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SUITABILITY OF *CHILO PARTELLUS*, *SESAMIA CALAMISTIS* AND *BUSSEOLA FUSCA* FOR THE DEVELOPMENT OF *COTESIA FLAVIPES* IN ETHIOPIA: IMPLICATION FOR BIOLOGICAL CONTROL

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ABSTRACT: *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson) are the most important cereal stemborers attacking maize and sorghum in Africa. Of these, *C. partellus* is an exotic species which invaded Africa sometime before 1930. A classical biological control program for the control of *C. partellus* in eastern and southern Africa using *Cotesia flavipes* (Cameron) was launched in 1991. The parasitoid has established in some countries like Kenya where it was released. In Ethiopia, the parasitoid has established without being released and reared from field collected *C. partellus*, *B. fusca* and *Sesamia calamistis*. *C. flavipes* created new association with African stemborers like *S. calamistis*, but its association with *B. fusca* under field condition was only reported from Ethiopia. The objective of the current study was to investigate if *C. flavipes*, collected from Ethiopia, could develop in different geographic populations of *B. fusca* under laboratory conditions. Ten populations of *B. fusca*, a single population of *C. partellus* and *S. calamistis* each were tested for suitability to *C. flavipes* collected from five locations in Ethiopia. Results indicate that 20% of the *B. fusca* populations existing in Ethiopia are likely to be suitable hosts to *C. flavipes* which further strengthens the hope that *C. flavipes* will play a significant role in the management of cereal stemborers in Ethiopia.

Key words/phrases: Biological control, *Cotesia flavipes*, Geographic population, Host range expansion, Parasitoid, Stemborers

INTRODUCTION

Of the stemborers attacking maize and sorghum in Africa, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) are the most important (Overholt *et al.*, 1997). *C. partellus* is an exotic stemborer which invaded Africa sometime before 1930 when it was first recorded in Malawi (Tams, 1932). Since arriving in Africa, *C. partellus* has spread to nearly all countries in east and southern Africa, often becoming the most damaging

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stemborer of maize and sorghum (Nye, 1960; van Hamburg, 1980). Indigenous parasitoids of African stemborers expanded their host range to include the exotic stemborer, but did not appear to effectively regulate densities at acceptable levels (Oloo and Ogedah, 1990; Kfir, 1992). Because of its economic importance, *C. partellus* has been a target of several classical biological control programs in Africa, but only *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) has recently established. *C. flavipes* is an Asian origin endo-larval gregarious parasitoid of Asian stemborers such as *C. partellus* (Overholt *et al.,* 1997). *C. flavipes* has been released in several eastern and southern African countries. It was first released in coastal Kenya in 1993 for the biological control of *C. partellus*. *C. flavipes* was planned to be released in Ethiopia at selected sites in 2000 to develop a model that could be used to optimise its future release either in Ethiopia or elsewhere in Africa. Prior to any release, a countrywide survey had been conducted in 1999 to study the stemborer distribution as well as that of their natural enemies. During the survey, six stemborer species and 20 primary parasitoids along with other groups of natural enemies were recorded (Emana Getu *et al.*, 2001, 2002 and 2003a). However, *C. partellus*, *B. fusca* and *S. calamistis* were the most widely distributed. *Cotesia flavipes,* which was not previously released in Ethiopia, was found to be the most widely distributed parasitoid contributing to 66% of the total parasitism (Emana Getu *et al.*, 2001). *Cotesia flavipes* was recorded on *C. partellus*, *S. calamistis* and *B. fusca* in Ethiopia (Emana Getu *et al.*, 2001). The acceptance and suitability of some stemborers occurring in Africa including *C. partellus*, *B. fusca* and *S. calamistis* for ovipostion and development of *C. flavipes* were studied in Kenya (Ngi-Song *et al.,* 1995) and West Africa (Haile Michael Yosef *et al.,* 1997). All stemborers were accepted by *C. flavipes* for ovipostion, but development only occurred in *C. partellus* and *S. calamistis*. The eggs of *C. flavipes* were encapsulated in *B. fusca,* indicating that *B. fusca* was not a suitable host. The development of *C. flavipes* in the Ethiopian population of *B. fusca* may indicate the existence of geographic variation in the immune response of *B. fusca* in Ethiopia (Mochiah *et al.*, 2001). It is not possible to make definite conclusions based only on field observation, as other immune breaking mechanisms such as multiple parasitisms might have occurred in the process. Ngi-Song *et al.* (2001) and Sallam (1998) indicated the existence of multiple parasitism when *C. sesamiae* and *C. flavipes* occurred together. Field surveys in Ethiopia indicated that both *C. sesamiae* and *C. flavipes* occurred sympatrically which might provide opportunities for multiple parasitism. Hence, the objective of the current study was to investigate if *C. flavipes* could develop

in different Ethiopian populations of *B. fusca* in comparison to its suitable hosts, *C. partellus* and *S. calamistis*, under ambient laboratory condition.

MATERIALS AND METHODS

Host insect rearing

C. partellus: Larvae of *C. partellus* (Cp) were collected from Melkasa Agricultural Research Centre experimental field and reared for one generation (progenies developed from the field collected insects) on natural diet (maize shoots and stems) in the laboratory. Fourth instar larvae of the second generation were used for the experiment.

S. calamistis: Larvae of *S. calamistis* (Sc) were collected from Meiso Agricultural Research Center experimental field and reared for one generation (progenies developed from the field collected insects) on natural diet (maize shoots and stems) in the laboratory. The susceptible stage of the stemborers' larvae, fourth instar larvae of the second generation was used for the experiment.

B. fusca: Larvae of *B. fusca* were collected from farmers fields (Fig. 1) at Awassa (Bfaw), Bako (Bfba), Ambo (Bfam), Jijiga (Bfjig), Desie (Bfde), Walayta (Bfwa), Yergalem (bfye), Jimma (BfJi), Weliso (Bfwe) and Alemaya (Bfal) and individually reared for one generation (progenies developed from the field collected insects) on natural diet (maize shoots and stems) in the laboratory. Fourth instar larvae of the second generation were used for the experiment.

Parasitoid rearing

Five populations of *C. flavipes* from different parts of Ethiopia were collected during the survey of 1999 and maintained in the laboratory at Melkasa Agricultural Research Centre in Ethiopia. These were Awassa population (Cfaw), Melkasa population (Cfml), Gedole population (Cfgd), Meiso population (Cfmei) and Lubuqaqaba population (Cflub) (Fig 2).

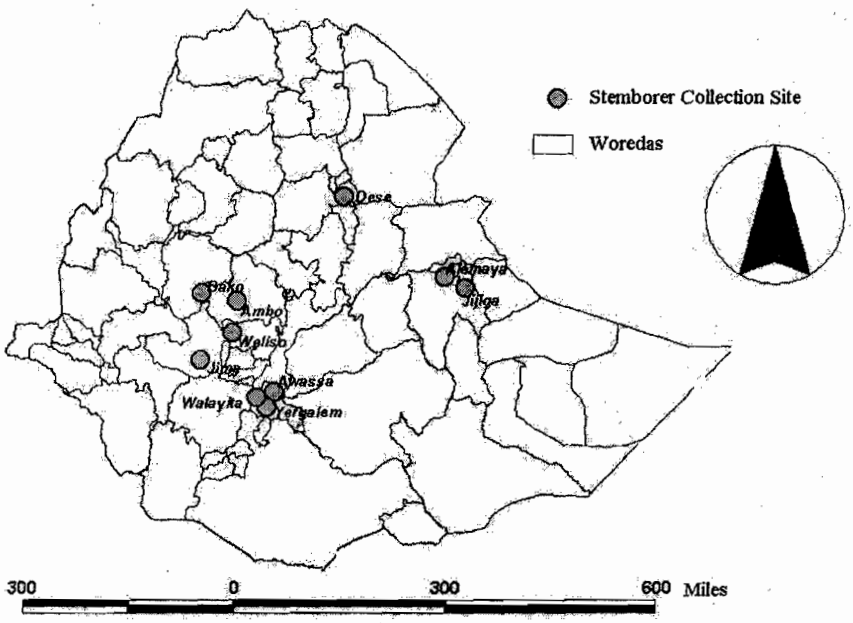

Fig. 1. Stemborer populations collection sites in Ethiopia.

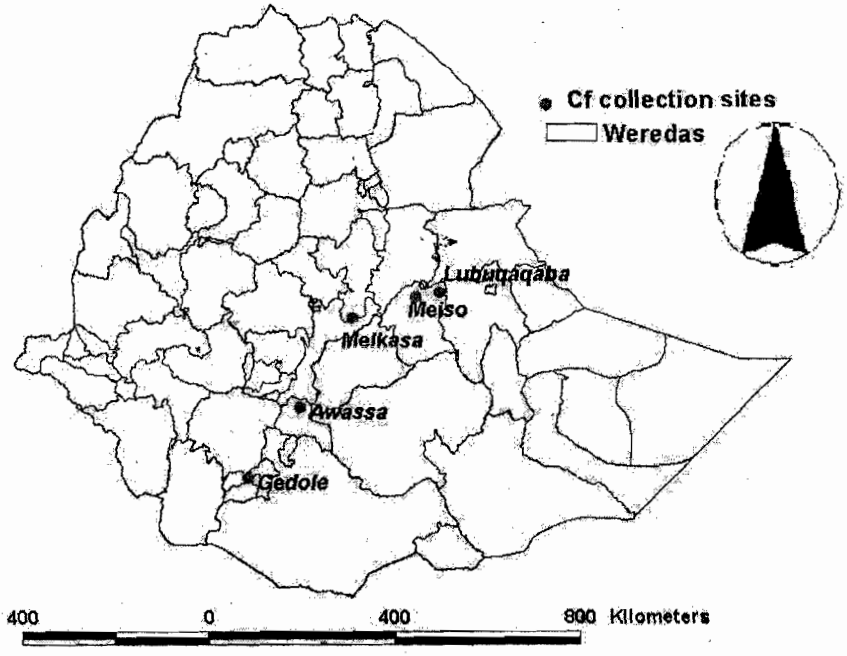

Fig. 2. Collection sites of *Cotesia flavipes* populations

Bioassay

Fourth instar larvae of each host were exposed to 12 hour old adult females from each parasitoid population using a hand stinging method described by Overholt *et al.* (1994). Stung larvae were individually placed in a petri-dish and given fresh pieces of maize stem as food. Each female parasitoid was provided with two-host larvae per day until its death. Observations were made daily until cocoon formation, host death or pupation. Cocoons from each petri-dish were separately transferred into a glass vial. Once the cocoons turned black, a small piece of cotton wool soaked in a 20% honey/water solution was placed in the vial. Dates of exposure, cocoon formation and adult emergence, number of dead larvae inside the host, number of dead cocoons, number of last instar larvae coming out of the host, number of progeny and sex of the progeny were recorded.

The experiment was conducted under a 12:12 (L:D) h photoperiod at ambient temperature ranging from 8±2^{O}C minimum night temperature to 27±3^{O}C maximum day temperature. The relative humidity ranged from 45 to 55%. The experiment was designed in a completely randomized design in 30 replications per host per parasitoid combination.

Data Analysis

Data of eight populations of *B. fusca* (Bfaw, Bfba, Bfam, Bfde, bfye, BfJi, Bfwe, Bfal) were excluded from the analysis since *C. flavipes* did not emerge from these populations. All count data were transformed to a logarithmic scale and percent female progeny was arcsine transformed before statistical analysis. However, results are reported with back transformed data (Gomez and Gomez, 1984). Analyses of variance were performed using PROC GLM, SAS computer software (SAS, 1999). Tukey's studentized range (HSD) was used to separate significant means. Abbot's formula was used to correct for the natural mortality (Abbot, 1925). Natural mortality was determined by comparing 30 unexposed (non-stung) and 30 exposed (stung) fourth instar larvae of each population of stemborers.

RESULT

Parasitism

Parasitism was significantly affected by the host ($F_{3,38} = 80.72$, $p = 0.001$) and the interaction of the host and the parasitoid populations ($F_{12,38} = 3.19$, p

= 0.003). Parasitism by all populations of the parasitoid was highest when Cp was the host and lowest when the hosts were Bfaw and Bfjig (Table 1).

Table 1 Comparison of percent parasitism of stemborer species (mean ± se) by different *Cotesia flavipes* populations from Ethiopia.

Stemborers	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	93.89±14a	95.65±17a	90.30±15a	90.59±15a	87.05±15a
Sc	80.92±17a	73.24±13b	38.72±6b	59.05±11b	73.03±13ab
Bfaw	36.29±9b	28.53±4c	40.78±5b	43.19±5b	42.13±5b
Bfjig	53.62±12b	60.64±11b	68.21±9ab	66.68±9ab	67.36±9ab

Means followed by the same letter (s) within a column are not significantly different (HSD), 5%; Cp = *C. partellus*, Sc = *S. calamistis*, Bfaw = *B. fusca* from Awasa, Bfjig = *B. fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cflub = *C. flavipes* from Lubuqaqaba, Cfaw = *C. flavipes* from Awasa, Cfmei = *C. flavipes* from Meiso.

Host and parasitoid mortality

The natural mortality of the stemborer populations used in the experiment is shown in Fig. 3. The highest natural mortality (> 40%) was observed in *B. fusca* populations and the lowest (about 20%) was recorded in *C. partellus*. *C. flavipes* larvae, dead in the host, significantly differed among parasitoid populations ($F_{4,552} = 4.10$, $p = 0.003$), hosts ($F_{3,552} = 347.84$, $p = 0.001$) and with the interaction between the host and the parasitoid ($F_{12,552} = 3.03$, $p = 0.0004$). The highest mortality of parasitoid immatures were recorded when the hosts were Bfaw and Bfjig for all the parasitoid populations (Table 2). Parasitoid mortality in the cocoon stage was only affected by the host ($F_{3,552} = 32.37$, $p = 0.0001$) and the mortality was highest in Bfaw and Bfjig (Table 3).

Table 2 Mean number of dead *C. flavipes* (mean ± se) immatures in larvae of different stemborer species.

Stemborers	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	5.20±0.7b	5.45±0.7b	4.83±0.4b	4.13±0.4b	3.77±0.3b
Sc	6.20±0.7b	9.13±1b	8.53±1b	10.37±0.8b	6.63±0.6b
Bfaw	12.97±2.3a	14.53±4a	15.47±4a	15.63±4a	15.87±4a
Bfjig	15.50±4a	14.53±4a	15.77±4a	15.60±4a	15.60±4a

Means followed by the same letter (s) within a column are not significantly different (HSD), 5%; Cp = *C. partellus*, Sc = *S. calamistis*, Bfaw = *B. fusca* from Awasa, Bfjeg = *B. fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cflub = *C. flavipes* from Lubuqaqaba, Cfaw = *C. flavipes* from Awasa, Cfmei = *C. flavipes* from Meiso.

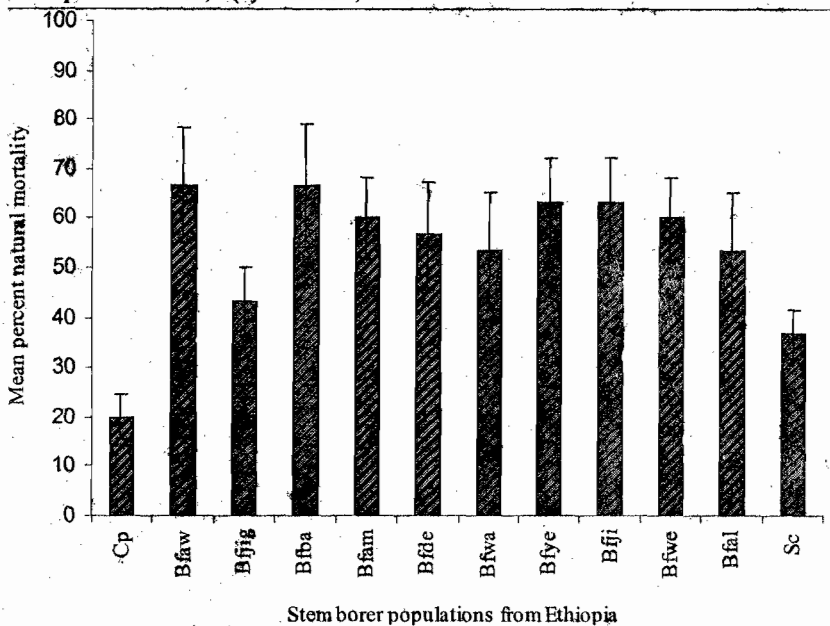

Fig. 3. Natural mortality of different stem borer populations used in suitablity study of different stem borers from Ethiopia for different populations of *C. flavipes* from Ethiopia.

Table 3 Mean number of dead *C. flavipes* cocoons (mean ± se) in different stemborer species

Stemborers	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	0.67±0.1b	1.73±0.1b	1.23±0.1b	0.7±0.1b	1.27±0.1b
Sc	1.3±0.1b	0.29±0.1b	0.93±0.1b	1.1±0.1b	1.2±0.1b
Bfaw	1.87± 0.1a	2.2±0.1a	1.67±0.1a	1.57±0.1a	2.1±0.1a
Bfjig	2.47±0.2a	2.73±0.2a	2.8±0.2a	2.23±0.1a	2.4±0.2a

Means followed by the same letter (s) within a column are not significantly different (HSD), 5%;

Cp = *C. partellus*, Sc = *S. calamistis*, Bfaw = *B. fusca* from Awasa, Bfjeg = *B. fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cflub = *C. flavipes* from Lubuqaqaba, Cfaw = *C. flavipes* from Awasa, Cfmei = *C. flavipes* from Meiso.

Progeny production

The number of last larval instar emerging from the host was only affected by the host ($F_{3, 552} = 76.36$, $p = 0.0001$). The highest number of parasitoid larvae emerged when the hosts were Cp and Sc (Table 4). The percentage of parasitoid females in progeny was affected by the host ($F_{3, 552} = 93.66$, $p = 0.0001$) with highest female progeny recorded from Cp and Sc (Table 5). The parasitoid total progeny was only affected by the host ($F_{3,552} = 132.21$, $p = 0.0001$) and the highest total progeny per host was recorded when the hosts were Cp and Sc (Table 6).

Table 4 Comparison of (mean ± se) *Cotesia flavipes* last larval instar successfully emerging

Stemborers	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	25±3.8a	38±5.5a	31±4a	30±4.1a	30±4.1a
Sc	19±3.2b	22±3.3b	19±3.2b	18±3.1b	20±3b
Bfaw	7±0.9c	10±1.1c	7±0.9c	5±0.4c	8±1c
Bfjig	9±1.1c	6±0.5c	9±1.1c	7±0.9c	9±1.1c

Means followed by the same letter (s) within a column are not significantly different (HSD), 5%; Cp = *C. partellus*, Sc = *S. calamistis*, Bfaw = *B. fusca* from Awasa, Bfjeg = *B. fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cflub = *C. flavipes* from Lubuqaqaba, Cfaw = *C. flavipes* from Awasa, Cfmei = *C. flavipes* from Meiso

Table 5 Percentage of *C. flavipes* females (mean ± se) in the total progeny emerging from different host species.

Hosts	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	60.43±8a	59.61±8a	59.74±8a	58.02±8a	54.12±7a
Sc	47.91±6a	59.13±8a	46.19±6a	30.67±4b	42.63±6b
Bfaw	7.93±0.9b	11.8±0.9b	11.79±0.9b	8.76±0.9c	13.93±1c
Bfjig	10.83±0.9b	5.55±0.5b	13.03±1ab	9.62±0.9c	13.75±1c

Means followed by the same letter (s) within a column are not significantly different (HSD), 5%; Cp= *C. partellus*, Sc = *S. calamistis*; Bfaw = *B. fusca* from Awasa, Bfiig = *B. fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cfmei = *C. flavipes* from Meiso.

Table 6 Comparison of stemborers (mean ± Se) for total *Cotesia flavipes* progeny per host

Hosts	Parasitoids				
	Cfml	Cfgd	Cflub	Cfaw	Cfmei
Cp	22.6±3a	34.9±5a	28.87±4a	27.63±4a	27.90±4a
Sc	15.63±2a	17.13±2b	15.4±2b	12.17±2b	15.33±2b
Bfaw	2.77±0.2b	6.57±0.5c	5.0±0.5c	2.1±0.1c	4.83±0.5c
Bfjig	4.63±0.5b	2.07±0.1c	5.27±0.5c	3.63±0.3c	5.3±0.5c

Means within a column for each parameters followed by the same letter (s) are not significantly different (HSD), 5%; Cp= *C. partellus*, Sc = *S. calamistis*; Bfaw = *B. fusca* from Awasa, Bfiig = *B.fusca* from Jijiga, Cfml = *C. flavipes* from Melkasa, Cfgd = *C. flavipes* from Gedole, Cfmei = *C. flavipes* from Meiso.

DISCUSSION

The current study suggests the existence of two biotypes of *B. fusca* in Ethiopia, one that is suitable for the development of *C. flavipes* and another that is not. Out of 10 populations of *B. fusca* tested in this study, only 2 populations supported the development of all populations of *C. flavipes*. In contrast to the findings of Ngi-Song *et al.* (1995) and Hailemichael Yosef

et al. (1997), both the parasitoid population and the host species/type affected percent parasitism and the number of dead *C. flavipes* larvae in the host. This implied the existence of population differences among the parasitoid populations as well. Variations in life history among different populations of *C. flavipes* between India and North Pakistan have been reported (Emana Getu *et al.*, 2003 a and b; 2004). The number of dead cocoons, last instar larvae emerging from the host, total progeny and ratio of females of different *C. flavipes* populations were strongly affected by the host and all were higher when the hosts were Cp and Sc in comparison to Bfaw and Bfjeg. The highest total progeny and percent female emerging from Cp and Sc indicate the high suitability of these stemborers to *C. flavipes,* which agrees with the findings of Ngi-Song *et al.* (1995) and Hailemichael Yosef *et al.* (1997). The mean percent female recorded by these hosts agrees with Potting (1996) who reported on the female-biased nature of mated *C. flavipes*. Though 20% of the *B. fusca* populations tested were suitable hosts to all populations of *C. flavipes*, the total progeny per host and percent female was very low, suggesting that *B. fusca* was not a suitable host as *C. partellus* and *S. calamistis,* and the need to look for other control options for the management of *B. fusca* in Ethiopia. Realized fecundity and percent female are the two most important life history processes considered to measure parameters for the implementation of biological control (Driesche and Bellows, 1996). Generally, *C. partellus and S. calamistis* were the most suitable hosts for *C. flavipes* in Ethiopia suggesting the possibility of using this parasitoid for the successful control of these pests in the country. The association of *C. flavipes* with *C. partellus* is old and its association with *S. calamistis* and *B. fusca* is new. In Ethiopia, more than 50% of the stemborer populations consist of *B. fusca* and the indigenous natural enemies do not keep the population below economic injury level (Emana Getu *et al.*, 2001). In addition, chemical control cannot be affordable by poor farmers.

Survey of stemborers and their natural enemies were conducted in 1997 and 1998 in Ethiopia (Mullugetta Negeri, 2001) and he did not report *C. flavipes* in Ethiopia. It was recorded for the first time in 1999 in all regions of the country (Emana Getu *et al.* 2001). The likely invasion of the parasitoid is from the Somalia release of 1997 in an area bordering eastern Ethiopia. If this speculation is correct, *C. flavipes* might disperse more than 2000 km away from its release site in Somali within a period of three years. Omwega *et al.* (1997) estimated that *C. flavipes* dispersed at the rate of 60 km per year. There could be some factors facilitating the high dispersal rate of *C.*

flavipes in Ethiopia, among which the presence of suitable population of *B. fusca* could be one. It took several years for *C. flavipes* to show significant suppression of stemborers in areas where it was released (Zhou *et al.* 2001). In some areas, establishment has not been reported. In Ethiopia, there is good evidence that *C. flavipes* can significantly reduce the population of stemborers and ultimately reduce yield losses by stemborers (Emana Getu *et al.* 2003). Successful parasitism of *B. fusca* may allow greater population growth of *C. flavipes*, and then greater overall suppression of the entire stemborer complex in an area. The fact that the Ethiopian *C. flavipes* population attacks *B. fusca* is an advantage. One of the evidence for the success of *C. flavipes* in Ethiopia is its host range expansion by including some populations of *B. fusca*. The occurrence of biotypes within insect fauna is common (Gutierrez, 1987). Ngi-Song *et al.* (1995) reported the existence of two biotypes of *C. sesamiae* in Kenya. Laboratory investigation should continue to aim at understanding the differential abilities of the two populations in suppressing *B. fusca* immune system.

From the current work, it can be summarized that *C. flavipes* has expanded its host range by including some biotypes of *B. fusca*, which is one of the dominant stemborer species in Ethiopia. Parasitism of *C. flavipes* had significantly increased from 7% in 1999 to 31% in 2003 (Emana Getu *et al.*, 2004). Parasitism by *C. flavipes* in 2005 reached as high as 58% (unpublished data). The suitability of the three stemborers to different populations of *C. flavipes* in Ethiopia is the best indicator of the best stemborer management option i.e., the use of biological control. As *C. flavipes* is already established in maize/sorghum agro-ecosystem in Ethiopia, augmentative release and conservation are the two possible biological control approaches to be applied. These approaches are already implemented and in certain areas, it has increased parasitism to 90% (unpublished data). The impact of parasitism on yield of maize and sorghum should be measured in order to justify the contribution of the Ethiopian population of *C. flavipes*. Suitability of more populations of *B. fusca* should also be investigated further.

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