Natural Enemies of the Fall Armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) in Ghana

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Natural enemies of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Ghana

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an invasive insect pest attacking maize in Ghana and sub-Saharan Africa countries. Biological control will need to be an important management strategy, and a first step was to identify potential natural enemies. Sampling was conducted in different localities of the 10 regions of Ghana from May to Nov 2017. A total of 1,062 larvae were collected from 106 maize farms, and the presence of natural enemies was recorded in 18 (17.0%) farms. Among natural enemies recorded, 7 species were parasitoids: *Chelonus bifoveolatus* Szpligeti, *Coccygidium luteum* (Brull), *Cotesia icipe* Fernandez, *Meteoridea testacea* (Granger), and *Bracon* sp. (all Hymenoptera: Braconidae), *Anatrichus erinaceus* Loew (Diptera: Chloropidae), and an undetermined tachinid fly (Diptera: Tachinidae). The parasitism rate was 3.58%. Three predator species were collected: *Pheidole megacephala* (F.) (Hymenoptera: Formicidae), *Haematochares obscuripennis* Stål, and *Peprius nodulipes* (Signoret) (both Heteroptera: Reduviidae). The 2 most abundant parasitoids were *C. bifoveolatus* and *C. luteum* with a relative abundance of 29.0% and 23.7%, respectively, and a parasitism rate of 1.04% and 0.85%, respectively. However, *C. bifoveolatus* was the most dispersed parasitoid, found in 6.6% of the inspected sites within all the agroecological zones of Ghana. This species is a good candidate as a biological control agent for fall armyworm in Africa. The predator that was most abundant (46.0%) and dispersed (3.8% of the farms) was *P. megacephala*.

Key Words: biological control; *Cotesia bifoveolatus*; *Pheidole megacephala*; *Coccygidium*

Resumen

El cogollero, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), es una plaga de insectos invasora que ataca el maíz en Ghana y los países de África subsahariana. El control biológico deberá ser una estrategia de manejo importante, y un primer paso es identificar los enemigos naturales potenciales. Se realizó el muestreo en diferentes localidades de las 10 regiones de Ghana desde mayo hasta noviembre del 2017. Se recolectó un total de 1.062 larvas de 106 granjas de maíz, y se registró la presencia de enemigos naturales en 18 granjas (17.0%). Entre los enemigos naturales registrados, 7 especies fueron parasitoides: *Chelonus bifoveolatus* Szpligeti, *Coccygidium luteum* (Brull), *Cotesia icipe* Fernandez, *Meteoridea testacea* (Granger) y *Bracon* sp. (todos los Hymenoptera: Braconidae), *Anatrichus erinaceus* Loew (Diptera: Chloropidae) y una mosca taquinida indeterminada (Diptera: Tachinidae). La tasa de parasitismo fue del 3.58%. Se recolectaron tres especies de depredadores: *Pheidole megacephala* (F.) (Hymenoptera: Formicidae), *Haematochares obscuripennis* Stål y *Peprius nodulipes* (Signoret) (ambos Heteroptera: Reduviidae). Los 2 parasitoides más abundantes fueron *C. bifoveolatus* y *C. luteum* con una abundancia relativa del 29.0% y 23.7%, respectivamente, y una tasa de parasitismo del 1.04% y 0.85%, respectivamente. Sin embargo, *C. bifoveotus* fue el parasitoide más disperso, encontrado en el 6.6% de los sitios inspeccionados dentro de todas las zonas agroecológicas de Ghana. Esta especie es un buen candidato como agente de control biológico para el cogollero en África. El depredador que fue más abundante (46.0%) y disperso (3.8% de las granjas) fue *P. megacephala*.

Palabras Claves: control biológico; *Cotesia bifoveolatus*; *Pheidole megacephala*; *Coccygidium*

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an important pest of many crops including maize (*Zea mays* L.; Poaceae), sorghum (*Sorghum bicolor* [L.] Moench; Poaceae), cotton (*Gossypium hirsutum* L.; Malvaceae), and diverse pasture grasses. It is widely distributed in the Americas (Sparks 1979) and recently has become a pest of concern in Africa (Goergen et al. 2016; Nagoshi et al. 2017). Given the importance of maize in these regions, this pest has become one of the most serious problems on both continents. The yield losses caused by fall armyworm vary and depend on various factors, but can range from 19 to 100% in Brazilian plantations (De Almeida Sarmento et al. 2002). After severe outbreaks in Africa, control of fall armyworm has been based principally on the use of chemical insecticides. However, biological control is a highly desirable management alternative for controlling this pest in the long term. The success of any biological control project depends on appropriate biological, ecological, and population studies of the species involved (Miller 1983).

There have been surveys for natural enemies of fall armyworm in the USA (Hogg et al. 1982; Ashley 1986; Meagher et al. 2016), Mexico and Central America (Castro & Pitre 1989; Molina-Ochoa et al. 2003), and South America (Beserra et al. 2002; Murúa et al. 2009). Although some species exist throughout the Western Hemisphere, most species'

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distribution generally is defined by their geographic areas (Ashley 1979). Since fall armyworm is new to Africa, it is not known which local natural enemy species will use it as a host, although a recent report identifies parasitoids found in eastern Africa (Sisay et al. 2018). Successful biological control management programs for fall armyworm will be those that incorporate multiple natural enemy species (Gross & Pair 1986; Riggin et al. 1993; Figueiredo et al. 2006; Wyckhuys & O'Neil 2006, 2007).

Materials and Methods

SAMPLING SITES

Surveys were conducted twice in 2017 to cover both maize seasons. The first period covered May to Jul, and the second period covered Aug to Nov. A total of 106 maize sites or farms were sampled from different localities of the 10 regions of Ghana. The sites were geographically positioned between latitudes 4.7333333°N and 11.1833333°N, and between longitudes 3.1833333°W and 1.1833333°E (MoFA 2016), with the highest point being Mount Afadjato (880 masl). The sandy coastline is backed by plains and scrubs, intersected by several rivers and streams. A tropical rain forest belt (central and east), interrupted by heavily forested hills and many streams and rivers, extends northward from the coastline. The north of the country varies from 91 to 396 masl and is covered by low bush, park-like savanna, and scattered grassy plains. The country is divided into 6 agroecological zones: Sudan Savannah, Guinea Savannah, Forest Transitional, Deciduous Forest, Rain Forest, and Coastal Savannah (MoFA 2016). The natural vegetation is determined by the different climatic conditions and is influenced by different soil types.

COLLECTION AND REARING OF NATURAL ENEMIES

Predators and parasitoids were collected from the field. Predators that were found preying on the larvae of *S. frugiperda* were directly collected and preserved in 70% ethanol; parasitoids were larval endoparasitoids which developed inside *S. frugiperda* larvae. Therefore, *S. frugiperda* larvae were collected from selected sites on infested maize plants, which were identified by the presence of larval feeding injury in the whorls and newly deposited frass.

Fall armyworm larvae collected from the field were morphologically identified and placed individually in conical transparent rearing containers (8.5 × 5.5 × 11 cm^3). Rearing containers were fitted with a black mesh cover at the top to allow ventilation and to prevent escape of larvae. Tissue paper was placed at the base of the containers to absorb moisture produced by the diet (maize leaves) and larvae (transpiration and frass). The larvae were reared in the laboratory under room conditions (27 ± 4 °C, 80 ± 8% RH, and 12:12 h [L:D] photoperiod). Parasitoids that emerged from the collected larvae were recorded every 24 h and placed in 70% ethanol. Natural enemies were identified by G. Goergen, Curator at the Biodiversity International Institute of Tropical Agriculture Benin.

DATA ANALYSIS

Larval mortality due to unknown factors, adult emergence rate and larval parasitism rate were calculated for all sites. The formula $Pr = \frac{Ni}{Nt}$ (Van Driesche 1983; Pair et al. 1986; Legaspi et al. 2001) was used to determinate the parasitism rate (Pr), where Ni is the number of parasitized individuals of species i, and Nt is the total number of individuals collected. Relative abundance (RA) of each natural enemy species was calculated using the following formula developed and used by Canal Daza (1993) and Molina-Ochoa et al. (2001, 2004): $RA = \frac{Ni}{Nt} \times 100$, where Ni is the number of individuals of species i, and Nt is the total number of all individuals collected. The different localities from which parasitoids were obtained were grouped according to the biogeographic region to which they belong (Table 1).

All analyses were conducted using SAS vers. 9.4 (SAS 2012). Data were first analyzed using Box-Cox (PROC TRANSREG) and PROC UNIVARIATE to find the optimal normalizing transformation (Osborne 2010). Differences in percent parasitism, mortality due to other factors, and adult emergence were first compared between growing seasons, then compared across regions using PROC GLM. In all analyses LSMEANS with an adjusted Tukey test was used to separate variable means.

Results

Fall armyworm larvae were collected from all maize fields across Ghana during both cropping seasons. Natural enemies of fall army-

Table 1. Localities with the collected natural enemies of *S. frugiperda*, Ghana 2017.

Region	Sites	Coordinates	Species (Order: Family)
Greater Accra	Legon	5.6477778°N, 0.1691667°W	*Coccygidium luteum*, *Bracon* sp., *Chelonus bifoveolatus* Szpligeti (Hymenoptera: Braconidae)
	Sege	6.4905556°N, 0.6138889°W	*Meteoridea testacea* (Granger) (Hymenoptera: Braconidae)
Central	Ayensu	5.0944444°N, 1.4291667°W	*Pheidole megacephala* (F.) (Hymenoptera: Formicidae); *C. bifoveolatus*
Western	Dunkwan	5.1108333°N, 1.6327778°W	*P. megacephala*, *M. testacea*
Ashanti	Kumasi	6.1913889°N, 2.0263889°W	*C. bifoveolatus*
	KNUST	7.1347222°N, 1.9747222°W	*Anatrichus erinaceus* Loew (Diptera: Chloropidae); *P. megacephala*
	Agogo Aburkyi	7.4030556°N, 1.1472222°W	*C. bifoveolatus*
Brong Ahafo	Ejura	7.4038889°N, 1.3455556°W	*C. bifoveolatus*
Eastern	Kpong	6.2316667°N, 0.1169444°W	*Bracon* sp., *Cotesia icipe*, *C. bifoveolatus, A. erinaceus*; *Haematochares obscuripennis* Stål, *Peprius nodulipes* (Signoret) (Heteroptera: Reduviidae); *P. megacephala*
Volta	Agove	6.8397222°N, 0.6419444°W	*C. bifoveolatus*
Northern	Tamale	9.6377778°N, 1.4733333°W	*Cotesia icipe*, *Coccygidium luteum*
	Savelugu	10.0716667°N, 1.4733333°W	*C. bifoveolatus*
	Kilompobile	9.6797222°N, 2.7866667°W	Undetermined sp. (Diptera: Tachinidae)
	Kukpehi	9.4163889°N, 0.9702778°W	*Coccygidium luteum*
	Sanga	9.4319444°N, 0.9488889°W	*Coccygidium luteum*
Upper East	Bolga1	11.4713889°N, 1.4508333°W	*A. erinaceus*
	Bolga2	11.4150000°N, 1.3058333°W	*Bracon* sp., *C. bifoveolatus*

worm occurred in 17.0% (18 of 106) of the inspected sites, including 8 sites that were sprayed with insecticides. For this study, a total of 1,062 *S. frugiperda* larvae were collected and 38 were parasitized, yielding a parasitism rate of 3.6%. There was no difference in parasitism between the major (4.8 ± 2.3%) and minor (3.2 ± 0.9%) cropping systems ($F_{1, 18}$ = 0.61; P = 0.4465). There also was no difference in parasitism among regions, with a range from 0% in the Upper West to 7.8% in the Northern region ($F_{9, 10}$ = 0.58; P = 0.7870) (Table 2).

The collection of fall armyworm during the major cropping season from May to Jul included 29 farms from the 10 regions, but natural enemies of fall armyworm were recorded only in 8, representing 27.6% of the sites. Even though parasitoids were not found in the Greater Accra, Central, Western, Brong Ahafo, and Upper West regions, a total of 306 larvae were collected from the field, and only 12 were parasitized giving a 3.9% parasitism rate. For the 8 sites that contained parasitoids, a parasitism rate of 17.4 ± 3.41% was recorded (Table 3).

The minor cropping season collections were carried out from the end of Aug to Nov on 77 farms from the 10 regions. Larval natural enemies of fall armyworm were recorded in 10 sites representing 13.0% of sampled farms. During this season, 756 *S. frugiperda* larvae were collected, and 26 larvae were parasitized representing a parasitism rate of 3.4%. Parasitoids were not found in the Upper West, Upper East, and Volta regions, but among sites that contained parasitoids, Agogo Aburkyi, Legon, Kpong, and Sanga were not sprayed with insecticides and had the highest parasitism rates of 60%, 55.6%, 33.3%, and 23.8%, respectively (Table 4).

Seven species of parasitoids were identified: *C. bifoveolatus*, *C. icipe, Coccygidium luteum*, *M. testacea*, and *Bracon* sp., *A. erinaceus*, and an undetermined Tachnidae species. The 2 most abundant parasitoids were *C. bifoveolatus* and *Coccygidium luteum*, with parasitism rates of 1.04% and 0.85%, respectively, and relative abundance of 29.0% and 23.7%, respectively. *Chelonus bifoveolatus* was the most dispersed parasitoid, found in 7 of the inspected sites (Table 5). Three species of predators were identified: *P. megacephala*, *H. obscuripennis*, and *P. nodulipes* (Table 1). The predator most abundant and most dispersed nationwide was *P. megacephala*, with a relative abundance of 46.0% collected from 4 of the inspected sites (Table 5).

In total, larval mortality due to unknown factors was 63.8%, and was not different between the major (65.6%) and minor (61.8%) cropping seasons ($F_{1,18}$ = 0.61; P = 0.4460). Larval mortality due to unknown factors ranged from 55.3% in the Eastern Region to 73.8% in the Greater Accra Region (Table 2); however, there was no difference among regions ($F_{9, 10}$ = 1.52; P = 0.2612; Table 2). Only 32.7% of larvae collected from fields completed development in the laboratory, and there was no difference between the major (29.5%) and minor (34.9%) cropping seasons ($F_{1,18}$ = 2.05; P = 0.1689). The Greater Accra region had the lowest development rate (20.6%), which was significantly lower than the Eastern (40.8%) and Upper West (42.6%) regions ($F_{9, 10}$ = 4.0; P = 0.0208; Table 2).

Discussion

The parasitism rate ranged from 6.25% at the Kwame Nkrumah University of Science and Technology site in the Ashanti Region, to 60% at the Bolga 2 site in the Upper East Region, and Agogo Aburkyi in the Ashanti Region. All 3 sites were not sprayed with chemical insecticides. The variations in parasitism is due to natural and cultural practices that can negatively or positively affect natural enemy populations (Kogan et al. 1999). The average parasitism across the country was lower than the previous findings in the Americas (8.1%, Ordòn̉ez-Garcia et al. 2015; 13.8%, Molina-Ochoa et al. 2004; 15.5%, Wheeler et al. 1989; 18.3%, Murúa et al. 2009; 28.3%, Meagher et al. 2016; 35%, Rios-Velasco et al. 2011; 39%, Murúa et al. 2009). This low parasitism rate of *S. frugiperda* is due to the aspect of new pest introductions in Ghana. Biological control of fall armyworm requires mass rearing of introduced parasitoids in the laboratories in Africa with field releases to increase parasitism. We believe that parasitism of fall armyworm will increase as the pest continues to be present. However, high applications of chemical insecticides will negatively affect the natural enemies. Therefore, parasitoids must be preserved (Pair et al. 1986; Molina-Ochoa et al. 2004) by using selective systemic insecticides (Figueiredo et al. 2006).

Chelonus bifoveolatus was the most abundant parasitoid collected from larvae. *Chelonus* spp. are typical of egg-larval solitary koinobiont endoparasitoids that attack Noctuidae and Pyralidae (Marsh 1978; Virla et al. 1999; Murúa et al. 2009) by ovipositing into host eggs (Pierce & Holloway 1912; Rechav & Orion 1975). The parasitized host larvae exhibit reduced growth rates and weight compared to unparasitized larvae (Ables & Vinson 1981; Ashley et al. 1983). In the Western Hemisphere, *Chelonus* spp. appear to be the most geographically dispersed parasitoid of fall armyworm (Ashley et al. 1982, 1983; Meagher et al. 2016) and were reported to be present in 12 countries of the Caribbean, and South and Central America (Molina-Ochoa et al. 2003). In many areas, *Chelonus* spp. were reported to be the most common species collected (Wheeler et al. 1989; Cortez-Mondaca et al. 2010, 2012; Rios-Velasco et al. 2011; Estrada-Virgen et al. 2013). *Chelonus bifoveolatus* was found in 7 of the 10 regions (Greater Accra, Central, Eastern, Volta, Ashanti, Brong Ahafo, and Northern) and was recorded in all agroecological zones of Ghana (Coastal Savannah, Evergreen, Equatorial Forest, Transition Zone, and Guinea Savannah). These results suggest that this species is adapted to all sub-Saharan Africa agroecological zones. Therefore, this species can be a good agent for biological control of

Table 2. Fate of fall armyworm larvae collected during the major and minor maize seasons in different regions of Ghana, 2017.

Region	Larvae collected	Mortality by parasitoids (%)	Mortality by other factors (%)	Emerged adults (%)
Greater Accra	107	6 (5.6)	79 (73.8)	22 (20.6)
Central	98	1 (1.0)	67 (68.4)	30 (30.6)
Western	107	3 (2.8)	68 (63.6)	36 (33.6)
Ashanti	136	5 (3.7)	90 (66.2)	41 (30.1)
Brong-Ahafo	90	1 (1.1)	56 (62.2)	33 (36.7)
Northern	166	13 (7.8)	108 (65.1)	45 (27.1)
Upper West	68	0 (0)	38 (55.9)	29 (42.6)
Upper East	67	4 (6.0)	42 (62.7)	21 (31.3)
Eastern	103	4 (3.9)	57 (55.3)	42 (40.8)
Volta	120	1 (0.8)	71 (59.2)	48 (40.0)
Total	1062	38 (3.6)	676 (63.6)	347 (32.7)

Table 3. Sites within regions in Ghana hosting parasitoids of fall armyworm collected during the major cropping season, 2017.

Region and locality	Maize stage	Insecticide application	Larvae collected	Number larvae parasitized	Parasitism rate (%)
ASHANTI					
Kumasi	Flower	Yes	14	1	7.1
KNUST	Flower	No	16	1	6.3
Total			30	2	6.7
NORTHERN					
Tamale	V8–10	No	18	3	16.7
Savelugu	V8–10	No	15	1	6.7
Total			33	4	12.1
UPPER EAST					
Bolga2	V4–6	No	10	3	30.0
Bolga1	V4–6	Yes	8	1	12.5
Total			18	4	22.2
EASTERN					
Kpong	Mature	Yes	4	1	25.0
VOLTA					
Agove	V10–12	No	9	1	11.1

fall armyworm in Africa. However, the most efficient biological control programs for fall armyworm are ones that use and amplify several parasitoid species rather than programs that rely on an individual natural enemy species (Riggin et al. 1993). Therefore, we believe that adding New World parasitoids that are known to attack fall armyworm (classical biological control), plus preserving the parasitoids that are already active in Africa (conservation biological control), will contribute to reducing pest populations. The other active parasitoids included *C. icipe*, which is the koinobiont endoparasitoid that attacks lepidopteran larvae (Quicke 1997; Whitfield 1997). *Cotesia* spp. also are reported parasitoids of fall armyworm in the Americas (Ashley 1983; Meagher et al. 2016). These species may parasitize both eggs (Ruberson & Whitfield 1996), and the first and second instar larvae (Loke et al. 1983). *Cotesia icipe* was reported to be a successful parasitoid of a major maize pest in West Africa and in Mediterranean countries (Kaiser et al. 2017). *Coccygidium luteum*, as all members of this genus, is an internal koinobiont parasitoid of larval Noctuidae (Sharkey et al. 2009). *Bracon* sp. is an idiobiont ectoparasitoid that attack larvae with hidden behaviors, such as cereal stem borers and cereal stored products borers (Moolman et al. 2013; Souobou et al. 2015). *Meteoridea testacae* is a gregarious endoparasitoid that was defined as an egg-larval parasitoid of Lepidoptera (Achterberg 1993). The grass fly, *A. erinaceus* is widespread in Africa, but its parasitic status report is controversial (Upadhyay et al. 2001). However, it is documented as a parasitoid of sugarcane borers. Tachinidae species are almost all parasitoids. In Africa, they are usually collected from cereal stem borer larvae (Moolman et al. 2013; Chinwada et al. 2014).

Pheidole megacephala, the most abundant and most dispersed predator, prefers humid forest habitats (Hoffmann et al. 1999; Wilson 2003; Burwell et al. 2012). When introduced into a new area, *P. mega-*

Table 4. Sites within regions in Ghana hosting parasitoids of *S. frugiperda* collected during the minor cropping season, 2017.

Region and locality	Maize stage	Insecticide application	Larvae collected	Number larvae parasitized	Parasitism rate (%)
GREATER ACCRA					
Legon	V10-12	No	9	5	55.6
Sege	Flower	Yes	8	1	12.5
Total			17	6	35.3
CENTRAL					
Ayensu	Flower	Yes	1	1	100
WESTERN					
Dunkwan	V8-10	No	18	3	16.7
BRONG AHAFO					
Ejura	V4-6	No	12	1	8.3
ASHANTI					
Agogo Aburkyi	Flower	Yes	5	3	60.0
NORTHERN					
Kilompobile	V10-12	Yes	17	2	11.8
Kukpehi	Mature	Yes	18	2	11.1
Sanga	Flower	No	21	5	23.8
Total			56	9	16.1
EASTERN					
Kpong	V10-12	No	9	3	33.3

Table 5. Parasitism rate, relative abundance, and dispersion of different parasitoid species of fall armyworm collected from maize fields in Ghana, 2017.

Species of natural enemies	Number of sites	Number of natural enemies	Parasitism rate	Relative abundance	Dispersion
Cotesia icipe	3	4	0.38	10.5	2.8
Coccygidium luteum	4	9	0.85	23.7	3.8
Bracon sp.	4	6	0.56	15.8	3.8
Chelonus bifoveolatus	7	11	1.04	29.0	6.6
Anatrichus erinaceus	3	3	0.28	7.9	2.8
Tachinidae	1	2	0.19	5.3	0.94
Meteoridea testacea	1	3	0.28	7.9	0.94
Pheidole megacephala	4	17	–	46.0	3.8
Haematochares obscuripennis	1	11	–	29.7	0.94
Peprius nodulipes	1	9	–	24.3	0.94

cephala expanded its range and invaded into the forest interiors, where it attacked and displaced other introduced and naturally occurring ant species (Hoffmann 1998; Burwell et al. 2012). But the efficacy of *P. megacephala* as a biological agent is a challenge due to its generalist behavior and ecological disaster. However, it is a good predator with an efficient nest mate recruitment that enables the species to dominate baits and to retrieve prey too large for single workers to carry (Dejean et al. 2007, 2008). Therefore, *P. megacephala* must be protected as a complementary biological agent of *S. frugiperda* in forest zones, as well as the other 2 species of predators: *H. obscuripennis* and *P. nodulipes*, which also were found in the Equatorial Forest of the Eastern Region. The 2 Reduviids were found in the collection of true bugs sampled from Lama Forest in southern Benin (Attignon 2004).

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