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Potential of indigenous Lepidopterans as biocontrol agents against the exotic mealybug species *Paracoccus marginatus* in Ghana

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ABSTRACT

The invasive mealybug species *Paracoccus marginatus* has caused severe damage to the papaya industry and the agricultural sector as a whole, since it was first reported in Ghana in 2009. It is now spreading to other African countries. In search of a sustainable and environment friendly control option against this pest, field survey and laboratory studies were undertaken in three districts in the Eastern region of Ghana to identify possible natural enemies against this pest. Fruit and leaf samples containing over 100,000 samples of *P. marginatus*, *Pseudococcus longispinus* (Targioni Tozzetti), as well as larvae of other insect species were incubated under controlled conditions. Various sampling techniques were used to sample parasitic wasps and predators in the field. A total of 25 different species of natural enemies of a number of papaya pests including 15 species of parasitoids were found, with four of these collected from *P. marginatus* incubated leaf and fruit samples. However, none of the parasitoids had the potential as biocontrol agent against *P. marginatus* as indicated by further studies. The carnivorous butterfly *Spalgis epius* (Westwood) was recorded for the first time in Africa as a potential biocontrol agent against *P. marginatus* as indicated by both field and laboratory studies. It should thus be considered for incorporation into *P. marginatus* biocontrol programmes. Other mealybug predators including the coleopteran *Crytolaemus montrouzieri* Mulsant, lacewings (*Hemerobius* and *Chrysoperla* sp.), Syrphid larvae and unidentified predatory Noctuid were also found to contribute in curbing the population of *P. marginatus* and can thus also play a role in its management.

KEYWORDS: *Paracoccus marginatus*, *Pseudococcus longispinus*, *Spalgis epius*, biocontrol, natural enemies, Ghana

INTRODUCTION

The non-traditional agricultural export sector is among the main contributors in the agricultural sector that accounts for about 35% of the Ghanaian economy [1] with papaya being one of the major contributors in the fruit sector [2]. Papaya is produced on a commercial scale in the Greater Accra, Eastern, Volta, and the Central regions of Ghana [3]. It is thought to have originated from Central America and/or Mexico [3-5], the suspected originating region of the papaya mealybug [6, 7]. *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 [7].

The papaya mealybug has been recorded in many countries around the world. It causes damage to a large number of tropical and sub-tropical fruits,

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vegetables and ornamental plants [6, 8, 9]. Since its invasion into Ghana lately in 2009 [10], about 85% of all the papaya farms in papaya growing regions have been devastated causing average yield losses of 65% [11]. It has also been found to infest other important economic crops such as cassava, egg plants, Jatropha, mango and cocoa [12, 13]. 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). Additionally, 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 in the bushes 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 [14]. Since mealybugs are capable of becoming resistant to insecticides, management strategies aimed at reducing or averting resistance 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 [9] with over 99% control achieved in some areas [8]. 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 investigate on the indigenous natural enemies that might have formed new associations with this invasive mealybug species in Ghana. This study provides data not only on natural enemies of *P. marginatus*, but also on natural enemies of other mealybugs species and parasitoids of other pests of papaya in the Eastern region of Ghana. Most importantly, it provides evidence on the role of indigenous lepidopterans in the control of *P. marginatus* in Ghana.

MATERIALS AND METHODS

Study sites and selection of experimental farms

The search for natural enemies of the papaya mealybug was carried out in the Eastern region of Ghana [15]. The Eastern region is one of the main papaya producing regions and it lies in the forest zone. Three districts- Suhum-Kraboa-Coalta, Akuapim South, and West Akim 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. As such, papaya plants taller than 6 m were not considered.

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 4 quadrates. In each quadrate, 4 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/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 distribution on leaves and fruits across the different seasons [16]. The leaf and fruit sampled were placed in paper bags, and transported in large hard cartons to ARPPIS laboratory, University of Ghana, Legon, for incubation.

Laboratory incubations

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 leaf 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 high abundance during this period with over 90% occurring on fruits samples [12]. 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 x 30 cm and 30 x 40 cm rectangular thick Perspex cages depending on the sizes of the fruit or leaf samples. Each of the cages had an opening with a fine netting system for ventilation. Larvae of other insect species were incubated using Petri dishes and Perspex cages. The temperature within the facility was maintained at 25 ± 2.0 °C with a relative humidity of 65 ± 5% and a photoperiod of 12h : 12h (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% ethanol 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 of Compere [17], Gauthier *et al.* [18], Allemand *et al*. [19], Jose and Fernandez-Triana [20], Rajmohana and Talukdar [21], and Begum *et al*. [22]. Other predators like Lepidopterans were identified using wing markings, colour and other morphological features based on Carter [23] and Venkatesha *et al*. [24]. Dipterans, lacewings and ladybeetles were identified using morphological keys of Bland [25], Scholtz and Holm [26]. Technical expertise of the insect taxonomist of DABCS was sought for confirmation and/or re-identification of the collected samples.

Field sampling of natural enemies

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 to move up the papaya plant close to the fruit samples before carrying out sweep netting. Sampling of less mobile predators was done using beat sheets (1 m x 1 m). The sheet was placed between two trees and the leaves were struck four times with a 2 m long stick, and predators collected. Hand picking was also used to collect less mobile predators hiding on fruits and under leaves such as coleopterans, lacewing larvae, and larvae of Lepidopterans. All the predators collected were stunned using a killing jar, and later transferred into separate collection boxes while parasitoids were preserved in vials containing 70% ethanol. The samples were later processed and identified using the method described above.

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 on both fruits and leaves (Table 1). Majority (62%) were obtained from incubated fruit samples while the rest (38%) were from leaf samples. However, individual incubation of *P. marginatus* samples yielded negative results.

In March, when *P. longispinus* was dominant on fruits [16] the following parasitoids 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 (*Chrysoperla rufilabris* and *Hemerobius* sp.) larvae were also observed to prey on *P. marginatus* in laboratory incubated samples. The predators were in general highly abundant in the dry than in the wet season with *S. epius*, the noctuid and lacewing larvae significantly higher in density than the Syrphid larvae (Figure 1). About 80% of *S. epius* and the noctuid for example were collected during the dry months (between January and March) while the rest were collected from incubated samples during the rainy months (between September and December). Although studies on

Table 1. Parasitoids collected during the incubation of mealybug samples.

Date	Host	Parasitoids						
		Ade	Scu	Enc	Aph	Div	Psi	Apa
Sep 2010	PM	0	0	0	0	0	0	0
Oct 2010	PM	0	0	0	0	0	0	0
Nov 2010	PM	0	0	0	0	0	0	0
Dec 2010	PM	2	1	1	0	0	0	0
Jan 2011	PM	4	3	2	0	0	0	0
Feb 2011	PM	2	1	1	1	0	0	0
Mar 2011	LM	4	0	4	7	13	12	3
Total		**12**	**5**	**8**	**8**	**13**	**12**	**3**

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.

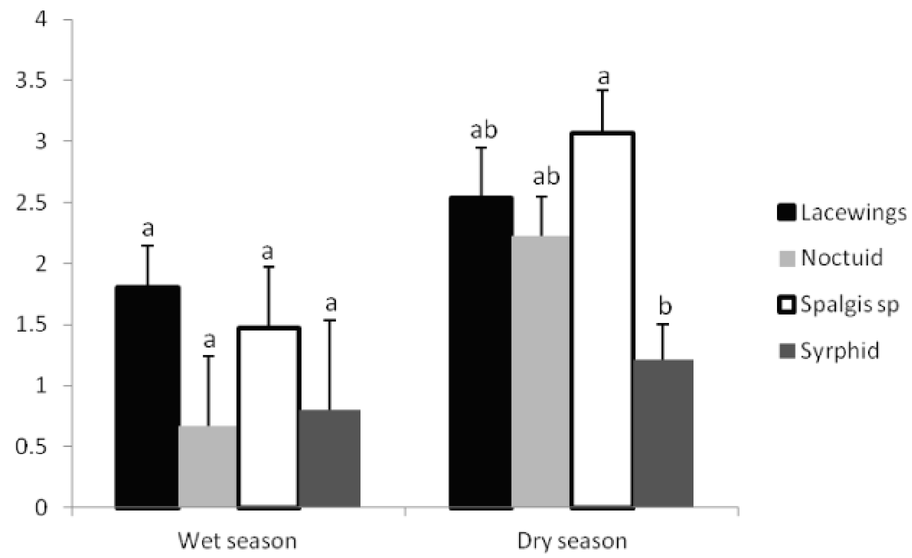

Figure 1. Mean numbers (square root transformed) of predators collected from *P. marginatus* incubated leave samples during the wet and dry months. Bars within the same season followed by different letters (example a and b) are significantly different at P < 0.05.

the efficiency of *S. epius* was not carried out, it was observed that two *S. epius* larvae were able to completely prey on 60 ± 8 mealybug nymphs and adults on a single fruit, or on 20 cm^2 papaya leaf within a week before pupating (Plate 1). Similar predatory activities were observed in the field for both *S. epius* and the noctuid predator (Plate 2).

Field samples of natural enemies

In Suhum-Kraboa-Coaltar district in the Eastern region of Ghana, a total of 17 different species of natural enemies belonging to 6 orders and 13 families, excluding spiders, were sampled (Table 2). Of these, the order Hymenoptera was the highest with 10 different species in 7 families, while the

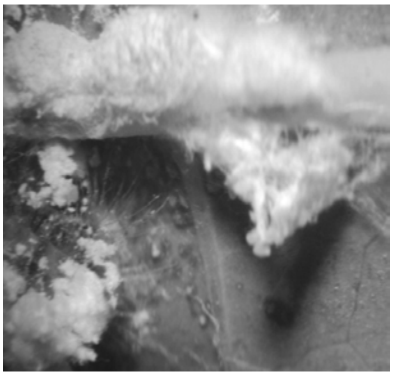

Plate 1. *Spalgis epius* larva preying on *P. marginatus* in laboratory incubated samples.

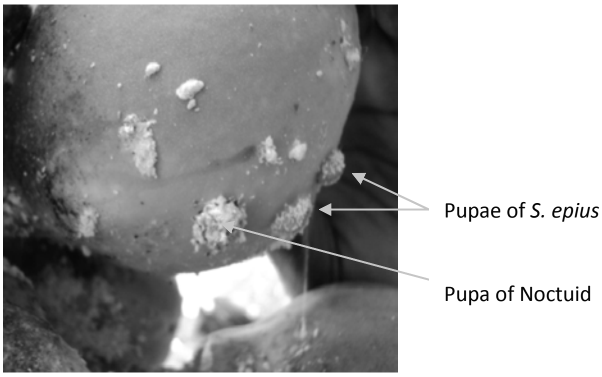

Plate 2. Prepupae stages of *S. epius* and pupa case of the noctuid on fruit.

Lepidopteran and Dipteran orders had the lowest number of individual 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 5 orders excluding spiders (Table 2). The order Hymenoptera was the highest with a total of 11 different species, in 9 families. The West Akim district had the lowest diversity of natural enemies in the Eastern region of Ghana with 11 species belonging to 9 families in 5 orders (Table 2). Of these, the order Hymenoptera was the single largest group with a total of 7 different species in 6 families, followed by the coleopterans. The carnivorous butterfly species *S. epius* was widely distributed across all the districts of the Eastern region.

DISCUSSION

The emergence of parasitoids (*Adelencyrtus* sp., *Scutellista* sp., *Encarsia* sp. and an Aphelinid) from cages incubated with *P. marginatus* seems to suggest that these parasitoids might be associated with *P. marginatus*. The fact that species of *Adelencyrtus, Encarsa,* and an Aphelinid were also reared from samples of *P. longispinus* collected from the same host plant as *P. marginatus* supports this claim. However, since no parasitoid was recovered from the incubation of individual *P. marginatus* samples, the association is not completely certain. Trjapitzin and Myartseva [27] 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 [22] which are all pests of papaya, as well as mealybugs of the family Pseudococcidae [28]. *Encarsia* spp. are known to parasitize whiteflies [22] while *Scutellista* sp. have been reported as effective parasitoids of soft body insects in Egypt [29]. The *Apanteles* sp. collected might have emerged from the larvae of a Lepidoptera [20] since lepidopterans (larvae in particular) were associated with *P. marginatus.* The high relative density of parasitoids on fruits compared to leaves might be due to the more exposed nature of mealybugs on fruits. This agrees with findings by Daane *et al.* [30] who studied 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* [16]. It is, however, expected that new associations will be formed between *P. marginatus* and parasitoids of other mealybug species such as *P. longispinus* which share the same host plants with *P. marginatus*. Other parasitoids collected from *P. longispinus* incubated samples such as *Diversinervus* sp. had been reported as parasitoid of scales by Bartlett and Medved [31], while *Psix* spp. have been known to parasitize Heteropterans [21].

The continuous use of synthetic chemicals such as Cydim super (cypermethrim and dimethoate), dimethoate, imidacloprid, cypermethrin, and

Table 2. Diversity of natural enemies of *P. marginatus* and other pests of papaya in the Eastern region of Ghana.

	Order	Family	Species	Location
Predators				
	Coleoptera	Coccinelidae	*Cheilomenes lunata* (Fabricius)	AS
	Coleoptera	Coccinelidae	*Cryptolaemus montrouzieri* Mulsant	AS
	Coleoptera	Coccinelidae	*Exocomus flavi pes* (Thnb)	AS, SKC, W
	Coleoptera	Coccinelidae	*Chilocorus schioedtei* Mulsant	AS, SKC, W
	Coleoptera	Staphylinidae	*Paederus* sp.	AS
	Neuroptera	Chrysopidae	*Chrysoperla rufilabris* (Burmeister)	AS, SKC
	Neuroptera	Hemerobiidae	*Hemerobius* sp.	AS, SKC, W
	Lepidoptera	Lycaenidae	*Spalgis epius* (Westwood)	AS, SKC, W
	Lepidoptera	Noctuidae	Unidentified	AS, SKC
	Diptera	Syrphidae	Unidentified	AS, SKC
Parasitoids				
	Hymenoptera	Encyrtidae	*Diversinervus* sp.	AS
	Hymenoptera	Encyrtidae	*Adelencyrtus* sp.	AS, SKC, W
	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

AS = Akwapim South district, SKC = Suhum-Kraboa-Coaltar district, W = West Akim district.

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. Further studies are, however, needed 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*. [32], who reported *S. epius* as an efficient predator of *P. marginatus* in India. Dinesh and Venkatesha [33] also reported similar findings on prey consumption of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) by *S. epius*. *Spalgis epius* have the advantage as biocontrol agent because their larvae are myrmecophilous (associated with ants) which give them an edge over other natural enemies which have to compete with ants for food and space [34]. Even when they are not myrmecophilous, they may be protected against ant aggression by a suite of ant-associated adaptations [35]. The predatory activity of lacewings and the Syrphid larvae observed were not surprising since they have also been reported [36]. Although found to have an effect on the control of *P. marginatus*, the predatory noctuid has not been reported as a biological control agent in the reviewed literature. Further study is however needed to determine its true identity and actual role in the ecosystem.

The low numbers of natural enemies collected between October and November compared to the dry months (December to March) might be due to the corresponding low host densities. The mealybugs, *P. marginatus* and *P. longispinus* for example have been reported to occur in high densities during the dry season compared to the rainy season [16] which is probably one reason for the low predator density during the rainy season.

Field sampling records showed that mealybugs and other pests of papaya in Ghana are under control by several natural enemies, both predators and parasitoids including spiders (which may contribute in the control of the winged males of *P. marginatus* and other pests). Most of the field sampled parasitoids were also collected during lab incubation as indicated above. Others were parasitoids of Lepidopterans, e.g. *Copidosoma* sp. [37] and *Choeras* sp. [38], of Dipterans e.g. the Figitids, *Leptopilina* sp. parasitoid of *Drosophiia* [19], 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.

CONCLUSION

In this study, *S. epius* was recorded for the first time in Africa as a potential predator of *P. marginatus*. A new possible potential predator was included in the list of predators against *P. marginatus*. No potential parasitoid was found associated with *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 5 orders excluding spiders were sampled, with the family Hymenoptera representing the largest group.

To enhance the performance of the Lepidopteran predators in the control of *P. marginatus*, farmers should be advised to adjust their management strategies so as to conserve these important indigenous predators of *P. marginatus* and *P. longispinus* present in the ecosystem. Further studies on the effectiveness of *S. epius* and the predatory noctuid in the control of *P. marginatus* as well as their ecological interaction is needed to determine their actual role in relation to other crops.

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CONFLICT OF INTEREST STATEMENT

All authors are in accord with the way this article has been presented and are not aware of any affiliations, memberships, or any other factor that might interfere with the publication of this article.

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