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Establishment of *Telenomus isis* polaszek (Hymenoptera: Scelionidae), an exotic egg parasitoid of noctuid stem borer pests in maize production systems in Kenya

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Abstract
The noctuid *Busseola fusca* forms a major proportion of the stem borer pest community in Kenya and various management initiatives including importation and release of egg parasitoids have been undertaken. *Telenomus isis* Polaszek, a noctuid stem borer egg parasitoid endemic to West and Central Africa was released in Kenya in 2005 to manage *B. fusca* in Wundanyi and Eldoret. Since the releases, their establishment status remains unknown. This study was undertaken to assess the parasitoid's establishment and fields with maize at pre-tasselling stage around the release sites were inspected. Three egg parasitoid species; the indigenous *Telenomus busseolae* Gahan and *Trichogrammatoidea lutea* Girault and the exotic *T. isis* were recovered from survey sites. The establishment of *T. isis* in Kenya is hereby confirmed, though the parasitoid was only present in release fields in both localities. Results of this study also revealed changes in egg parasitism (from parasitoid pre-release figures) with a significant reduction in Eldoret (V=326; p=0.00072). In Wundanyi, a significant increase in egg parasitism was recorded (V=211; p=0.00035). Mean egg parasitism was significantly different between the localities (W=57; p=6.8e^{-10}). Mean discovery efficiency in Wundanyi was significantly higher (55.4±6.1%) than in Eldoret (8.9±2.2%) (W=87; p=6.08e^{-09}5). There was no significant difference in parasitism efficiency between the two localities (W=397, p=0.061).

Keywords: *Biological control, Noctuidae, augmentative releases, synergistic, redistribution, egg parasitism*

1. Introduction

Maize is one of the most important cereal crop in Sub-Saharan Africa (SSA) [39, 13] and an important staple food for more than 1.2 billion people in the region. Introduced into Africa in the 1500s, maize recruited indigenous pests some of which currently constrain its production [23, 30, 33]. Important among the recruited indigenous pests are the phytophagous lepidopteran stem borers [5, 24] which generally vary in their distribution among different regions in Africa [17. 28]. In Eastern and Southern Africa, important stem borer pests are *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson (Family: Noctuidae), *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Family: Crambidae) [33, 40, 30]. Among the aforementioned stem borer pests, *B. fusca* and *C. partellus* constitutes a major proportion of the stem borer pest community in Kenya [38, 4, 42, 45] and thus results in considerable maize yield losses.

Due to losses associated with stem borer pest infestations, different management approaches have been initiated to keep pest populations below economically damaging levels. For stem borer pest management, egg parasitoids can be an important source of mortality before the pest reaches the most destructive larval stage [43]. Focus on the management of *B. fusca* in Kenya led to the importation of an egg parasitoid, *Telenomus isis* Polaszek (Hymenoptera: Scelionidae), from West Africa in 2005 [36, 44]. *Telenomus isis* is a solitary egg parasitoid of noctuid stem borers in West and Central Africa [41. 20. 21, 37, 9]. In West and Central Africa *T. isis* co-exists with *Telenomus busseola* Gahan (Hymenoptera: Scelionidae) in the *T. busseolae* complex where together, they suppress populations of noctuid stem borers; *B. fusca* and *S. calamistis* [41] .However, of the species in the *T. busseolae* complex found in West Africa, only *T. busseolae* exists in East Africa. It is on this background that *T. isis* was released in Kenya in late 2005 to synergistically work with *T. busseolae* to suppress populations of the dominant *B. fusca* in mid altitude / highland zones [36, 44].

Importation and release of *T. isis* was part of a redistribution approach initiated by IITA with an aim of exchanging natural enemies and strains between African regions. However, as with other biological control agents released in new environments, questions on the spread and establishment of *T. isis* in East Africa remain unanswered. A post-release survey done in 2006, reported overall egg parasitism rates of about 37.9±1.7% in Wundanyi. However, the aforementioned post release survey was done barely one year after the release. This may in turn have resulted in erroneous conclusions and recommendations. No post release survey has been carried out in Eldoret, another *T. isis* release site. This study was thus undertaken to assess the establishment and dispersal of *T. isis*, and determine changes in egg parasitism in midland and highland zones in Kenya.

2. Materials and Methods

2.1 Description of the study area

Surveys were carried out in two localities, Eldoret and Wundanyi; localities at which *T. isis* was released in 2005 (Fig. 1). Eldoret is a high altitude area (1500-2400masl) while Wundanyi stretches across mid to high altitude areas. In Eldoret, *T. isis* releases were done on 13 farms (Fig. 1). These farms were however close to one another and were collapsed during sampling to avoid overlaps among transects. In

Wundanyi, *T. isis* was released at three sites: Josa, Wesu and at the Wundanyi prison's farm (Fig. 1). This study was undertaken during the month of April 2014, the period during which stem borer eggs were considerably abundant in the field (Yaovi *pers. com*).

2.2 Sampling protocol
Maize farms with pre-tasseling plants were identified radially along the four cardinal compass directions from points of parasitoid release and marked for sampling. A total of 52 and 21 fields were sampled in Eldoret and Wundanyi respectively. During sampling, marked fields were divided into five subplots; one at each corner and one at the centre of the field. All plants in each subplot were inspected for noctuid stem borer eggs. The number of plants inspected, number of egg batches per plant and the number of eggs per batch were recorded. Egg batches were collected and individually placed in glass vials, plugged with cotton wool and labelled. Collected eggs were taken to the laboratory at *icipe* where they were incubated at 25±1°C and 60-70% relative humidity. Incubated eggs were checked and recorded each day for emergence of stem borer larvae or parasitoids. Recovered parasitoids were identified using keys developed by [32].

2.3 Statistical analyses
Number of eggs and egg batches were subjected to Wilcoxon rank sum test to test for differences between the localities. Three kinds of parasitization parameters were computed: (a) mean egg parasitism per field was calculated as percentage of eggs parasitized within an individual egg batch averaged over all egg batches in the field. (b) Discovery efficiency was calculated as the percentage of egg batches with parasitoids per field [3]. (c) Parasitism efficiency was calculated as the percentage of eggs parasitized within discovered egg batches averaged over all egg batches per field [3]. Egg parasitism, discovery efficiency and parasitism efficiency constituted proportion data and were analysed using Wilcoxon rank sum test (W) to differentiate between localities and one sample Wilcoxon test (V) to separate between results obtained before and after parasitoid release. Statistical analyses were performed in SAS [35] programme.

3. Results
3.1 Egg parasitoid community and their relative contribution
Three egg parasitoid species, the indigenous *T. busseolae* and *Trichogrammatoidea lutea* Girault (Hymenoptera: Trichogrammatidae) and the exotic *T. isis* were identified. Though these parasitoids were present in the two localities, there was only a single instance in Eldoret where there was multiple species parasitism in one egg batch. The observed multiple parasitism involved *T. busseolae* and *Tr. lutea*.
In terms of species importance, *T. busseolae* was recovered from 21 fields, *T. isis* from 3 fields and *Tr. lutea* from 6 fields in Eldoret (Fig. 1). In Wundanyi, *T. busseolae* was recovered from 20 fields, *T. isis* from 1 field and *Tr. lutea* from 2 fields (Fig. 1). Of the three parasitoid species, *T. busseolae* dominated the parasitoid community constituting 89.9 and 97.8% of the total parasitoids collected in Eldoret and Wundanyi respectively. *Trichogrammatoidea lutea* constituted 6.8 and 0.2% in Eldoret and Wundanyi respectively while *T. isis* constituted 3.3 and 2.1% in Eldoret and Wundanyi respectively. *Telenomus isis* was only recovered from fields where releases had been done in 2005.

3.2 Stem borer egg batches and general parasitism
Collected number of egg batches varied among the sampled localities, Eldoret (1,168) and Wundanyi (683), with each locality giving a total of 51,944 and 15,529 eggs respectively. The mean number of egg batches per field varied significantly between the localities ($W = 292$, $p<0.05$) with relatively higher mean observed in Wundanyi (32.5±5.2) compared to Eldoret (22.5±6.3) (Table 1). In Eldoret, mean number of egg batches recovered (22.5±6.3) was significantly higher than in pre-release period (2004) (2.1±0.2) (V=1147, $p<0.05$). This pattern was also depicted in Wundanyi where mean number of egg batches recovered during this study (32.5±5.2) was significantly higher than in 2004 (1.0±0.1) (V=228, $p<0.05$) (Table 1). Egg batch size varied between the two localities. In Eldoret, the sizes fluctuated between 0 to 196 eggs with a variance of 660.4 eggs while in Wundanyi, it fluctuated between 2 to 144 eggs with a variance of 176.23 eggs.
Differences were also observed in the mean number of eggs per batch (Table 1). Mean number of eggs per batch were significantly higher in Eldoret (30.4±3.0) compared to Wundanyi (21.3±0.7) (W=292, $p<0.05$). In Eldoret, mean number of eggs per batch (30.4±3.0) was significantly lower than pre-release period (34.2±1.4) (V=1198, $p<0.05$) while in Wundanyi, mean number of eggs per batch collected during this study was significantly lower (V=228, $p<0.05$) (Table 1).
Generally, there was evidence of differences in egg parasitism between the localities. Of all egg batches collected in Wundanyi, 361 were parasitized compared to 83 found parasitized in Eldoret. This was consistent with mean egg parasitism observed in Eldoret (7.31±2.04%) and Wundanyi (52±5.79%) which were significantly different (W=57, $p<0.05$) (Table 2). These were however different from parasitism values observed in respective localities in 2004 before release of *T. isis*. In Eldoret, egg parasitism prior to release of *T. isis* (10.0±0.45%) was significantly higher than after the release (7.31±2.04%) (V=326, $p<0.05$) while in Wundanyi, it was significantly higher after *T. isis* release (52±5.79%) compared to pre-release (25.9±1.96%) (V=211, $p<0.05$) (Table 2).
The observed variations in egg parasitism were similar to variations in discovery efficiency (Table 2). The mean discovery efficiency observed in Wundanyi (55.4±6.11%) was significantly higher compared to observations made in Eldoret (8.9±2.23%) (W=87, $p<0.05$). This however was different from observations made in respective localities in 2004. In Eldoret, mean discovery efficiency in 2004 was significantly higher (14.2±1.9%) than what was recorded during this study (8.9±2.2%) (V=299, $p<0.05$). In contrast, mean discovery efficiency in Wundanyi during this study was significantly higher (55.4±6.1%) compared to pre-release results (32.9±1.7%) (V=199, $p<0.05$).
Results of this study show that parasitism efficiency was relatively low with no significant difference between Eldoret (7.31±2.04%) and Wundanyi (2.41±0.51%) ($W = 397$, $p > 0.05$) (Table 2). However, observed parasitism efficiency in Eldoret (7.31±2.04) was lower compared to pre-release period (63.6±1.53%) (V=0, $p<0.05$). Similar difference was observed in Wundanyi where the observed parasitism efficiency (2.41±0.51%) was lower compared to the pre-release period (79.9±1.15%) (V=0, $p<0.05$).

4. Discussion

This study revealed the presence of three egg parasitoids, *T. busseolae*, *T. isis* and *Tr. lutea* in the noctuid stem borer eggs in Kenya. Presence of these parasitoids corroborates findings by [44] who in addition to these three species reported *Telenomus spp* and *Tr. bournieri* in Wundanyi. The existence of *T. isis* in the egg parasitoid community in Eldoret and Wundanyi confirms its establishment in localities in which it was released in 2005. Before the release of *T. isis* in Kenya, key factors such as climatic tolerance and host suitability were studied as both biotic and abiotic factors are involved in determining species establishment [14, 15, 31, 7]. Based on results of these studies, predictions regarding favourable areas in East Africa were made. Predicted favourable areas included the mid and high altitude zones [7, 44]. Presence of *T. isis* in the release areas as witnessed in this study confirms that predicted release areas were actually favourable for *T. isis*.

Parasitoids have been reported to experience complex interactions with other parasitoids and hyperparasitoids [11]. Such interactions may in some cases result in multiple species parasitism. There were concerns before release of *T. isis* that it would face competition from indigenous egg parasitoids through such interactions that would in turn limit its establishment. These concerns emanated from results of detailed studies of extrinsic and intrinsic competition between egg parasitoids, especially between *T. busseolae* and *T. isis* [1]. Contrary to above fears, this study indicates that there is no multiple parasitism involving *T. isis*. The lack of multiple species parasitism cases by *T. isis* may be explained by its characteristic avoidance mechanism. Studies show that *T. isis* and its conspecific, *T. busseolae*, strongly avoid multiple species parasitism indicating interspecific host discrimination [1]. Of the three parasitoids collected in the two localities, Eldoret and Wundanyi, *T. busseolae* dominated the parasitoid community. Similar dominance of parasitoid community by *T. busseolae* was noted by [34, 37, 44] who attributed it's the dominance to faster development and its competitive superiority in cases of superparasitism [6, 7]. Despite the dominance of *T. busseolae*, results of this study indicate that *T. isis* has established though it was only present in fields of previous release.

Despite *T. isis* establishment, this study showed persistence of stem borer pests in crop fields. This is evident in the observed number of stem borer egg batches on maize plants with majority of plants having single egg batches. Single egg batches observed on maize plants is not unique to this study as such a trend was reported in similar study [44]. This observation is attributed to the female noctuid stem borer oviposition characteristic in which they are able to recognize and avoid plants with egg batches [41. 22, 8]. This behaviour is used to reduce competition for the food resource and increased mortality associated with emigration to other food sources.

The observed mean number of eggs per batch was higher in Eldoret compared to Wundanyi. This may be explained by the difference in farming practices in the two localities. In Eldoret, most fields are monocultures of maize grown for commercial purposes while in Wundanyi there is mixed cropping as most households farm for domestic consumption. The variation in egg batch sizes may be explained by the clutch size plasticity hypothesis. The theory hypothesizes that female lepidoptera preferentially oviposit larger egg clutches in habitats that ensure survival of F1 generation [12]. Larger maize monocultures in Eldoret provide more food compared to smaller farms in Wundanyi where mixed cropping is practised. In contrast, the mean number of egg batches per field was generally higher in Wundanyi compared to Eldoret. Variation in the number egg batches between Wundanyi and Eldoret may be attributed to differential adjustment in female response to habitat quality. Studies on oviposition plasticity and number of batches show that female's decision to allocate her reproductive investment is based upon variation in quality of encountered plants [14]. Habitat quality in Wundanyi is relatively poor compared to Eldoret and female moths must thus lay several egg batches to ensure survival of the progeny.

Contrary to expectations of the biological control programme, a decrease registered in Eldoret and the difference in activity of *T. isis* in the two localities may be attributed to prevailing cropping systems. In Eldoret, there is only one maize growing season that runs over seven months. The main host, *B. fusca* enters into diapause in the absence of the main crop host (maize) [18] and this limits availability of eggs that could otherwise host *T. isis.* This may explain the observed low parasitoid action. In contrast, Wundanyi is characterized by two maize growing seasons and this ensures availability of eggs to host *T. isis* for a longer period in the year. In addition to favourable season, Wundanyi is endowed with a network of rivers which are used by farmers for irrigation during the dry season. This ensures availability of maize plants [44] and thus *T. isis* hosts, stem borer eggs. This explains the higher egg parasitism in Wundanyi and validates predictions by [7] who projected that the availability of host eggs during off season would aid in *T. isis* establishment in mid-altitude areas. Besides the cropping systems, availability of other suitable hosts of *T. isis* resulted in increased parasitism. Non-diapausing *S. calamistis*, is found in mid-altitude Wundanyi where it is able to support the parasitoid population when the main host may not be available. This validates the suggestion that presence of significant populations of suitable, non-diapausing non-target species would increase the chance of establishment of *T. isis* in Kenya [44]. Studies in other regions have demonstrated that maintaining noctuid populations all year round can lead to *T. isis* outcompeting *T. busseolae* [10]. Witholding the conditions in Wundanyi (that necessitate for host availability all year round) and if *T. isis* reaches the spreading phase, this may be achieved.

The difference observed in parasitism parameters computed for the localities may have also arisen from average size of egg batches. Larger egg batches recorded in Eldoret resulted in lower parasitism as in such, central eggs are inaccessible for parasitization. In contrary, smaller egg batches recovered from Wundanyi resulted in higher parasitism as a larger number of the eggs were accessible to the parasitoid. Parasitization starts from peripheral eggs such that smaller egg batches are easily reached into by the parasitoid compared to large egg batches whose central rows may be left unparasitized [2].

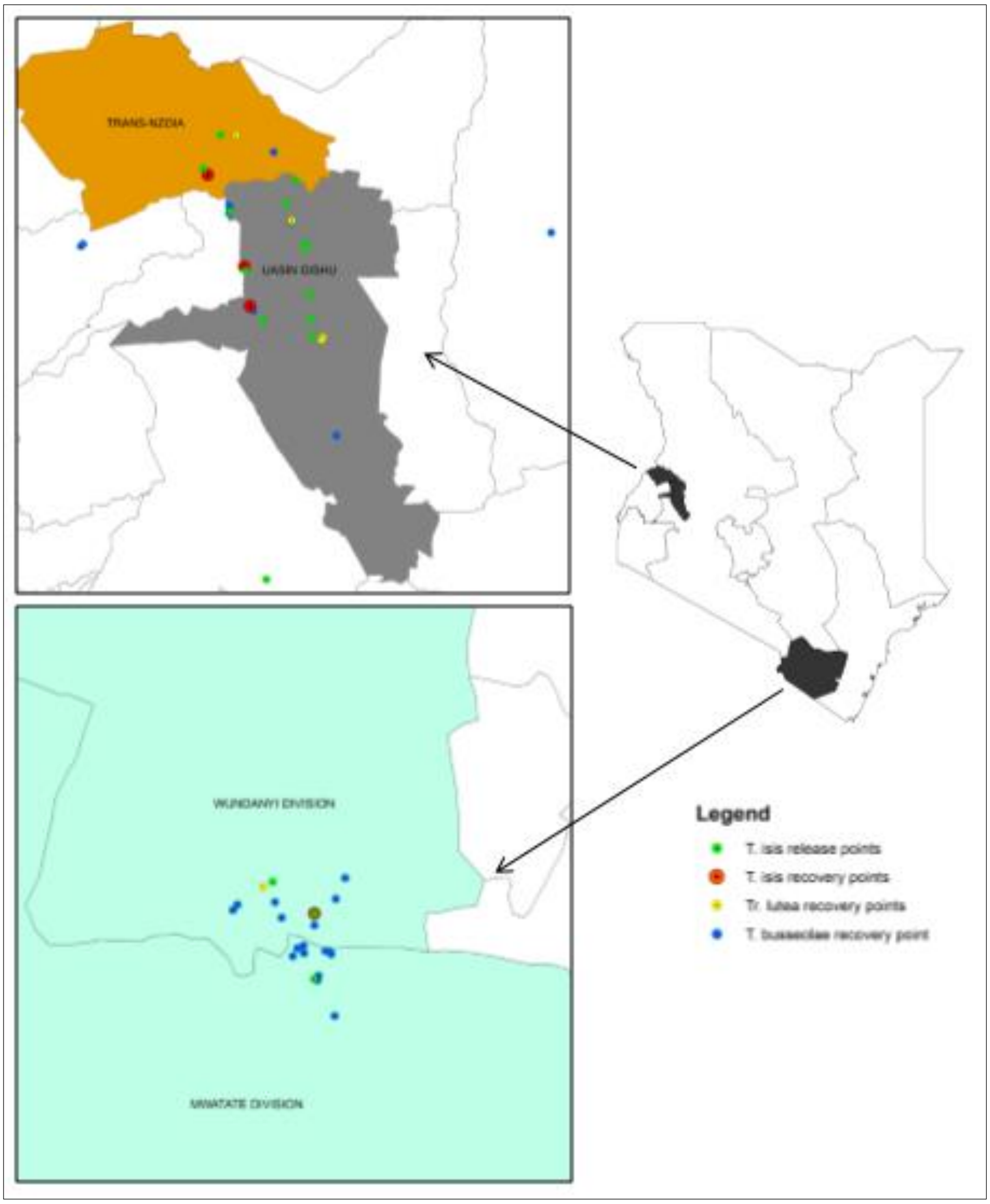

Fig 1: Points in Uasin Gishu and Trans-Nzoia counties and Wundanyi and Mwatate subcounties where egg parasitoids of noctuid stem borers were released and recovered.

Table 1: Mean number of eggs per batch and egg batches per field of egg parasitoids in Eldoret and Wundanyi.

	Period	Mean number of eggs/batch	Mean number of egg batches/field
Eldoret	2004	34.2 ± 1.4^{a}	2.1 ± 0.2^{a}
	2015	30.4 ± 3.0^{b}	22.5 ± 6.3^{b}
	V-value	1198	1147
	p-value	3.41e $^{-06}$	2.93e $^{-05}$
Wundanyi	2004	25.7 ± 1.4^{a}	1.0 ± 0.1^{a}
	2015	21.3 ± 0.7^{b}	32.5 ± 5.2^{b}
	V-value	228	228
	p-value	4.77e $^{-06}$	9.87e $^{-05}$
Eldoret Wundanyi	2015	30.4 ± 3.0^{a}	22.5 ± 6.3^{a}
	2015	21.3 ± 0.7^{a}	32.5 ± 5.2^{b}
	W-value	416	292
	p-value	0.113	0.0019

Mean (±SE) within columns followed by the same lower case superscripts are not significantly different ($p>0.05$). Pre-release period is denoted by 2004 while post-release period is denoted by 2015

Table 2: Egg parasitism, discovery and parasitism efficiency of egg parasitoids in Eldoret and Wundanyi.

Locality	Period	Egg parasitism	Discovery Efficiency	Parasitism efficiency
Eldoret	2004	10.0 ± 0.5^{a}	14.2 ± 1.9^{a}	63.6 ± 1.5^{a}
	2015	7.3 ± 2.04^{b}	8.9 ± 2.2^{b}	7.3 ± 2.04^{b}
	V-value	326	299	0
	p-value	0.0007223	0.0002803	1.35e $^{-10}$
Wundanyi	2004	25.9 ± 2.0^{a}	32.9 ± 1.7^{a}	79.9 ± 1.2^{a}
	2015	52 ± 5.8^{b}	55.4 ± 6.1^{b}	2.4 ± 0.5^{b}
	V-value	211	199	0
	p-value	0.0003538	0.003913	9.54e^{-07}
Eldoret Wundanyi	2015	7.3 ± 2.0^{a}	8.9 ± 2.2^{a}	7.3 ± 2.0^{a}
	2015	52 ± 5.8^{b}	55.4 ± 6.1^{b}	2.4 ± 0.5^{a}
	W-value	57	87	397
	p-value	6.80e^{-10}	6.08e^{-09}	0.061

Mean (±SE) within columns followed by the same lower case superscripts are not significantly different ($p>0.05$). Pre-release period is denoted by 2004 while post-release period is denoted by 2015

5. Conclusion

Through this study, it is evident that the introduction of *T. isis* in Kenya was a success as the parasitoid has established. However, it is probable that further spread and impact of *T. isis* may have been achieved if releases had been done repeatedly over a period of time. This has been practiced in other classical biological control programmes such as *Cotesia flavipes* Cameron which then resulted in extensive spread [25. 26. 27. 29]. Similar release practices were proposed by [19] who asserted that species introduced in large and consistent quantities are more likely to survive unlike species introduced in small numbers with only a few release events. Like in other organisms, establishment of a new species is followed by spread whereby the organism becomes part of the new habitat's fauna before expanding its range to produce significant impact in composition, structure and ecosystem processes. This study thus projects that levels of *T. isis* will rise and spread to favourable areas as there is evidence of steady increase in egg parasitism. It is recommended that extensive releases be done in suitable areas in order to increase populations of *T. isis* and to realize impact on noctuid stem borer pest population in Kenya.

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