Field and Laboratory Studies on Natural Enemies Associated with the Newly Invasive Mealybug Species, *Paracoccus marginatus,* and other Pests of Papaya in the Eastern and Greater Accra Regions of Ghana

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Abstract

Field and laboratory studies on natural enemies of the papaya mealybug, *Paracoccus marginatus*, and other pests of papaya were undertaken in three districts in the Eastern and Greater Accra regions of Ghana from September 2010 to March 2011. Fruit and leaf samples of over 100,000 specimens of *P. marginatus, Pseudococcus longispinus* (Targioni Tozzetti), as well as samples containing larvae of different insect species, were incubated at 25 ± 1.0 °C and 65 ± 5% RH. Various sampling techniques were used to sample parasitic wasps and predators in the field. A total of 25 different species of natural enemies, including 15 different species of parasitoids, were found with four of these collected from *P. marginatus* incubated samples. Several potential mealybug predators, including the carnivorous butterfly species, *Spalgis epius* (Westwood), coleopterans, including *Crytolaemus montrouzieri* Mulsant, lacewings (*Hemerobius* and *Chrysoperla* sp.), Syrphid larvae and unidentified predatory Noctuid, not yet listed in available literature, were found. Conservation and enhancement of the activities of these indigenous natural enemies will help improve their role as biocontrol agents.

Introduction

The non-traditional agricultural export sector is among the main contributors in the agricultural sector, which accounts for about 35% of the Ghanaian economy (ISSER, 2009), with papaya being one of the major contributors in the fruit sector (Jaeger, 2008). Papaya is produced on a commercial scale in the Greater Accra, Eastern, Volta, and the Central regions of Ghana (Afreh-Nuamah, 2007). It is thought to have originated from Central America and, or Mexico (Tweneboah, 2000; Afreh-Nuamah, 2007; Crane, 2008), the suspected origin of the papaya mealybug (Walker *et al*., 2003; Muniappan *et al*., 2006). *Paracoccus marginatus* has never gained status as a serious pest in Mexico and Central America, probably due to the presence of an endemic natural enemy complex (Walker *et al*., 2003).

The papaya melybug has invaded many countries around the world, causing serious damage to a large number of tropical and sub-tropical fruits, vegetables and ornamental plants (Meyerdirk *et al*., 2004; Muniappan *et al*., 2006; Amarasekare *et al*.,

2009). Since its invasion in Ghana lately in 2009 (Pelican News, 2009), about 85% of all papaya farms in the papaya growing region have been devastated, causing average yield losses of 65% (IITA, 2011). It has also been found to infest other important economic crops such as cassava, egg plants, Jatropha, mango and cocoa (Cham, 2011; Owusu *et al*., 2011). In an attempt to manage *P. marginatus*, most papaya growers have resorted to the use of different mixtures of insecticides, such as Cydim Super (cypermethrin + dimethoate), dimethoate, imidacloprid, cypermethrin, and thiamethoxam (actara). Farmers who cannot afford the high cost of chemical control are either cutting down their papaya plantations and replacing them with crops such as maize, cassava, plantains or oil palms, or abandoning their papaya farms due to frustration.

The waxy nature of mealybugs and problems associated with chemical control such as environmental pollution, residues in fruit and vegetables, as well as adverse effect on non-target beneficial insects, make chemical control a less desirable control option (Charles, 2004). Since mealybugs are capable of becoming resistant to insecticides, management strategies aimed at reducing or averting resistance and the adverse effects of these synthetic chemicals, biological control will be the best control option against *P. marginatus*.

Biological control has been proven to be an effective control strategy against the papaya mealybug in many parts of the world (Amarasekare *et al*., 2009) with over 99% control achieved in some areas (Meyerdirk *et al*., 2004). During biological control, exotic natural enemies in most cases often work alongside indigenous natural enemies in the control of exotic pests. It is, therefore, necessary, to carry out a study on the indigenous natural enemies of the papaya mealybug in Ghana. The study will provide data not only on natural enemies that may have formed new association with *P. marginatus*, but also on natural enemies of other mealybugs species and parasitoids associated with other pests of papaya in the Eastern and Greater Accra regions of Ghana.

Materials and methods

Study area

The search for natural enemies of the papaya mealybug was carried out in the Eastern and Greater Accra regions of Ghana. The Eastern Region lies in the forest zone, while most parts of Greater Accra are in the coastal savanna zone (Modern Ghana, 2010). In the Eastern Region, three districts. Suhum-Kraboa-Coalta, Akuapim South and West Akim districts were selected for the study. Within each district three farms, without any control measure, were randomly selected. Site selection was based on the availability of papaya farms, pest infestation levels and accessibility. Papaya plants taller than 6 m were not considered. In the Greater Accra Region, the study was carried out on the campus of the University of Ghana, Legon. The focus in the Greater Accra Region was to search for natural enemies on other alternative hosts.

Leaf and fruit sampling

Papaya leaf and fruit collection, and incubation for parasitoids took 7 months (between December 2010 and March 2011). The selected plantations were divided into four quadrates. In each quadrate, four papaya plants were selected for leaf and fruit

sampling. For each selected plant, a leaf was randomly sampled from the bottom canopy during the wet season and between the bottom and middle leaf canopy in the dry season. In the case of fruits, one random sample each was collected from the bottom during the wet season, and from the top or middle fruit bearing canopy during the dry season from each of the randomly selected 16 plants in each plantation. Canopy selection was based on mealybug density. The leaf and fruit sampled were placed into paper bags and transported in large hard cartons to ARPPIS laboratory, University of Ghana, Legon, for incubation.

Laboratory studies

Laboratory studies on natural enemies of *P. marginatus* were carried out by incubating both leaf and fruit samples. Before incubation, large papaya leaves were shaped into 10 cm^2 area around the petiole while random samples of *P. marginatus* were collected from the remaining leave patches and placed on the shaped leaves. Throughout the study period, about 288 leaves and 392 fruits were incubated. In March, incubation was on fruit samples of *P. longispinus*, since it is known to occur in abundant during this period, with over 90% occurring on fruits samples (Cham, 2011). Larvae of other insect species associated with *P. marginatus* were also incubated together with samples of *P. marginatus* and their activities monitored daily.

The samples were incubated within transparent 20 cm^2, 20 cm × 30 cm and 30 cm × 40 cm rectangular thick Perspex cages, depending on the sizes of the fruit or leaf samples. Each of the cages had an opening made of a fine netting system for ventilation. Larvae of different insect species were incubated using Petri dishes and Perspex cages. The temperature within the facility was maintained at 25 ± 1.0 °C, with a relative humidity of 65 ± 5% and a photoperiod of 12 h: 12 h (L:D). Incubation took a period of 4 weeks after which the samples were discarded. This was repeated every month throughout the sampling period. Parasitoids and adults of other insect species (in the case of samples containing larvae of other insect species) that emerged were collected, counted and recorded.

All parasitoid collected were preserved in 70% alcohol and later transferred to the Department of Animal Biology and Conservation Science (DABCS), University of Ghana, Legon, where they were processed and mounted on microscope slides using Canada Balsam and identified using morphological keys based on Compere (1931), Gauthier *et al*. (2000), Allemand *et al.* (2002), Jose & Fernandez-Triana (2010), Rajmohana & Talukdar (2010), and Begum *et al*. (2011). Other predators like Lepidopterans were identified using wing markings and other morphological features based on Carter (1992) and Venkatesha *et al*. (2004). Dipterans, lacewings and ladybeetles were also identified using morphological keys by Bland (1978) and Scholtz & Holm (1985).

Field studies

Field sampling of natural enemies such as parasitic wasps on papaya plants was carried out with sweep nets. In each of the six papaya plantations in the two districts, 40 random sweeps were made, 10 in each quadrate of

the plantation. For tall papaya plants a ladder was used before sweep netting was carried out. Sampling of less mobile predators was done using beat sheets (1 m × 1 m). The sheet was placed between two trees and the leaves were stroke four times with a 2-m long stick, and predators collected. Hand-picking was used to collect less mobile predators such as coleopterans, lacewing larvae, and larvae of other insect species (e.g. Lepidopterans) hiding on fruits and under the leaves.

On ornamental plants at the University of Ghana, Legon, sweep net was also used in the sampling of parasitic wasps. The number of sweeps per site depended on the size of the sample site. Beat sheets (1 m × 1 m) were placed under flowers or hedge, and four strokes made to collect less mobile predators. Hand-picking was used to sample other less mobile predators hiding on leaves or flowers. All the predators collected were stunned using a killing jar, and later transferred into separate collection boxes, while parasitoids were preserved in 70% alcohol containing vials. The samples were later processed and identified using the method described earlier.

Results

Parasitoids collected from incubated samples

From over 100,000 samples of *P. marginatus* analysed, no parasitoid was collected between September and November. However, between December 2010 and February 2011, eight parasitoids of *Adelencyrtus* sp. (Hymenoptera: Encyrtidae), five *Scutellista* sp. (Hymenoptera: Pteromalidae), four *Encarsia* sp. (Hymenoptera: Aphelinidae), and one unidentified Aphelinid, were collected from the incubated mealybug samples (Table 1). Majority (62%) were obtained from incubated fruit samples while the rest (38%) were from leaf samples.

In March 2011, when *P. longispinus* was dominant on fruits, the following parasitoids

TABLE 1
Natural enemies collected during the incubation of mealybugs samples

Date	*Host*	*Parasitoids*					*Predators*					
		Ade	*Scu*	*Enc*	*Aph*	*Div*	*Psi*	*Apa*	*Spa*	*Noc*	*Syr*	*Lac*
Sep 2010	PM	0	0	0	0	0	0	0	1	0	0	2
Oct 2010	PM	0	0	0	0	0	0	0	2	0	2	2
Nov 2010	PM	0	0	0	0	0	0	0	1	1	0	4
Dec 2010	PM	2	1	1	0	0	0	0	4	1	1	4
Jan 2011	PM	4	3	2	0	0	0	0	11	4	2	8
Feb 2011	PM	2	1	1	1	0	0	0	8	6	1	5
Mar 2011	LM	4	0	4	7	13	12	3	5	1	3	3
Total		12	5	8	8	13	12	3	32	13	9	28

PM = Papaya mealybug, LM = Longtail mealybug, Ade = *Adelencyrtus* sp., Scu = *Scutellista* sp., Enc = *Encarsia* sp., Aph = Aphelinid, Div = *Diversinervus* sp., Psi = *Psix* sp., Apa = *Apanteles* sp., Spa = *Spalgis* sp., Noc = Noctuid, Syr =syrphid fly, Lac = Lacewings

were collected from incubated fruit samples; 13 *Diversinervus* sp. (Hymenoptera: Encyrtidae), 12 *Psix* sp. (Hymenoptera: Scelionidae), seven parasitoid species of the family Aphelinidae including four *Encarsia* sp., four *Adelencyrtus* sp. (Hymenoptera: Encyrtidae), and three *Apanteles* sp. (Hymenoptera: Braconidae) (Table 1).

Predators of P. marginatus *collected during incubation*

Two important predators of *P. marginatus*; the carnivorous butterfly species *S. epius* and a moth (Lepidoptera: Noctuidae), were collected from the incubated samples. Syrphid and lacewing larvae were also observed to prey on *P. marginatus* in the laboratory incubated samples. A total of 35 *S. epius* were collected, with about 80% collected between January and February. Only 13 individuals of the noctuid were collected, with more than 90% collected between January and February (Table 1). Although studies on the efficiency of *S. epius* were not carried out, it was observed that two *S. epius* larvae were able to completely prey on *P. marginatus* on a single fruit, or on 20 cm^2 papaya leaf within a week before pupating (Fig. 1). Similarly, predation was observed in the field for both *S. epius* and the noctuid predator (Fig. 2).

Natural enemies collected from field samples

In the Suhum-Kraboa-Coaltar district in the Eastern Region of Ghana, a total of 17 different species of natural enemies belonging to six orders and 13 families, excluding spiders, were sampled (Table 2). Of these, the order Hymenoptera was the highest with 10 different species in seven families, while the Lepidopteran and Dipteran orders had the lowest number of species. The Akwapim South District, on the other hand, had the highest diversity of natural enemies with a total of 21 different species belonging to 16 families in five orders excluding spiders (Table 2). The order Hymenoptera was the highest with a total of 11different species in nine families. *Spalgis epius* and the Noctuid species were also sampled. The West Akim District had the lowest diversity of natural enemies in the Eastern Region of Ghana, with 11 species belonging to nine families in five orders (Table 2). Of these, the order Hymenoptera was the single largest group (seven species in six families), followed by the Coleoptera. The carnivorous butterfly species *S. epius* was also sampled.

At the University of Ghana, Legon campus, seven species of natural enemies belonging to five orders and five families, excluding spiders, were sampled (Table 2). Of these, Coleopterans had the highest number (four different species in four families) including the mealybug predator, *Cryptolaemus montrouzieri*.

Mean occurrence of natural enemies

Besides spiders, which had a very high mean occurrence of 10, other predators such as the carnivorous butterfly species *S. epius*, lacewings (*Hemerobius* sp. and *Chrysoperla rufilabris*), and the coleopteran *C. schioedtei*, also had a high mean occurrence of nine, eight, seven and nine, respectively (Fig. 3). Syrphid flies had an average mean occurrence of six, while the rest of the predators, including the mealybug predator *C. montrouzieri* were rare. In the case of parasitoids, *Encarsia* sp. and *Apanteles* sp.

Fig. 1: *Spalgis epius* larva preying on *P. marginatus* in laboratory incubated samples

Fig. 2. Prepupae stages of *S. epius* and pupa case of the noctuid on fruit

TABLE 2
Diversity of natural enemies associated with P. marginatus and other pests of papaya in the Eastern and Legon in the Greater Accra Region of Ghana

	Order	*Family*	*Species*	*Location*
Predators				
	Coleoptera	Coccinelidae	*Cheilomenes lunata* (Fabricius)	AS, L
	Coleoptera	Coccinelidae	*Cryptolaemus montrouzieri* Mulsant	L
	Coleoptera	Coccinelidae	*Exocomus flavi pes* (Thnb)	AS, SKC, W, L
	Coleoptera	Coccinelidae	*Chilocorus schioedtei* Mulsant	AS, SKC, W, L
	Coleoptera	Staphylinidae	*Paederus* sp.	AS,
	Neuroptera	Chrysopidae	*Chrysoperla rufilabris* (Burmeister)	AS, SKC
	Neuroptera	Hemerobiidae	*Hemerobius* sp.	AS, SKC, W, L
	Lepidoptera	Lycaenidae	*Spalgis epius* (Westwood)	AS, SKC, W, L
	Lepidoptera	Noctuidae	Unidentified	AS, SKC
	Diptera	Syrphidae	Unidentified	AS, SKC, L
Parasitoids				
	Hymenoptera	Encyrtidae	*Diversinervus* sp	AS,
	Hymenoptera	Encyrtidae	*Adelencyrtus* sp.	AS, SKC, W, L
	Hymenoptera	Encyrtidae	*Copidosoma* sp.	AS,
	Hymenoptera	Scelionidae	*Psix* sp.	AS, SKC, W
	Hymenoptera	Signiphoridae	*Chartocerus* sp.	AS, SKC, W
	Hymenoptera	Eulophidae	*Meruana* sp.	AS, SKC, W
	Hymenoptera	Eulophidae	*Phymasticus* sp.	SKC
	Hymenoptera	Platygastridae	*Fidiobia* sp.	AS,
	Hymenoptera	Braconidae	*Apanteles* sp.	AS, SKC, W
	Hymenoptera	Braconidae	*Psytallia* sp.	SKC
	Hymenoptera	Braconidae	*Choeras* sp.	SKC
	Hymenoptera	Aphelinidae	*Encarsia* sp.	AS, SKC
	Hymenoptera	Aphelinidae	Unidentified	AS,
	Hymenoptera	Pteromalidae	*Scutellista* sp.	AS, SKC, W
	Hymenoptera	Figitidae	*Leptopilina* sp.	AS, W
Others				
	Arachnida (spiders)			AS, SKC, W, L

AS = Akwapim South District, SKC = Suhum-Kraboa-Coaltar District, W = West Akim District, L = Legon

had the highest mean occurrence of nine followed by *Adelencyrtus* sp., *Psix* sp. and *Scutellista* sp. (Fig. 4). *Chartocerus* and *Muruana* spp. also had a fairly high mean occurrence of six and five, respectively. The number of *Diversinervus* sp., *Choeras* sp., and the Aphelinid collected were four, while the rest of the parasitoids were rare species.

Discussion

The emergence of parasitoids (*Adelencyrtus* sp., *Scutellista* sp., *Encarsia* sp. and an Aphelinid), from cages incubated with *P. marginatus*, are clear indications that these parasitoids might be associated with *P. marginatus*. The fact that species of *Adelencyrtus*, *Encarsa*, and an Aphelinid

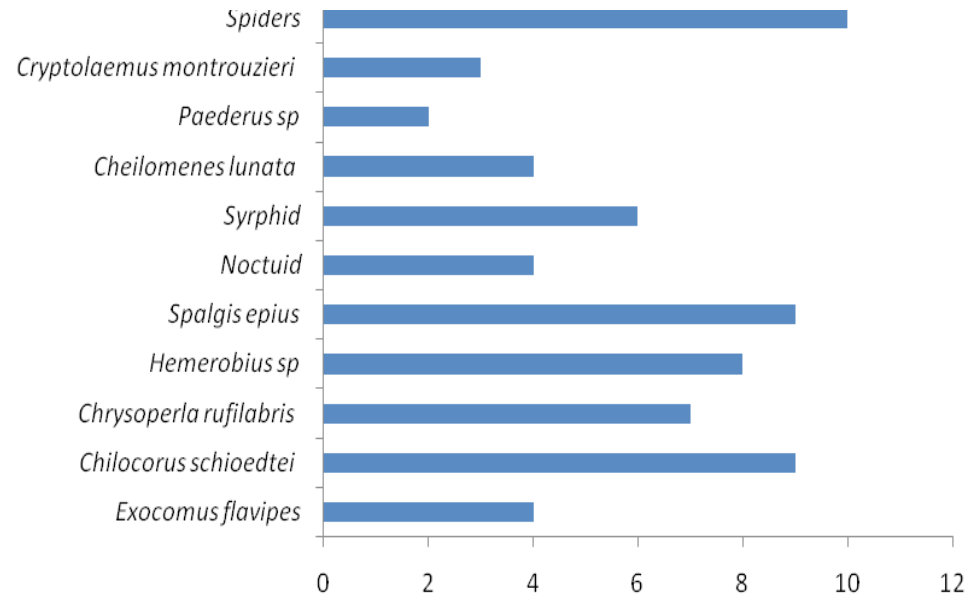

Fig. 3. Mean occurrence of predators associated with *P. marginatus* and other mealybug species

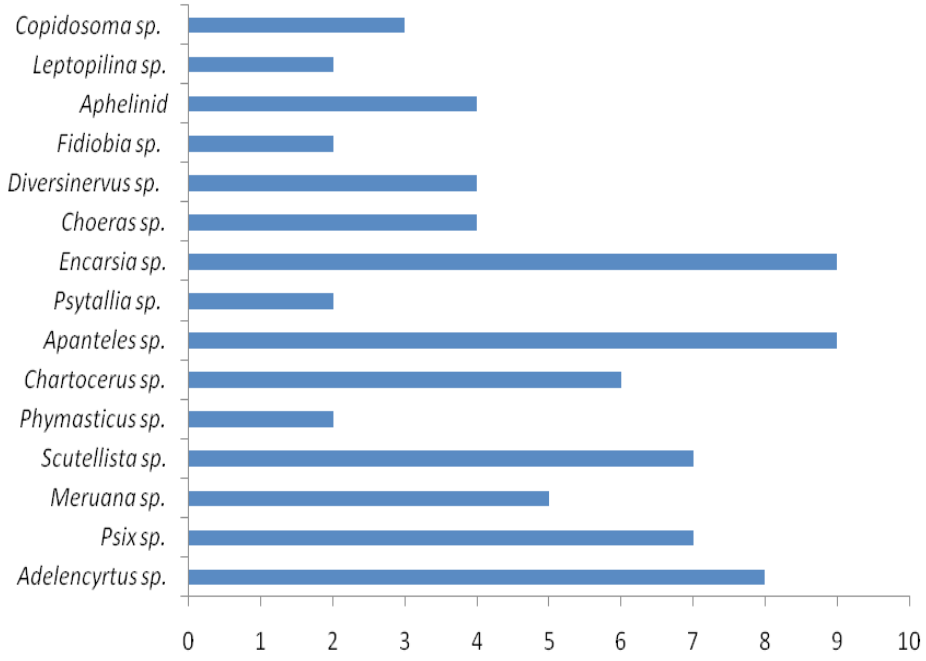

Fig. 4. Mean occurrence of parasitoids associated with insect pests of papaya in the Eastern Region of Ghana

were also reared from samples of *P. longispinus,* collected from the same host plant as *P. marginatus,* support this claim. Trjapitzin & Myartseva (2001) reported species of *Adelencyrtus* as being parasitoids of the scale *Aonidiella aurantii* Maskell (Homoptera: Diaspididae). Besides coccids, Aphelinid parasitoids have also been known to parasitize aphids and whiteflies (Begum *et al*., 2011), which are all pests of papaya, as well as mealybugs of the family Pseudococcidae (Fernanado & Kanagaratnam, 1987). *Encarsia* spp. are known to parasitize whiteflies (Begum *et al*., 2011) while *Scutellista* sp. have been reported as effective parasitoids of soft body insects in Egypt (Badary & Abd-Rabou, 2011). The above parasitoids were not found associated with *P. marginatus* in available publications.

The *Apanteles* sp. collected might have emerged from the larvae of a Lepidoptera (Jose & Fernandez-Triana, 2010) since lepidopterans, especially larvae were associated with *P. marginatus*. The fact that only few species of parasitoids were found associated with *P. marginatus* is not surprising since *P. marginatus* is a recently invasive mealybug species in Ghana. The high relative density of parasitoids on fruits compared to leaves might be due to the fact that the fruits are more exposed to the mealybugs. This agrees with findings by Daane *et al*. (2003) on the study of the population dynamics of the vine mealybug and its natural enemies.

The relative diversity and abundance of parasitoids reared from incubated *P. longispinus* samples was expected since it has existed in the ecosystem for long. This also explains why *P. longispinus* had low relative density in the field compared with *P. marginatus*. It is, however, expected that new associations will be formed between *P. marginatus* and other parasitoids, especially those already associated with *P. longispinus* when it becomes established with time. Other parasitoids collected from *P. longispinus* incubated samples such as *Diversinervus* sp. had been reported as parasitoid of scale insects (Bartlett & Medved, 1966), while *Psix* spp. had been known to parasitize Heteropterans (Rajmohana & Talukdar, 2010).

The continuous use of synthetic chemicals such as Cydim super (cypermethrim and dimethoate), dimethoate, imidacloprid, cypermethrin and thiamethoxam (actara) by farmers in the Eastern Region might have a detrimental effect on parasitoids, and may lead to *P. longispinus*, and other minor pests becoming a major problem to papaya production in the future if nothing is done. Studies are, however, needed in order to confirm the association of these parasitoids with *P. marginatus, P. longispinus* and other hosts species, and the effect of these synthetic chemicals on them.

Laboratory studies indicated that *S. epius* and the predatory noctuid can be used as biocontrol agents against *P. marginatus*. Field observations on their effects on *P. marginatus* also confirmed this claim. These findings confirm that of Thangamalar *et al*. (2010), who stated that during the whole larval period, the predatory larvae of *S. epius* could devour about 42–53 ovisacs, and 196–222 nymphs and adults of *P. marginatus*. Dinesh & Venkatesha (2011) also reported similar findings on prey consumption of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), by *S. epius*. *Spalgis epius*

has the advantage as biocontrol agent because their larvae are myrmecophilous (associated with ants) by secreting honeydew (Venkatesha, 2005). Even when they are not myrmecophilous, they may be protected against ant aggression by a suite of ant-associated adaptations (Pierce *et al*., 2002). The predatory activity of lacewings and the Syrphid larvae observed were not surprising since they have all been reported (Wong, 2006). Although found to have an effect on the control of *P. marginatus*, the predatory noctuid have not been reported as a biological control agent in the reviewed literature. Further study is, however, needed to determine its actual role in the ecosystem.

Many insecticides have been found to have negative impact on *S. epius* (Venkatesha *et al*., 2004). This, therefore, implies that, in other to preserve this important predator and other predators of *P. marginatus*, papaya farmers in the Eastern Region should adjust their management strategies. The low numbers of natural enemies collected between October and November compared to the dry months (December–March), might be due to the low mealybug density during this period as a result of rainfall (Cham, 2011), which tends to wash them away.

Field sampling records showed that mealybugs and other pests of papaya in Ghana were associated with several natural enemies, both predatory and parasitoids, including spiders (which might have some control of the winged males of *P. marginatus* and other pests). All the parasitoids that were collected from incubated samples of *P. marginatus* and *P. longispinus* were sampled in various sampling sites in the Eastern and Greater Accra regions of Ghana. Most of the parasitoids have been reported as parasitoids of mealybug, scales insects, white flies, aphids, coccids and other soft body insects and of Heteropteran eggs in the cited references. Others were parasitoids of Lepidopterans, e.g *Copidosoma* sp. (El-Heneidy & Abbas, 2009), and *Choeras* sp. (Walker, 1996) of Dipterans, e.g the Figitids, *Leptopilina* sp. parasitoid of *Drosophila* sp. (Allemand *et al*., 2002), and of diverse species most of which are pests of papaya in Ghana. The presence of hyperparasitoids such as the *Chartocerus* sp. is certainly no good news since it might become a problem to some of the already present parasitoids.

Although natural enemy density and diversity was less in Legon as compared to the Eastern Region, two important natural enemies of *P. marginatus* (*S. Epius* and *C. montrouzieri*) were sampled. Other coleopterans were also wide spread and occurred more frequently on ornamentals. The wide spread nature of *S. epius*, including the brown lacewing (*Hemerobius* sp.) and green lacewing (*C. Rufilabris*), shows their potential to spread across ecological zones. Parasitoids, on the other hand, were more abundant and diverse in the Eastern Region than at Legon in the Greater Accra Region. This probably might be due to the availability of suitable hosts and or environment, since most parts of the Eastern Region lies in the forest zone as opposed to Legon in the coastal savanna. The high mean occurrence of *Encarsia* sp., *Apanteles* sp., and *Adelencyrtus* sp., and other parasitoids, is an indication of the wide spread nature of their respective hosts.

Conclusion

Although four different parasitoid species were collected from *P. marginatus* incubated

samples, further studies are required to confirm their association with *P. marginatus*. Several potential mealybug predators, including the carnivorous butterfly species *S. epius* were found already associated with the newly invasive *P. marginatus*. A new possible potential predator was included in the list of predators against *P. marginatus*. Besides *P. marginatus* and *P. longispinus*, other pests of papaya were found to be associated with a wide range of parasitoids. A total of 25 different species of natural enemies in 16 families and five orders, excluding spiders, were sampled with the family Hymenoptera representing the largest group.

To facilitate the introduction of parasitoids against *P. marginatus*, farmers should be advised to adjust their management strategies in other to conserve the indigenous predators of *P. marginatus* and *P. longispinus*, and parasitoids associated with other pests of papaya present in the ecosystem. Further studies on the effectiveness of the carnivorous butterfly species *S. epius* and the predatory noctuid on the control of *P. marginatus*, as well as their ecological interaction, are needed to determine their actual role in relation to other crops.

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