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Do parasitoids reduce cereal losses? Estimating the impact of parasitoids on stem borer pest infestation and maize yield

Esther Adhiambo Abonyo[1,2], George Otieno Ongámo[1,2], Gideon Nyamasyo[1], Catherine Lukhoba[1], Gerphas Ogola[2], Zoul Midingoyi[3], Hippolyte Affognon[3], Bruno Le Ru[2,4]

[1]College of Biological and Physical Sciences, University of Nairobi, P. O. Box, 30197, Nairobi, Kenya

[2]International Centre of Insect Physiology and Ecology, *icipe*, P. O. Box 30772-00100, Nairobi, Kenya

[3]International Crops Research Institute for the Semi-Arid Tropics, BP 320, Bamako, Mali

[4]UMR IRD 247, EGCE, Evolution, Génomes, Comportement et Ecologie, CNRS, 91198 – Gif-sur-Yvette, France and Université de Paris-Sud, 91405 - Orsay, France.

Corresponding author: Esther Adhiambo Abonyo E-mail address: e_abonyo@yahoo.com

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ABSTRACT

Chilo partellus (spotted stem borer) is an economically important stem borer pest in Eastern Africa causing high yield losses in maize and sorghum. The larval endoparasitoid, *Cotesia flavipes* (parasitic wasp) was released in Kenya to manage *C. partellus* and post release studies showed it had established and stem borer parasitism was steadily increasing. Despite the rise in parasitism, doubts on whether parasitoids can effectively reduce stem borer populations have been raised.

Objective: This study was carried out to estimate the impact of *C. flavipes* on stem borer infestation and the resultant effect on maize yield in Kenya.

Methodology and results: Using insecticidal check method, selective insecticides were used to exclude stem borer pests and natural enemies in Treatments A and B respectively with controls designated as Treatment C. Results revealed significant differences in stem borer infestation ($F_{2,9}$=5.835; p<0.05) and parasitism ($F_{2,9}$ =91.97; p<0.0001) among the three treatments. Significantly higher infestation was recorded in treatment B (2.0±0.2%) compared to A (0.5±0.3%) and C (1.5±0.4%). Parasitism was significantly higher in treatment C (23.5±6.8%) compared to A (0.0±0.0%) and B (0.3±0.1%). However, there was no significant difference in yield among treatments showing that parasitoid action did not translate into changes in yield.

Conclusions and application of findings: The parasitoid *C. flavipes* has made an important contribution towards suppressing stem borer population. This was shown by high infestation recorded in treatment B which was as a result of partial elimination of parasitoids. This deduction was further supported by the results which showed that pest exclusion using chemicals did not have a significant difference from parasitoid action as shown by insignificant difference in infestation between treatment A and C fields. Percentage infestation and parasitism results obtained from this study thus provide quantitative data on the impact of *C. flavipes.* Release of *C. flavipes* can therefore be done in other areas both within and beyond the country's borders where *C. partellus* is still a problem. However, since reduced infestation and increased parasitism did not translate into yield increase, more suppressive effect may be achieved if the pest's multiple developmental stages are targeted, most especially before it reaches the destructive larval stage. There is need to carry out further research on *C. partellus'* egg parasitoids. Optimum yield may then be realized.

Key words*:* Classical biological control; *Chilo partellus ; Cotesia flavipes;* parasitism*;* insecticidal check technique; Kenya

INTRODUCTION

Parasitoids have been used in different agro-ecosystems as biological control agents for many years (Rodriguez and Hawkins, 2000; Gullan and Cranston, 2005; Sanda and Sunusi, 2014). In classical biological control (CBC), parasitoids are released in areas where previously harmless insects have become pests after accidental introduction outside their native range (Greathead, 1990; DeBach and Rosen, 1991; Mohyuddin, 1991). *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the exotic insects that became an important pest in Eastern and Southern Africa (ESA). Accidentally introduced in the 1930's from Asia (Tams, 1932), *C. partellus* infestation has resulted in yield losses estimated at 73% in maize and 80-100% in sorghum (Seshu Reddy, 1983; Reddy and Walker, 1990; Kfir, 1994). As a result of these losses, *C. partellus* has been a target of various management strategies. The exotic larval parasitoid, *Cotesia flavipes* Cameron (Hymenoptera, Braconidae), is among biological control agents used in management of *C. partellus*. Two *C. flavipes* releases have been made in East and Southern Africa. In the first release, *C. flavipes* from Rawalpindi, Pakistan, was introduced in Uganda, Kenya and Tanzania by the Commonwealth Institute of Biological Control (CIBC) (now International Institute of Biological Control) between 1968 and 1972. Post release studies showed that the parasitoid did not establish (CIBC, 1968-1972). In the second release, *C. flavipes* from Sindh region in Pakistan was introduced at three sites in Southern Coastal area of Kenya in 1993 (Overholt *et al*., 1994). Following the second attempt, surveys revealed that *C. flavipes* had established (Overholt *et al*., 1994). Subsequent surveys showed that parasitism by *C. flavipes* was rising steadily. This was reflected in initial recoveries made in 1995 and 1996 during which parasitism was estimated at 0.05-3% (Overholt *et al*., 1997), 7.1% in 1997 and 13% in 1999 (Zhou *et al*., 2001). Though there was a general increase in parasitism, there are doubts on whether parasitoids can effectively reduce stem borer pest populations below economically damaging levels (Kfir, 1997; Jiang *et al*., 2006; Kipkoech *et al*., 2009). These doubts arise due to the fact that there is limited quantitative data on impact of parasitoids on stem borer pest population and the resulting cereal yields (Kfir *et al*., 2002a; Cugala *et al*., 2006). The limited quantitative data available on the impact of parasitoids on lepidopteran pest populations is attributed to methodological challenges associated with low densities, mobility of the lepidopteran pests and the endophagous behaviour of the larvae (Kfir, 2004). Due to these challenges, very few studies using insecticidal check method have been undertaken (Kfir, 2002b; Cugala *et al.,* 2006). Insecticidal check method, first described by DeBach (1946), is considered as a good experimental technique for evaluating the efficacy of natural enemies (Jones, 1982, Kenmore *et al*., 1984; DeBach and Rosen, 1991; Luck *et al*., 1999). This method has been used to estimate the impact of parasitoids on stem borer pest population in South Africa (Kfir *et al*., 2002a) and Mozambique (Cugala *et al*., 2006). Studies were done in regions characterized by stem borer community dominated by *Busseola fusca* (Fuller) and *C. partellus* respectively. They both observed significantly higher infestation in plots sprayed with a selective insecticide, dimethoate and higher parasitism in unsprayed plots, results they both attributed to partial elimination of parasitoids. These studies were however done on small plots measuring 333m^2 in South Africa and 100m^2 in Mozambique. Results from such small plots may not be effectively used to determine the effect of natural enemies on pest population and the yields in large farmers' fields because results are likely to be blurred by movement of natural enemies (van Driesche and Bellows, 1996). Despite limitations associated with aforementioned exclusion experiments (Kfir, 2002b; Cugala *et al.,* 2006), no such study has been undertaken in East Africa, a region where *C. flavipes* was released more than 20 years ago. Extrapolation of results from the above exclusion experiments in the East African context may not be feasible due to ecological differences. It is on this background that this study was initiated in the moist mid-altitude agro-ecological zone (AEZ) in Kenya, a region dominated by *C. partellus* with an aim of assessing the impact of *C. flavipes* on stem borer pest population and maize yield.

METHODOLOGY

Description of the study area: The study was carried out in twelve farmers' fields during the short rain-growing season between September 2015 and February, 2016 in Oluch village, Homa-Bay County (Table 1). Oluch village is located in moist mid-altitude AEZ, at an elevation of 1000-1800masl between 0°27.949′ to 0°28.001′ S and 34°33.192′ to 34° 33.919′E. The area is characterised by temperatures ranging from 17 to 32°C with bimodal rainfall ranging between 500 and 1000mm annually. Bimodal rainfall received in the area allows cultivation of maize in both long and short rainy season.

Table 1: Estimated number of maize plants in different experimental fields

Treatment	Replication	Farm size (Acres)	No. of plants	Estimated no. of plants/acre
A (Stem borer excluded)	I	0.503	6760	13439
	II	0.178	3440	19326
	III	0.600	10472	17453
	IV	0.611	2808	4596
B (Parasitoid excluded)	I	0.842	10360	12304
	II	0.687	6800	9898
	III	0.438	5016	11452
	IV	0.306	2200	7190
C (Control)	I	1.191	8580	7204
	II	1.058	12470	11786
	III	0.531	6630	12485
	IV	0.574	2890	5034

Study design and insecticide treatment: The twelve fields were purposively selected within a radius of one kilometre so as to limit differences that may arise with respect to ecological characteristics. Selected fields were assigned three different insecticide treatments (A, B and C), and replicated four times. Four maize fields designated for Treatment A were sprayed with stem borer pesticide, Thunder® 145 O-TEQ, to exclude stem borers, while fields designated for Treatment B were sprayed with Dimethoate 40 EC to exclude parasitoids. Maize plants in fields designated as Treatment C were not treated with any insecticide and were thus used as controls. Two millilitres of the stem borer pesticide, Thunder® 145 O-TEQ, was mixed with one litre of water and sprayed onto pre-selected fields designated as A to exclude stem borers from maize plants. The two active ingredients in Thunder Imidacloprid and Beta-Cyfluthrin, act through quick knock-down effect by blocking acetylcholine receptors in insect nerve cells and by keeping sodium channels in membranes of nerve cells open respectively (Bayer Crop Science, 2013). To exclude stem borer parasitoids, two millilitres of the parasitoid insecticide, Dimethoate 40 EC, was mixed with one litre of water and sprayed on maize plants in pre-selected fields in Treatment B. Dimethoate 40 EC, is a selective organophosphate compound with both systemic and contact action. Dimethoate inhibits the enzyme acetylcholinesterase resulting in nerve damage and death (Fukuto, 1990). The first stem borer pesticide spraying was done three weeks after germination of maize plants and three subsequent applications done after every two weeks. The first dimethoate application was done four weeks after germination and two subsequent treatments were done after every two weeks.

Sampling protocol: Sampling for stem borer infestation, densities and parasitism was done once during the 5th week after germination. During each sampling session, all plants in respective treatment fields were inspected for stem borer infestation. The observed number of infested plants were expressed as a percentage of total plants inspected in respective fields. Ten infested plants in each field were sampled and dissected. All stem borer larvae collected after dissection were identified and recorded in three different size categories; small (1st and 2nd instars), medium (3rd and 4th instars) and large (5th instar). The total number of stem borer larvae collected was used to estimate larval densities in respective plots. Collected larvae were placed individually in glass vials (2.5cm diameter x 7.5cm depth) containing artificial diet (Onyango & Ochieng-Odero, 1994) and plugged with cotton wool. Collected larvae together with all other stem borer life stages were taken to the laboratory at International Centre of Insect Physiology and Ecology (*icipe*) where they were reared at ambient temperatures of 24-25^{0}C and a relative humidity of 55-65%, with a 12:12 light: dark photoperiod until pupation. Resulting pupae were placed individually in plastic jars lined at the bottom with moist paper towel while the remaining larvae were maintained in vials with artificial diet. Humidity in plastic jars was maintained by moistening paper towels using a few drops of distilled water once after every 2 days. Vials were inspected each day for parasitoid cocoon spinning and pupation, while pupae jars were inspected for adult moth emergence. Emerging parasitoids were identified and recorded while moths were used to confirm stem borer species identity. Parasitism (%) was estimated from the number of stem borer larvae that gave cocoons from the appropriate larval stage and expressed as the proportion of respective plot densities. At the end of the study, dry maize cobs were harvested and their respective weights recorded.

Estimating effect of infestation and parasitism on maize yield: The effect of infestation and parasitism on maize yield was estimated using a hypothetical approach (Songa *et al*., 2001). In this approach, ten infested and non-infested maize plants were identified and tagged in all the farmers' experimental fields. The following parameters were recorded from the tagged plants during harvest: height and diameter, tunnelling length, number of exit holes, number of cocoon cases and maize cob weights with husks. These parameters were statistically compared to determine their respective effects on observed yields.

Statistical analyses: Data on the percentage infestation, larval density, stem borer parasitism and cob weight (with husk) from individual fields, were pooled in respective treatments and used as replicates during analysis. Before analysis, the aforementioned parameters were tested for normality using Shapiro-Wilk's test. Percentage infestation, larval density and yield were normally distributed and One-way ANOVA was thus used to compare variations among treatments. Tukey's post-hoc test was used to separate means where treatments were found to be significantly different (p<0.05). Percentage parasitism was log transformed $(Log_{10}x+1)$ before subjection to One-way ANOVA and means separated using Tukey's post-hoc test. Number of exit holes, tunnelling length, plant height and stem diameter were analysed to test their respective effects on cob weight. Plant height, stem diameter, number of nodes and yield from non-infested and infested plants were compared using Mann-Whitney test. Yield data was standardized to yield per acre in order to correct for differences in farm sizes. Mean yield between infested and non-infested plants were compared using student's t-test while Pearson's correlation test was used to determine the effect of tunnel length, exit holes and cocoon cases on maize yield. GLM was used to determine the effect of plant height, stem diameter, mean larval density, infestation and parasitism on maize yield.

RESULTS

Stem borer diversity, infestation and mean larval density: *Chilo partellus* was the only stem borer species found occurring in the fields during this study. Stem borer infestation varied among treatments (A, B and C) ($F_{2,9}$=5.835; p<0.05; Table 2). Infestation in treatment A (0.5 ± 0.3%) was significantly lower compared to treatment B (2.0±0.2%) and C (1.5± 0.4%). Like infestation levels, there was evidence of variation in mean larval density among treatments ($F_{2,9}$=8.887; p<0.05; Table 2). Larval density was significantly lower in Treatment A (0.5±0.4) compared to Treatment B and C where densities were 2.4±0.4 and 2.7±0.4 respectively (Table 2).

Parasitoid species composition and pest parasitism: A total of 118 cocoon masses were recovered from parasitized stem borers during the study. *Cotesia flavipes* was the only parasitoid species recovered and thus constituted 100% of parasitoid community. However, there was evidence of variation in level of parasitism among treatments ($F_{2,9}$ =91.97; p<0.0001). Parasitism was significantly high in treatment C (23.5±6.8%) compared to Treatment A (00.00±0.00) and B (0.3±0.1) where populations of stem borers and parasitoids were manipulated respectively (Table 2).

Table 2: Mean $(\bar{x} \pm SE)$ stem borer infestation, larval density, parasitism and maize yield among different insecticide treatments

Treatment	Infestation (%)	Larval density	Parasitism (%)	Yield (kg/acre)
A	0.5±0.3^{a}	0.5±0.4^{a}	0.0±0.0^{a}	915.3±294.2^{a}
B	2.0±0.2^{b}	2.4±0.4^{b}	0.3±0.1^{a}	841.3±223.9^{a}
C	1.5±0.4^{ab}	2.7±0.4^{b}	23.5±6.8^{b}	587.0±047.7^{a}
$F_{2,9}$	5.835	8.887	91.97	0.641
p-value	<0.05	<0.01	<0.0001	>0.05

Mean $(\bar{x} \pm SE)$ within columns followed by the same lower case superscripts are not significantly different (p>0.05) (Tukey's post hoc test).

Maize yield: After correcting for difference in farm sizes, Treatment A produced relatively higher yield (915.3±294.2kg/acre) followed by Treatment B (841.3±223.9kg/acre) and Treatment C (587.0±47.7kg/acre). However, statistical comparison did not reveal any differences in mean yield among the treatments ($F_{2,9}$=0.64; p>0.05) (Table 3).

i). Effects of plant diameter, plant height and number of nodes on maize yield

Comparison of infested and non-infested maize plants: On average, infested maize plants were significantly shorter (171.6±4.3cm) compared to non-infested plants (202.6±3.5cm) (W=4033; p<0.05) (Table 3). Significant variations were also observed in the number of nodes between infested (10.4±0.2) and non-infested plants (11.3±0.2) (W=4814.5; p<0.05).However, plant diameter did not vary between infested (2.1±0.0) and non-infested plants (2.1±0.0) (W=6003; p>0.05) (Table 3). Statistical comparison of yield weights revealed significant variation between infested (100.9±8.1) and non-infested plants (199.1±6.4) (W=2579.5; p<0.05).

Table 3: Mean $(\bar{x} \pm SE)$ plant height, number of nodes, diameter and yield weight of infested and non-infested plants.

Infestation status	Plant height(cm)	Number of nodes	Plant diameter (cm)	Yield weight(Kg)
Infested plants	171.6±4.3^{a}	10.4±0.2^{a}	2.1±0.1^{a}	100.849±8.1^{a}
Non-infested plants	202.6±3.5^{b}	11.3±0.2^{b}	2.1±0.0^{a}	199.056±6.4^{b}
W value	4033	4814	6003	2579.5
p-value	<0.001	< 0.001	> 0.05	< 0.001

Mean (± SE) within columns followed by the same lower case superscripts are not significantly different (p>0.05)

ii) Effect of infestation and parasitism on cob weights: Regardless of the status of infestation, GLM analysis revealed that performance of maize plant is positively affected by maize plant height (b=0.51; t=4.20; p<0.0001) and diameter (b=48.15; t=4.01; p<0.0001). Further GLM analysis of stem borer infestation parameters (tunnelling length and exit holes) revealed their respective negative effect on cob weights (Table 4). Tunnelling length caused during stem borer feeding negatively affected cob weights (b=1.65; t=-3,524.01; p<0.05). The number of exit holes indicating number of stem borers that completed their life cycle within the maize stems, also negatively affected cob weight though insignificantly (b=-7.08; t=-1.86; p>0.05)

(Table 4). The number of cocoon cases did not have a significant effect on cob weight (b=-4.34; t= -0.49; p>0.05).

Table 4: Estimated effect of different growth, infestation and parasitism parameters on cob weights

Term	b	SE	T	*p*(T)
(Intercept)	-47.209	24.558	-1.92	0.05582
Height of maize plant (cm)	0.510	0.121	4.20	0.00004
Maize plant diameter (cm)	48.154	11.997	4.01	0.00008
Tunnelling length (cm)	-1.650	0.469	-3.52	0.00052
Number of exit holes	-7.083	3.800	-1.86	0.06362
Number of cocoon cases	-4.338	8.936	-0.49	0.62783

DISCUSSION

Chilo partellus was the only stem borer pest collected during the study contrary to results from previous surveys in which *B. fusca* reportedly dominated the stem borer community (Seshu Reddy, 1983; Ongámo *et al*., 2006a, b). The predominance of *C. partellus* had previously been reported in low altitude areas (Nye, 1960; van Hamburg, 1979; Seshu Reddy, 1983), an observation attributable to its competitive advantage over indigenous stem borer species. However, with climate change, predictive models showed that *C. partellus* was likely to expand its distribution range beyond low altitude areas (Khadioli *et al.,* 2014; Mwalusepo *et al*., 2015). Recovery of *C. partellus* from mid-altitude zone during this study validated the predictive models. Other researchers have reported similar range expansion by *C. partellus* into mid and high –altitude areas (Kfir, 1997; Kfir *et al*., 2002b; Songa, 1999; Overholt *et al.,* 2000). Found associated with *C. partellus*, was its old association parasitoid, *C. flavipes* whose establishment is hereby confirmed in moist mid-altitude zones despite there being no official release (Omwega *et al*., 1995). Its presence is attributed to a population of parasitoids which escaped from a laboratory at *icipe*, Mbita point field station where they had been quarantined for pre-release studies (Omwega *et al*., 1995). *Cotesia flavipes* was the only parasitoid recovered contrary to earlier surveys during which the native, gregarious larval endoparasitoid *Cotesia sesamiae* Cameron was also recovered (Zhou and Overholt, 2001). The direct effect of stem borer infestation on maize production and contribution of biological control agents have rarely been quantified with two documented studies having been done on small plots (Kfir *et al.,* 2002b; Cugala *et al*., 2006). The high cost involved in replicating experiments using larger plots was cited as a constraint and thus plots measuring 333m^2 in South Africa (Kfir *et al.,* 2002a) and 100m^2 in Mozambique (Cugala *et al*., 2006) were used, 10 years after the initial release of *C. flavipes* in the latter. However, small plots have been found to be ineffective in determining the effect of natural enemies on pests because results are blurred by movement of natural enemies across plots (van Driesche and Bellows, 1996). Larger plots measuring between 720 to 4820m^2 were used in this study after 23 years since the initial release of *C. flavipes* in Kenya in order to overcome this challenge. Following the study, generated results provided evidence on the impact of C*. partellus* infestation on maize yield and contribution of *C. flavipes* in limiting losses associated with infestations. Low infestation observed in Thunder treated fields were attributed to mortality induced by the insecticide which killed majority of first instar larvae with only a few that had penetrated into stems surviving. The higher percentage infestation recorded in treatment B was as a result of partial removal of natural enemies by dimethoate application, an observation that corroborated findings from South Africa and Mozambique (Kfir, 2002b; Cugala *et al*., 2006). In South Africa, direct effects of natural enemies on *B. fusca* and *C. partellus* populations were assessed and significantly higher infestation was recorded in plots sprayed with dimethoate with higher parasitism being recorded in unsprayed plots (Kfir, 2002b). In Mozambique, a similar experiment was carried out on *C. partellus*, *B. fusca* and *S. calamistis* and similar results were obtained following natural enemy exclusion (Cugala *et al.,* 2006). In treatment B, parasitoid action was considerably reduced by dimethoate application. However, 100% elimination of natural enemies is often not achieved by insecticide application (Kidd and Jervies, 2005) and this explained the low parasitism rate in treatment B in comparison to treatment C. This is in agreement with results obtained from a similar study carried out by Cugala *et al*. (2006) where parasitism in control plots were higher than in stem borer and parasitoid-excluded fields. This study revealed a general trend in increase of parasitism in the region. In 1997, Khan *et al.* (1997) recorded about 3.5% parasitism while Ogeda recorded about 6.1% in 1999 (Ogeda, 1999). Results of this study demonstrate an increase in parasitism (23%) in control fields. Contrary to variations in stem borer pest infestation and parasitism among treatments, there was no evidence of variation in maize yield among treatments. This contradicted earlier reports that natural enemies (including *C. flavipes*) played a considerable role in stem borer population suppression resulting in increased maize yield (Cugala *et al.,* 2006). It is worth noting that maize yield is affected by both biotic and abiotic factors. Manipulated stem borer and parasitoid populations are only part of the biotic factors that may affect yield. However, comparison of yields from infested and non-infested plants showed that infestation contributes to yield loss depending on the health of the plant. Plant height and diameter played an important role towards yield as they together showed positive influence. In contrast, infestation parameters particularly tunnel length and number of exit holes negatively affected the yield. Tunnel length is considered a good indicator of stem borer damage as it results in destruction of the meristematic tissue (Polaszek, 1998; Cherry *et al*., 1999; Midega, 2013) leading to stem weakening and lodging (Santiago *et al*., 2003). The observed low yield in infested plants was thus attributed to stem tunnelling which interfered with translocation of water and nutrients to actively photosynthesizing parts of a plant resulting in reduced plant growth, seed setting and grain sizes (Kalule *et al*., 1997; Polaszek 1998; Malvar *et al*, 2008) thus reduced yield (Ajala and Saxena, 1994; Songa *et al*., 2001; Midega, 2013). The other infestation parameter, number of exit holes, showed a strong positive correlation to tunnelling length indicating that with an increase in the number of stem borers that completed their life cycle within the maize stem, there was an associated increase in crop damage.

Cocoon cases are an indication of parasitoid presence and action on the target pest. Parasitism being a numerical response, an increase in number of exit holes and tunnelling lengths was observed to be accompanied by an increase in cocoon cases. However, larval parasitoids attack 3rd, 4th or 5th instar larvae of the pest after damage had been done on maize. Results obtained in this study upheld reports that the number of stem borers, exit holes and tunnelling length are the most important factors affecting yield in maize crop (Songa *et al*., 2001). According to the same author, these were then followed by plant height and plant diameter in that order of importance. Generally, this study revealed a reduction in stem borer infestation levels in the area (1.45% in control fields) compared to earlier studies in which stem borer infestation levels were estimated at 8-100% (Seshu Reddy *et al.,* 1983) and 33.72% (Ongámo *et al*., 2006a). Reduced infestation levels were attributed to increase in parasitism by *C. flavipes*. These results demonstrate that International Centre of Insect Physiology and Ecology's Biological Control programme which initiated the importation and release of *C. flavipes* succeeded in the objective to reduce stem borer population in the study area. This biological control success story can thus be replicated in other areas within and beyond the nation's borders where a similar pest problem may occur. However, given the *modus operandi* of the parasitoid, decreased infestation coupled with increased parasitism did not translate into improved maize yield. Optimum yield may be realised when biological control involving both egg and larval parasitoids are used. Further research to identify egg parasitoids of *C. partellus* in the pest's native range or that have expanded their host range to include *C. partellus* in Kenya needs to be done.

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