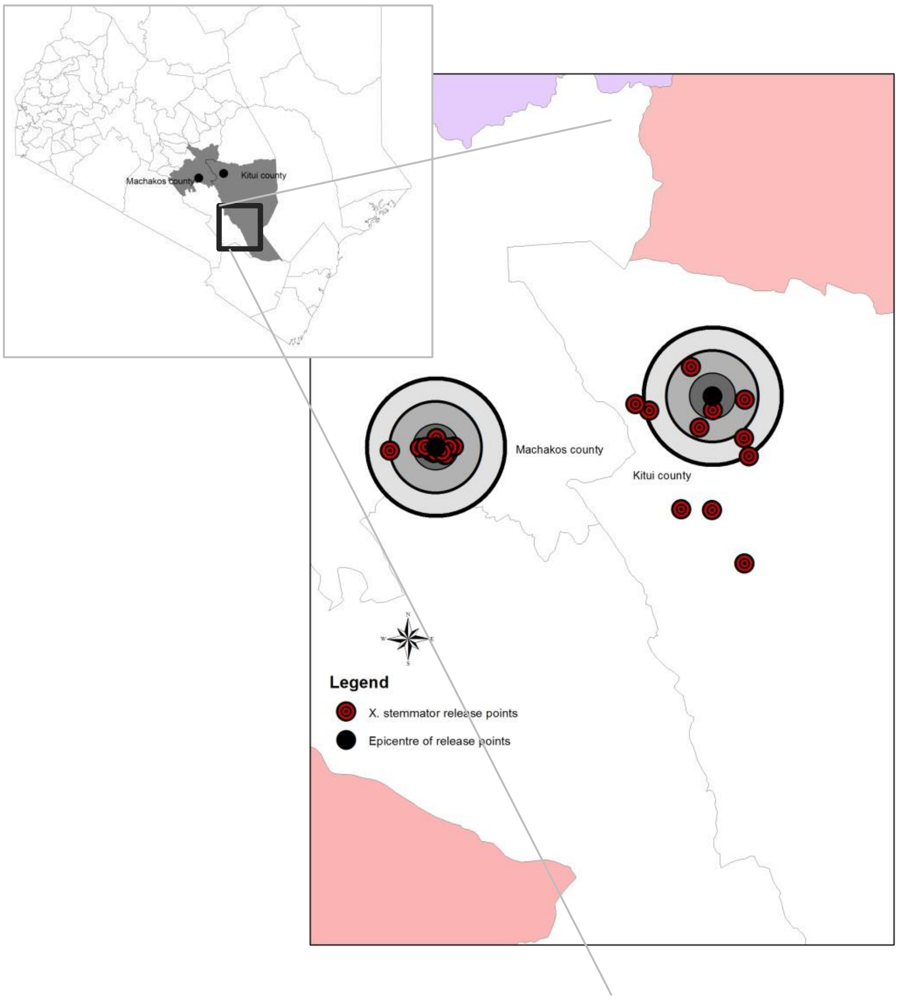

Figure 1: Release and sampled sites for *X. stemmator* in Machakos and Kitui counties, Eastern region of Kenya.

Sampling for stem borers

Maize farms around *X. stemmator* release sites were identified and marked for assessment of stem borer infestation and parasitism. Marked farms radiated outwards along transects in the four cardinal compass directions as the terrain allowed. Stem borer infestation levels were assessed in farms at intervals of 15, 30 and 45 km along transects laid in the cardinal directions from the release points (Figure 1).

In each farm, a total of 100 maize plants were inspected for stem borer infestation during which 10 infested maize stems were destructively sampled and dissected. Immature stem borer stages were collected, identified and categorized (as small {1st and 2nd instars}, medium, {3rd and 4th instars} and large {5th and 6th instars}). Identified larvae were placed individually in glass vials containing artificial diet (Onyango and Ochieng-Odero, 1994) 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, pupal parasitoid 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.

Statistical analyses

The number of infested plants was expressed as a percentage of the total plants inspected in respective fields and resulting data was used to compute percentage stem borer infestation. At each sampling distance, inspected farms were treated as replicates and the data pooled before analysis. Parasitoid cocoons that were spun from appropriate larval stages were expressed as a proportion of the respective field densities in order to compute percentage parasitism. Percentage infestation and parasitism were subjected to the normality test and data that failed the normality test were appropriately transformed before further analysis. Normally distributed data was analysed using One-Way ANOVA and significantly different means were separated using Tukey's HSD test. Data which failed the normality test was subjected to Kruskal-Wallis rank sum test and significantly different means were separated using Nemenyi post-hoc test ($p<0.05$). One sample *t*-test and Wilcoxon rank sum tests were used to compare mean infestation and parasitism levels obtained before and after parasitoid release.

Results

Stem borer species composition and diversity

A total of 5,500 maize plants were sampled from 55 farms surveyed during the study. Three stem borer species, *C. partellus, B. fusca* and *S. calamistis* were identified from the sampled larvae. *Chilo partellus* was the most dominant pest constituting 71.2% of the total stem borer population collected. The other two species, *S. calamistis* and *B. fusca,* were generally low constituting 26.0 and 2.8% of the total stem borer community respectively (Table 1).

Table 1: Stem borer species recovered, their percentage composition and pest density per infested plant in Eastern region

Stem borer species	% composition	Larval density ($\bar{x}$ ±SE)
Chilo partellus	71.2	2.4 ± 0.4^{b}
Sesamia calamistis	26	0.8 ± 0.2^{a}
Busseola fusca	2.8	0.1 ± 0.0^{a}
χ^2 value		74.92
df		2
p value		2.20E-16

Parasitoid and hyperparasitoid species composition and diversity

Seven parasitoid species were recovered during the survey with the ecological congeners, *C. flavipes* and *C. sesamiae* being the most abundant (Table 2). In addition to parasitoids, hyperparasitoid, *Aphagnomus fijiensis* (Ferriére) was also recovered from some larvae. Larval parasitoids dominated the stem borer natural enemy complex in the study area with only one pupal parasitoid, *Dentichasmias busseolae* Heinrich identified from the collection.

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Table 2: Parasitoids recovered, their percentage composition, guild and host species in Eastern region.

Parasitoid species	Composition (%)	Guild	Stem borer species parasitized
Cotesia flavipes (Cameron)	76.5	Larval	*C. partellus, S. calamistis, B. fusca*
Cotesia sesamiae (Cameron)	7.6	Larval	*C. partellus, S. calamistis, B. fusca*
Dolichogenidea polaszeki Walker	0.1	Larval	*S. calamistis*
Chelonus curvimaculatus (Cameron)	0.4	Larval	*C. partellus, B. fusca*
Atherigona sp (Rondani)	0.03	Larval	*C. partellus*
Dentichasmias busseolae Heinrich	0.2	Pupal	*C. partellus*
Sturmiopsis parasitica (Curran)	0.0	Larval/pupal	*B. fusca*
Aphanogmus fijiensis (Ferriére)	15.1	Hyperparasitoid	*C. partellus, S. calamistis*

Stem borer infestation and parasitism levels

During the survey, overall stem borer infestation was estimated to be 22.5±7.4% (Table 3). Further analysis revealed no difference in infestation across distances from parasitoid release points ($F_{3,51}$=0.4; p>0.05) (Table 3). Stem borer infestation levels significantly reduced after the release of *C. flavipes* and *X. stemmator* (t_{54}=41.6; p<0.05). Stem borer parasitism levels in this region were recorded at 25.3±3.3%. This was a significant increase from parasitism levels previously recorded following parasitoid release (V=1213; p<0.05). Pupal parasitism was estimated at 0.03±0.02% (Table 4).

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Table 3: Stem borer infestation and parasitism levels ($\bar{x} \pm SE$) across distances from release points.

Distance from release points	No. of farms	Infestation	Parasitism
0KM	10	22.4±6.7[a]	26.6±7.6[a]
15KM	20	24.8±2.1[a]	31.9±6.0[a]
30KM	14	20.6±2.6[a]	23.0±5.8[a]
45KM	11	20.7±3.3[a]	14.9±6.7[a]
df		3, 51	3, 51
F value		0.4	4.34
p-value		0.75	0.23

Table 4: Overall stem borer infestation before and after parasitoid release in Eastern region of Kenya

Period	Infestation (%)	Period	Parasitism (%)
Pre-release (1997)	92[a]	Pre-release (2001)	10[a]
2014	22.5±7.4[b]	2014	25.3±3.3[b]
t value	41.63	*V* value	1213
df	54		
p value	2.20E-16	*p* value	0.0002

Discussion

Suppressing stem borer pest population is considered an important factor in enhancing maize production in tropical Africa. However, stem borer management interventions and their successful implementation varies among regions depending on the dominant/target pest species. During this study, *C. partellus* dominated the pest community in Eastern Kenya followed by *B. fusca* and *S. calamistis*, an observation that corroborated findings of Songa *et al*. (2002a, b; 2007). Except for *C. partellus*, all other stem borer species in the pest community are indigenous to the Africa continent. As an exotic species, *C. partellus* recruited several native natural enemies a characteristic that can explain the high number of natural enemies found associated with its larvae during the study. Similar observations were made in other studies during which native natural enemies reportedly expanded their host range to include the exotic *C. partellus* (Oloo & Ogedah, 1990; Kfir, 1992). Results obtained also uphold reports that *C. partellus* has a larger number of parasitoids attacking it in comparison to native stem borers (Zhou *et al.*, 2003). Despite the high number of parasitoids associated with stem borers in this area, the list might not be exhaustive as the survey was limited to a certain distance and only on farms radiating from *X. stemmator* release points. Other researchers recovered much more parasitoids from the stem borer population in the same region (Songa *et al.*, 2002a).

Growing dominance of *C. partellus* in the region due to limited success of indigenous natural enemies in suppressing its population informed the decision to introduce additional exotic natural enemies in the region. Larval parasitoid, *C. flavipes*, and pupal parasitoid, *X. stemmator*, were released in Eastern Kenya as part of classical biological control to augment population of indigenous natural enemies in the region. Collective action by the stem borer natural enemy assemblage within the Eastern region resulted in reduction of overall stem borer infestation in comparison to levels observed before release of *C. flavipes* and *X. stemmator*. The observed reduction was consistent across all different sampled radii and similar patterns were observed with respect to parasitism levels. Generally, there was a considerable increase in parasitism compared to lower levels (0.1-5.69%) recorded before release of *X. stemmator* (Songa *et al.*, 2002a). Observations in the present study are consistent with findings of other studies that have shown an existence of positive relationship between the diversity of parasitoids and parasitism (Hawkins & Gagne, 1989; Hawkins & Gross, 1993). Despite the high diversity of parasitoids recovered during this survey, *D. busseolae* was the only pupal parasitoid recovered. It was however present in low numbers with significantly low resultant parasitism, results that are consistent with findings of previous studies (Mohyuddin & Greathead, 1970; Oloo & Ogedah, 1990). This study was undertaken 15 years after the release of *X. stemmator* in the region and contrary to research expectation, the study did not yield any *X. stemmator* specimen. This observation may or may not imply failure of *X. stemmator* to establish in the region.

Pre-release host suitability studies revealed that *X. stemmator* has a broad host range and could successfully parasitize and develop in *C. partellus*, *S. calamistis* and *B. fusca* (Gitau *et al*., 2005). A range of reasons (excluding host suitability) could be advanced in an attempt to explain the lack of recovery within the surveyed fine scale. First, the releases were done on ten farms during short rains of 2002 in Kitui. However, no repeat releases were carried out. In Machakos, *X. stemmator* releases were done in the short rains of 2002 and 2003 and during the long rains of 2003 on an average of seven farms each time. CBC proponents agree that multiple releases boost the natural enemy population after the initial introduction in a new environment (Sanda & Sunusi, 2014). This is because the establishment process is marred by both biotic and abiotic factors whose effects can be abated by pumping in more and fresher individuals (Sanda & Sunusi, 2014). Biological control agent releases may need to be repeated sometimes over years to increase chances of establishment. Non-recovery of *X. stemmator* is not unique to this study. In Mozambique, *X. stemmator* was only recovered during the release season and one year after its release but not in subsequent years (Cugala, 2007).

Secondly, the biological control programme's main objective that necessitated *X. stemmator*'s release was to suppress *C. partellus* population. *Chilo partellus* occurrence in wild habitat has been reported by various researchers (Songa *et al*., 2002a, Ong'amo *et al*. 2006b; Otieno *et al*. 2006; Mohamed *et al*., 2007). Country-wide surveys on wild host plants in Kenya revealed that more than 95% of *B. fusca* and *C. partellus* were found on wild sorghum species providing a suitable refugia for *X. stemmator*. The wild habitat was however not sampled during this survey and this study cannot confirm the presence of *X. stemmator* in the wild. However, this gap needs to be explored before further decisions regarding the use of *X. stemmator* in management of *C. partellus* is made as wild host plants play an important role in the stem borer pest and parasitoid perennation (Muturi *et al.*, 2005; Mailafiya *et al.*, 2010).

Thirdly, an aspect of competition within the parasitoid community whose differentiation was demonstrated by the attack method used, was shown to be an important criterion in parasitoid selection (Muli *et al.*, 2006). *Xanthopimpla stemmator* uses the "drill and sting" attack strategy (Smith *et al.*, 1993) whereby the parasitoid pierces the stem to gain access to pupa in pupal chamber. While comparing the different attack strategies employed by pupal parasitoids, the "ingress and sting" attack strategy whereby the parasitoid seeks and attacks the stem borer host within the tunnel was thought to be superior to the "drill and sting" strategy (Muli *et al.*, 2006). *Xanthopimpla stemmator's* ovipositor length is about 0.52cm (Muturi *et al.*, 2005) and thus stem borer pupae in thin stemmed plants such as sorghum, millet and rice would be much readily available than those in large stemmed plants such as maize and sugarcane (Hailemichael *et al.*, 1994). This emphasizes further, the importance of sampling alternative hosts in order make a clear decision on whether *X. stemmator* established in the region or not.

Biological control success and failure reports from various countries inform decision making processes. Though reports of failed establishment of *X. stemmator* were also been made in South Africa where releases were done on maize and sorghum fields (Moore & Kfir, 1996, Kfir, 1997), its non-recovery during this study cannot be regarded as a non-establishment until wild and/or alternative hosts are sampled. This is because it successfully managed *Eldana saccharina* and *Chilo sacchariphagus* in sugarcane in South Africa (Conlong, 1994), Mozambique, Mauritius and Reunion (Moutia & Courtis, 1952; Moore & Kfir 1996; Conlong & Goebel 2002). Though *X. stemmator* was not recovered in maize fields during the study, the hope to use it in management of *C. partellus* in the area is ignited by the recovery of two specimen in maize fields in Lunga Lunga along Kenya/Tanzania border in a separate study (Abonyo, unpublished data). This result corroborated findings by Bonhof *et al*. (1997) who reported the parasitoid along the Kenyan Coast. Presence of *X. stemmator* along the Kenyan coast is thought to have come from influx of parasitoid populations from Tanzania, Uganda, Ethiopia, Zanzibar and Eritrea (Mailafiya, 2009) where releases had been done. These possible influxes of *X. stemmator* from neighbouring countries indicate that populations may have established in the respective countries. This study is therefore recommending repeated release of *X. stemmator* in selected multiple sites using populations from neighbouring countries.

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