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Original Research paper

Survey and exclusion study to explore for natural enemies in Ghana for the Control of Aerial yam (*Dioscorea bulbifera* L.) in Florida, USA

ABSTRACT

Aims: A survey and exclusion study to establish the presence of D. bulbifera and its associated phytophagous insect fauna was conducted in Ghana between 2004 and 2006 to identify potential biological control agents for this invasive plant in Florida,
Study design: Survey covering five regions of Ghana based on herbarium records from three academic institutions and exclusion field trial fitted in Randomized Complete Block (RCBD).
Place and Duration of Study: Experimental fields of the Crops Research Institute, Kumasi between May 2004 and July 2008.
Methodology: Exploratory surveys were conducted in areas where aerial yams are cultivated in Ghana and exclusion experiments to measure impact of herbivores on plant performance
Results: A total of 40 phytophagous species in 9 orders were encountered on the plant with 24 species attacking D. bulbifera in two feeding guilds in Ghana; foliage and bulbil feeders. The impact of foliage feeding varied from species to species. The mean percent defoliation of aerial yam over all plots was 30%. Coleopterous species fed on only foliage and nearly all the leaves showed damage from these species. *Anomala sp.* and *Adoretus sp*. were the most important Coleoptera (Scarabaeidae) found feeding on the plant. *Anomala* species, exhibited a very narrow host range, attacking only D. alata together with D. bulbifera. Even though the damage it caused to aerial yam was relatively small, it appears a potential biological control agent because of its very narrow host range. Lepidopterous species, mainly the Arctiid moths Diacrisia and Estigmene species attack aerial yam leaves and bulbils. They caused considerable damage to the bulbils the principal planting material **Conclusion:** Although the Lepidopterous species exhibited a wide host range attacking other Dioscorea species and therefore do not appear to be good biological control candidates but considering the poor representation of Dioscorea species of economic importance in the United states of America they could be valuable

Keywords: invasive, aerial yam, evaluation, Estigemene, Diacrisia species, Dioscorea bulbifera

1. INTRODUCTION

Dioscorea bulbifera L. commonly called aerial yam, air potato or bitter yam belongs to the yam family (Dioscoreaceae). The genus *Dioscorea* (true yam) is economically important worldwide as a food crop particularly in West Africa. The origin of *D. bulbifera* is, however, uncertain. Some believe that the plant is native to both Asia and Africa [1, 2]. Whereas others believe that it is native to Asia and was subsequently introduced into Africa [3].
Dioscorea bulbifera is characterized by its aggressively high-climbing annual twining stems, large ovate leaves with prominent veins, and potato-like aerial tubers in the leaf axils [4; 5]. Production of large numbers of aerial tubers allows for rapid proliferation and colonization. The plants grow rapidly in full sun and can overgrow and kill native flora [6]. According to Morisawa [7], vines grow as rapidly as 20 cm per day, quickly spiraling up to tree tops to form dense masses that shade out trees and may eventually kill them. *D. bulbifera* was introduced to the Americas from Africa during the slave trade ~~(Coursey~~ [8], and specifically into Florida in 1905 for scientific study [1] and now constitutes one of the most aggressive weeds ever introduced to Florida [3]. It is found throughout the state of Florida and currently a widespread weed throughout many southern states [9; 10] and threatens the stability and biodiversity of native communities [11].

Dioscorea bulbifera is considered the most serious environmental threat, described as a category I weed by the Florida Exotic Plant Pest Council [11]. It was listed earlier on in 1999 as a noxious weed by the Florida Department of Agricultural and Consumer Services (FDACS).

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Because of its underground tuber and abundant bulbils (aerial tubers), it is difficult to eradicate [12] and according to Duxbury *et al*. [14], management of this vine is challenging, largely because of the plant's ability to grow from bulbils. To prevent its spread and suppression of natural plant communities, aerial yam requires active management. Current practices of manual and herbicide control are very labour intensive, expensive and inefficient [6]. As the aerial yam was introduced to Florida and without its natural control agent(s), there is the need to institute classical biological control to bring it under check. Schultz [6] indicated that there are no approved biological control agents for *D. bulbifera* in the United States of America ~~hence~~ the need to explore for biological control agents for this weed pest. Earlier investigations by scientists at the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI), Kumasi, Ghana revealed that some coleopterans inflict feeding damages on the leaves of the plant. Overholt *et al.* [15], using the chloroplast DNA technology - determined that Florida air potato is most likely to be of African origin. West Africa is also regarded as one of the origins of aerial yam [2; 3]. Ayensu and Coursey [16] reported the presence of wild and cultivated types in various parts of Ghana. The wild types are usually found at the peripheries of secondary forest [17]. Interestingly, however, it does not pose any threat to the vegetation here in Ghana. Preliminary investigations have revealed that insects attack both the bulbils and the leaves [17]. The probability of finding a suitable control agent clearly increases when searching in the target weed's native range. It therefore sounds reasonable to initiate efforts to explore for natural enemies since the above revelations clearly suggest that a probable natural enemy may be found in Ghana. The objectives of this study were to 1) determine locations of aerial yam and associated insect pests in Ghana and 2) conduct a study on a good arthropod candidate for possible control of this noxious weed in Florida, USA.

2. MATERIAL AND METHODS

2.1 Exploratory survey to determine locations of aerial yam and associated pests

Exploratory surveys were conducted in areas where aerial yams are cultivated in Ghana from May 2004 to August 2004. Wild types were also sampled along the major roads, specifically at the fringes of secondary forests. Information was sought on types or varieties grown and whether there were insect herbivores associated with the plant. The survey covered five regions of the country comprising the Ashanti, Brong Ahafo, Eastern, Central-and Upper West regions. Selected sites were based on herbarium records from the University of Ghana (UG), University of Cape Coast (UCC) and the Forestry Research Institute of Ghana (FORIG). In each selected region, twenty communities were purposively selected and studied. Five farmers were interviewed per community by the administration of a questionnaire. Additionally, the exact coordinates of every aerial yam plant sampled at each location was recorded using the etrex Garmin Global Positioning System (GPS). Data generated from the GPS was used to represent the various locations on the map of Ghana (Figure 1).

A total of 500 farmers were interviewed on their knowledge about cultivated and wild forms as well as pest problems associated with the *D. bulbifera*. Insects found on the vegetative parts and bulbils were collected for identification and further laboratory studies.

2.2 Exclusion experiments to measure impact of herbivores on plant performance

These experiments began in May 2005 and ended in October 2005 at the experimental fields of the Crops Research Institute (CRI) at Kwadaso, Kumasi (Lat. 6º 42N; Long. 1º 40W; 262m above sea level). It was repeated from ~~in~~ March 2006 to October 2006. The trial was set up with five accessions of cultivated types of aerial yams. Bulbils were collected from different locations and from some farmers in Upper West (Goyiri and Tuna), Ashanti (Anwia Nkwanta and Anyinamso), Central (Hweremoase and Kakum) and Eastern (Bunso) during the survey. The experimental design was a randomized complete block, with insecticide protection and no protection in three replications. Bulbils of similar sizes were planted individually in mounds of 50 cm in diameter and 40 cm high. Stakes were provided to direct and facilitate growth of vines in slanting fashion (Plate 2). Collection of agronomic data and chemical application started at 50% sprouting. No chemical was applied to the unprotected plots whilst a foliar insecticide Cymethoate Super (combination of 36 g Cypermethrin and

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400 g Dimethoate per litre) was applied at a rate of 4ml per litre of water to exclude herbivores from plants in the protected plots.

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2.3 Data collection

Data was taken on five plants in systematic sampling fashion. On each sampled plant, all leaves were counted as well as damaged ones. Vine length was measured from 10 cm above the mound with twine wrapped along the vine which was then measured with a measuring tape. The stem diameter was also measured at the 10 cm mark above the mound with veneer calipers. Percent defoliation was scored with a scoring scale at the upper, middle and lower portions of each plant. A score of 1 indicated 25% of total leaves showed perforations or damage by insects, 2 indicated 50% damaged leaves, 3 indicated 75% of leaves damaged and 4 indicated all leaves counted showed perforations. Number of bulbils damaged was also recorded. Plant biomass (dry weight of vines) was assessed at harvest. Performance of the plants under unprotected (control) and protected treatments were then compared.

2.4 Catalogue of insect herbivores

All invertebrate herbivores that visited the plants were documented and collected for identification and further investigation. Observations and collections were made twice a week during the day and at night starting in 2005 and continued throughout the growing season in 2006 (i.e from sprouting until the plants senesced) with the aid of a rechargeable lamp. The insects were collected with a pair of forceps or a camel hair brush into a kilner jar or into 30 ml glass vials containing 70% ethanol. Known insect species were documented whilst unknown species were sent to the laboratory and preserved for identification later. Samples of arthropods collected were curated according to standard procedures [17]. Specimens were identified by comparing species collected from the field with specimens in the insect museum of CRI to their respective family. Feeding guilds were also determined. Adults of moth species that could not be identified as above were sent to Michael G. Pogue at the Systematic Entomology Laboratory of the US Department of Agriculture and were identified as *Diacrisia rattrayi* Rothchild and Estigmene sp. (Lepidoptera: Acrtiidae) - . Voucher specimens of all arthropods collected have been kept at CSIR-CRI.

2.5 Data Analysis

Data were analyzed statistically using analysis of variance (ANOVA), percentage data were arcsine transformed while number counts were log (x + 1) transformed. Mean values were separated using the Student Newman Keul's (SNK) test at a significance level of $P \leq 0.05$. All data were analyzed using the SAS® program (release 9.2 for Windows [19].

3. RESULTS

Communities in all the five regions region visited during the survey are shown in Table 1.
Out of the five regions, only the Central region recorded both wild and cultivated types of aerial yams. In the Kakum forest from the Central region some coleopterans species were collected on *D. bulbifera* in the night which did not feed on the detached leaves of aerial yam and other Dioscorea species provided them until the insects died. The insects collected could probably be transient or were not pests of the plants tested.
In the Ashanti, Brong Ahafo and Eastern regions, both wild and cultivated types were present and were intercropped with cassava and plantain. The wild types were located along the fringes of the secondary forests. No insects were, however, found feeding or associated with the wild *D. bulbifera*. In the Eastern region, however, efforts were concentrated around Bunso, where several cultivated types were encountered. At Bunso, the beetles *Anomala* sp. and *Adoretus pullus* were observed feeding on the leaves of *D. bulbifera*. as well as on leaves of *D. alata*. In the Upper West region, only the cultivated types were encountered. The plant was also popular with the farmers since most of them emphasized that the crop was maintained to fight hunger during the lean seasons. No insect

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was found feeding on the plants during the survey in the Upper West region even though some plants showed damaged symptoms (perforated leaves and partly eaten bulbils).

Out of the 500 farmers interviewed only 10% did not know the plant. About 60% of those who knew the plant admitted seeing damage holes on the leaves but could not attribute the damage to any defoliator. A few farmers (6%) in the Central and Ashanti regions admitted seeing rodents feeding on the bulbils.

3.1 Catalogue of insect herbivores of D. bulbifera

The phytophagous herbivores associated with aerial yam during 2005 and 2006 are summarized in Table 2. A total of 40 species were collected on *D. bulbifera.* Majority of the insects belonged to the following Orders/Families; Coleoptera, Lepidoptera and Hemiptera. The rests were from Dermaptera, Manthodea, Diptera, Orthoptera, Hymenoptera and Isoptera. A few millipedes and molluscs were also found.

3.2 Exclusion Experiment to measure impact of herbivory on plant performance

For both 2005 and 2006 significant differences were not observed in number of leaves for treated and protected (control) plots (Figure 2). There were however, significant differences ($P \leq 0.05$) in the number of damaged leaves (Figure 3) but plant height, stem diameter, number of bulbils and number of damaged bulbils did not show any significant differences ($P \leq 0.05$) (Figure 4, 5, 6, and 7). Damage to leaves was significantly greater in the unprotected plots than the protected plots (Figure 2). The differences observed between the protected and unprotected crop for damaged leaves, however, did not translate into the growth parameters, thus no differences were observed in plant height and stem diameter.

Two members of the family Scarabaeidae, *Anomala* sp. (Plate 3) and *Adoretus pulus*, that caused damage to the leaves of *D. bulbifera*, previously reported in earlier studies by CRI scientists [17] were encountered. They fed on *D. bulbifera* and *D. alata* (Plates 6a and 6c), however, there was no infestation on leaves of *D. rotundata* (Plates 6b and 6c). The activities of these beetles were observed shortly after dusk. Many of the beetles were seen and collected during routine visits to the field between ~~1900~~ and ~~2000 hrs~~ GMT. These beetles were never encountered during day time. Even though *Anomala* sp. and *Adoretus* sp. were seen feeding on leaves in the field and caused most of the foliar damage, the beetles did not feed on bulbils, and leaves of *D. rotundata* yam varieties in the laboratory as well as in the field. It was also observed that the beetles preferred *D. bulbifera and D. alata* to rotundata yams (Figure 7).

In addition to the beetles, larvae of the two Lepidopteran insects, (*Estigmene* sp. and *Diacrisia* sp.) belonging to the family Arctiidae were found causing damage to both the leaves and bulbils of *D. bulbifera*. (Plates 4 and 5). They fed voraciously on leaves and bulbils particularly at night.

4.0 DISCUSSION

Surveys in this study confirmed an earlier assertion by Ayensu and Coursey [16]) that both cultivated and wild forms of the yam are found in several places in Ghana. The present study revealed that aerial yams in the wild are mostly found arund the fringes of forests. The study also showed a considerable number (40) phytophagous insect fauna associated with *D. bulbifera*, with 24 species attacking the foliage and bulbils. Similar surveys discovered several damaging herbivore species in Nepal [11]. Results gathered from the exploratory survey suggested that several factors could account for the low incidence of the aerial yam in Ghana. Among these factors are insects as well as rodents which might consume fallen bulbils.

To implement a classical biological control program successfully, it is important that ecological and biological information about the target species and its associated fauna be established both in its area of origin and the invaded area [20]. This study indicated the potential of some arthropods as biological control agents of aerial yam in Ghana. In an exclusion study for example, about 94% herbivory by native insects were recorded although the growth performance of the aerial yam were not affected. Two members of the family Scarabaeidae, *Anomala* sp. (Plate 3) and *Adoretus pullus* were observed to have caused damage to the leaves of *D. bulbifera* and *D. alata* (Plates 6a and 6c), however, there was no defoliation on leaves of *D. rotundata* (Plates 6b and c). Laboratory investigations confirmed the results obtained from the field as regards the non-pestiferous nature of the beetles on the *D.*

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rotundata yams (Figure 7). Both insect species consumed significant amount of leaves of aerial yam and water yam. Neither the stem diameter, plant height, number of leaves nor damage to bulbils as indicators for changes in plant development after herbivore attack showed a relation to beetle density.

The ecological and economic effects of aerial yam to the economy of the United States may require the consideration of the lepidopteran insect pests of aerial yam identified from this study since no Dioscorea species is cultivated commercially as food crops in Florida and the southern U. S. A. [12; 21].

The abundance and diverse insect fauna and other vertebrates such as rodents that feed on the bulbils constitute an important check on the growth and spread of aerial yam in Ghana. However, the relatively sparse fauna on the yam in the U. S. A. could probably contribute to its invasive habit and pest status there. From a biological control point of view, the defoliators and bulbil feeders are worth considering as potential control agents. It became evident that there was the needed to use alternative testing procedures that could reflect what really happens in nature. Furthermore, some authors have contended that during cage tests, if an insect is confined with a non host plant species it may be forced to accept the plant as survival instinct and may become habituated to inherent feeding deterrents [22; 23]. The larval host range in the laboratory was broader than the field host range for both Lepidoptera species. This situation according to Harris [24] arises when the field host range is determined by adult habit and host finding requirements. According to Thompson *et al*. [25], in laboratory tests, insects often accepted a broader range of hosts than in nature. In most insects the adult is responsible for selecting a suitable host since its larvae initially lack the necessary mobility. The female in most cases, is under selection pressure to oviposit on plants that optimize the survival of its progeny [24]. The larva, on the other hand, has to stay on the plant, which may involve distinguishing it from intermingled vegetation, and feed.

In a situation where the larva finds itself on a wrong plant, its best survival option is usually to try and feed on it. Consequently, the adult host preference tends to be narrower and more firmly held than that of the larva [24]. The results from this study indicated that all *Dioscorea* species tested including *D. bulbifera* and *D. alata* were acceptable hosts for the acrtiid moths, but it was observed in the field that *D. bulbifera* was the preferred hosts.

5. CONCLUSION

Both cultivated and wild forms of aerial yam exist in Ghana and a considerable number of phytophagous insect fauna have been observed attacking the foliage and bulbils of *D. bulbifera*. Two lepidopteran species (*Estigmene* and *Diacrisia*) were easily reared on aerial yam. The study demonstrated that all selected non-target species were attacked and were largely suitable for insect development. Larvae of both Lepidoptera species completely devoured all the *Dioscorea* species provided them and successfully completed their development on leaves and bulbils. Generally, *Estigmene* and *Diacrisia* species do not appear to be good biological control candidates but their consumption preference for bulbils, the main source of propagation and spread, and considering the poor representation of *Dioscorea* species of economic importance in the United States of America, they could be valuable.

Two species of beetles *Anomala* sp. and *Adoretus* sp, found to cause considerable damage to the Foliage. *Anomala* species, consumed *D. bulbifera* and *D. alata* but not *D. rotundata* yam species. However, the damage they caused to aerial yam was rather low. This species even though exhibited a very narrow host range, attacking only *D. alata* together with *D. bulbifera* may not be suitable candidate for the control of *D. bulbifera* because they fed only on the leaves and also their feeding could not impact negatively on the performance of the plant.

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Table1: Locations and other characteristics of cultivated and wild types of *Dioscorea bulbifera* in five regions of Ghana during 2004 survey

Region	**Town/village of plant attacked**	**Presence/ Absence of *D. bulbifera***	**Other *Dioscorea* species found**	**insect feeding on *D. bulbifera***	**Part**
Ashanti	Anyinamso	Present	Yes	No	-
	Mmoframfaadwene*	Present	Yes	No	-
	Amangoase	Present	Yes	Yes	Leaf
	Otaakrom*	Present	Yes	Yes	Leaf
	Nkansakrom	Absent	Yes	No	-
	Sakamukrom	Absent	No	No	-
	Ahenkro	Absent	No	No	-
	Anyinasuso	Present	No	Yes	Leaf
	Ejura/Hiawoanwu	Absent	Yes	No	-
	Mfensi	Absent	Yes	No	-
	Betinko*	Present	No	Yes	Bulbil
	Hwibaa	Absent	No	No	-
	Barniekrom	Present	No	No	-
	Ahwia Nkwanta	Absent	Yes	No	-
Brong Ahafo	Mmehame Nkwanta	Present	Yes	Yes	Leaf
	Goaso	Present	No	No	-
	Mim	Present	No	No	-
	Hiayeanimguase*	Present	Yes	Yes	Leaf
	Buokukruwa*	Present	No	No	-
Eastern	Bunso*	Present	Yes	Yes	Leaf
	Nkawkaw	Absent	Yes	No	-
	Pepease	Present	No	No	-
Central	Kakum	Present	No	Yes	Leaf
	Assin Manso	Present	No	Yes	Bulbil
	Hweremoase	Present	Yes	Yes	Bulbil
Upper West	Goyiri*	Present	Yes	No	-
	Tuna*	Present	Yes	No	-
	Dusie*	Present	Yes	Yes	Leaf
	Kolkpong*	Present	Yes	No	-

* Locations where *Dioscorea bulbifera* were cultivated

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Table 2. Invertebrate herbivores collected and other attributes from *D. bulbifera* in Ghana during the study period of 2004 – 2006

Insects Order	Family	Species	Stage	Part damaged	Specificity	Remarks
Coleoptera	Scarabaeidae	*Anomala* sp.	Adult	foliage	y	
Agent 1						
	Scarabaeidae	*Adoretus pullus*	Adult	foliage	y	
Agent 2						
	Scarabaeidae	*Pachnoda cordat*	Adult	bulbils	x	
	Bruchidae	*Callosobruchus maculatus*	Adult	unknown	b	
	Chrysomelidae	*Ootheca mutabilis*	Adult	foliage	b	
	Chrysomelidae	*Podagrica uniformis*	Adult	foliage	b	
	Coccinellidae	*Coccinella septempunctata*	Adult	beneficial	c	
	Lagriidae	*Lagria villosa*	Adult	unknown	b	
	Lagriidae	*Lagria cuspida*	Adult	unknown	b	
	Unidentified		Adult	unknown	b	
Hemiptera	Pentatomidae	*Nezara viridula*	Adult	unknown	b	
	Alydidae	*Riptortus dentipes*	Adult	unknown	b	
	Pyrrhocoridae	*Dysdercus superstitious*	Adult	unknown	b	
	Alydidae	*Anoplocnemis curvipes*	Adult/Nymph	unknown	b	
	Aphididae	*Aphis craccivora*	Adult	foliage	x	
	Aphididae	*Brevicoryne brassicae*	Adult	foliage	x	
	Aleyrodidae	*Bemisia tabaci*	Adult	foliage	x	
	Aleyrodidae	*Aleurodicus dispersus*	Adult	foliage	x	
	Cicadellidae	*Empoasca fabae*	Adult	unknown	b	
	Pseudococcidae	*Phenacoccus manihoti*	Adult	foliage	x	
Dermaptera	Forficulidae	*Forficula auricularia*	Adult	unknown	b	
Mantodea	Mantidae	*Sphodromantis viridis*	Adult & Nymph	beneficial	c	
	Mantidae	*Mantis religiosa*	Adult & Nymph	beneficial	c	
Diptera	Muscidae	*Musca domestica*	Adult	unknown	b	
Orthoptera	Pyrgomorphidae	*Zonocerus variegatus*	Adult	foliage	x	
	Acrididae	Not identified	Adult	unknown	b	
	Tetrigidae	Not identified	Adult	unknown	b	
	Tettigonidae	Not identified	Adult	foliage	x	

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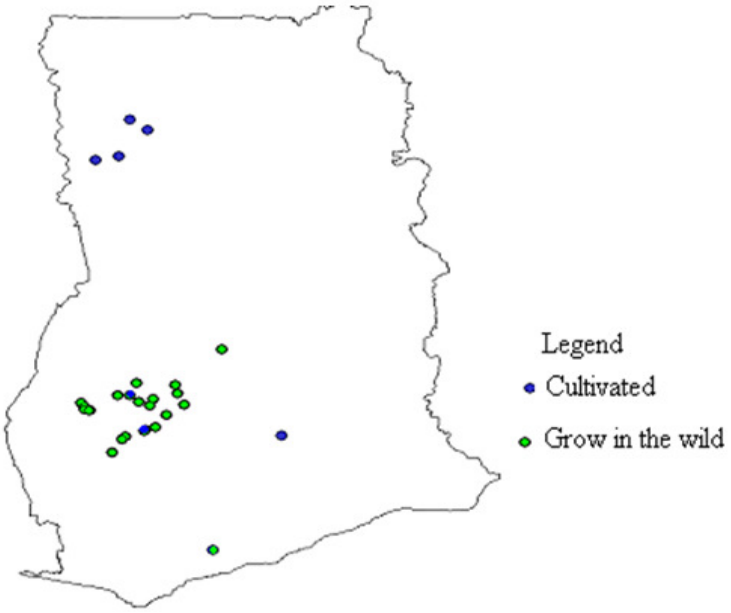

Figure 1: Distribution of *Dioscorea bulbifera* sampled areas in Ghana

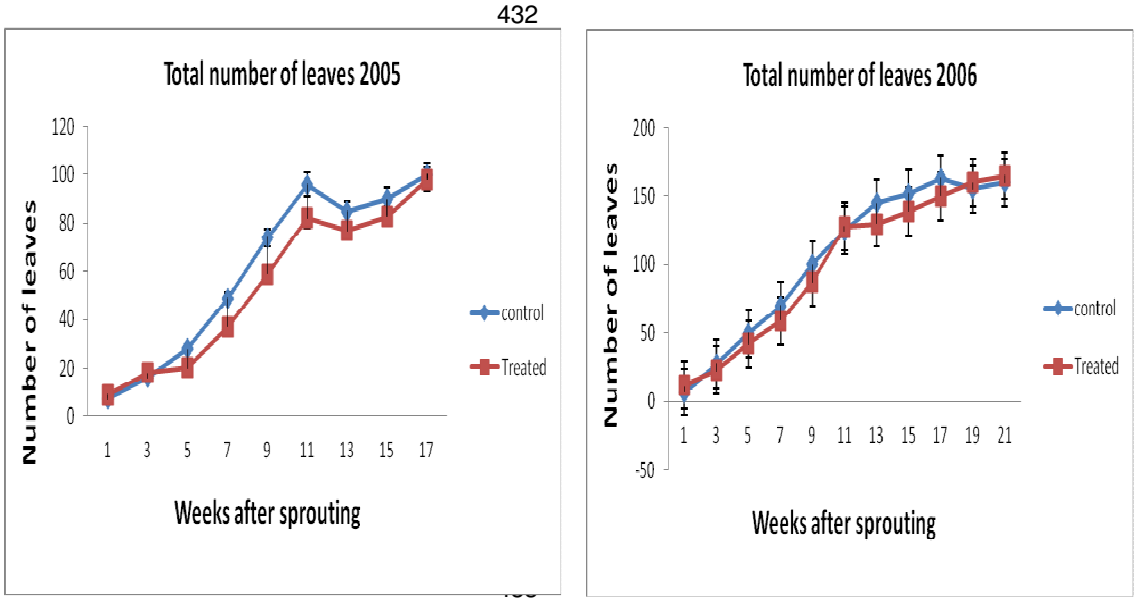

Figure 2. Effect of Cymethoate Super and water on mean number of insect-damaged leaves of *D. bulbifera* in the field at Kwadaso, Kumasi, Ghana

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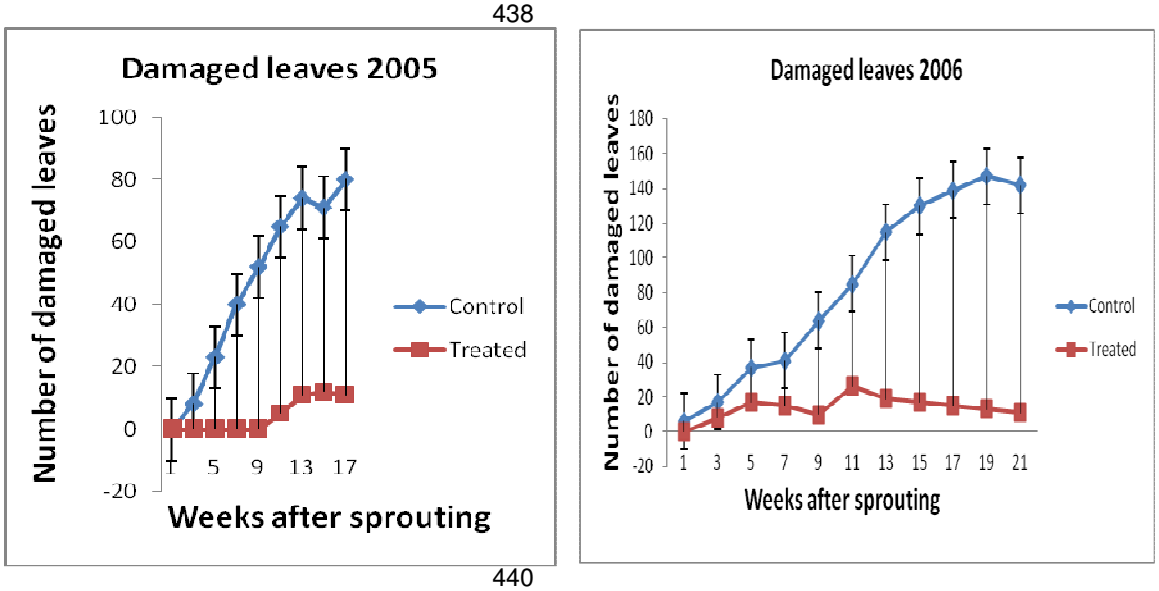

Figure 3. Effect of Cymethoate Super and water on mean number of insect-damaged leaves of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana.

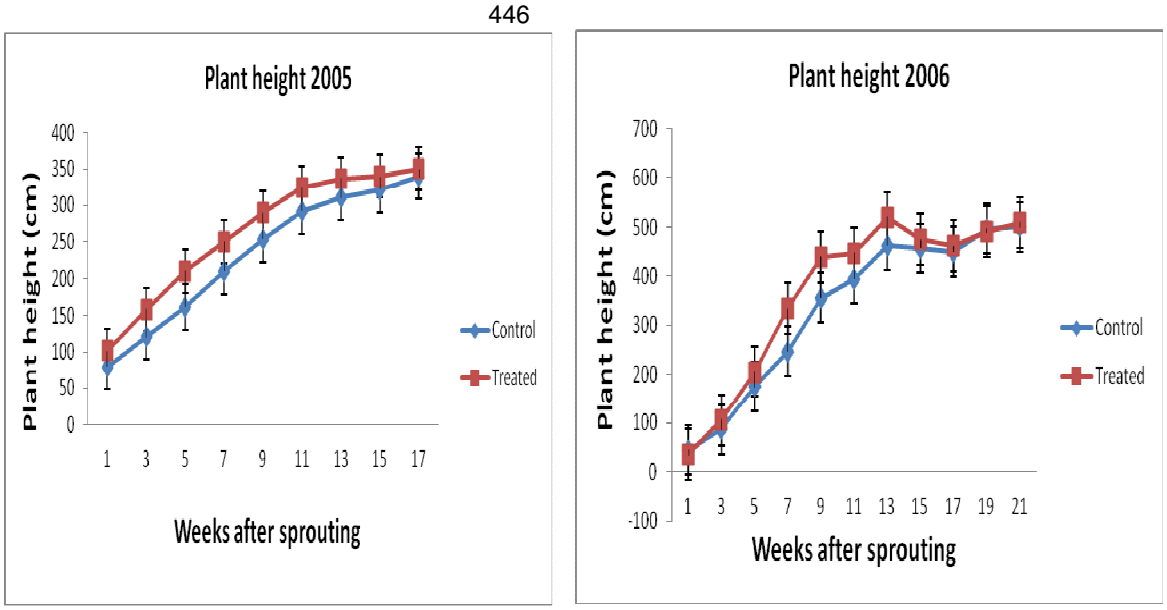

Figure 4. Effect of Cymethoate Super and water on mean plant height (cm) of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana

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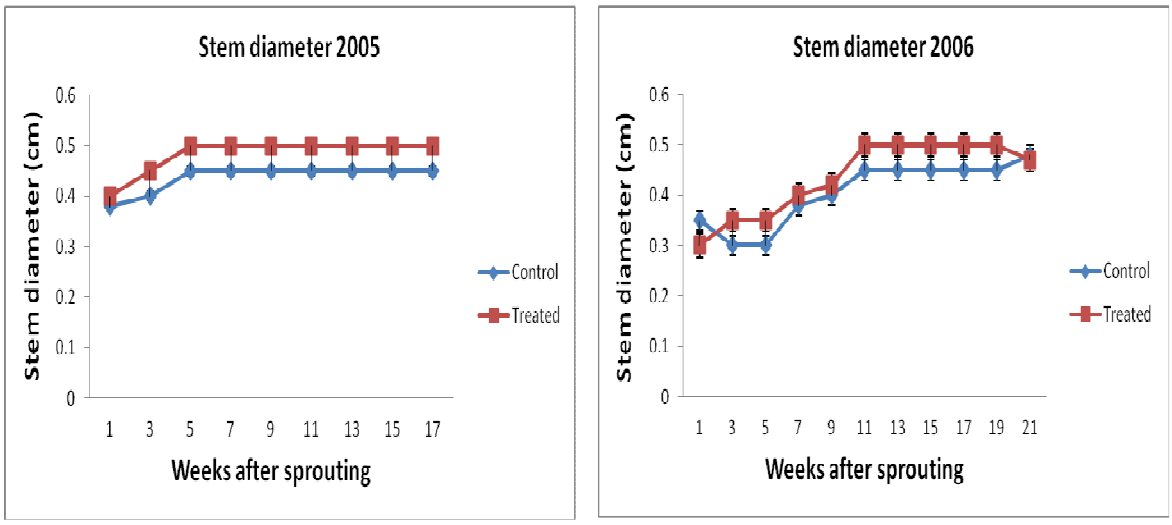


Figure 5. Effect of Cymethoate Super and water on mean stem diameter (cm) of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana.

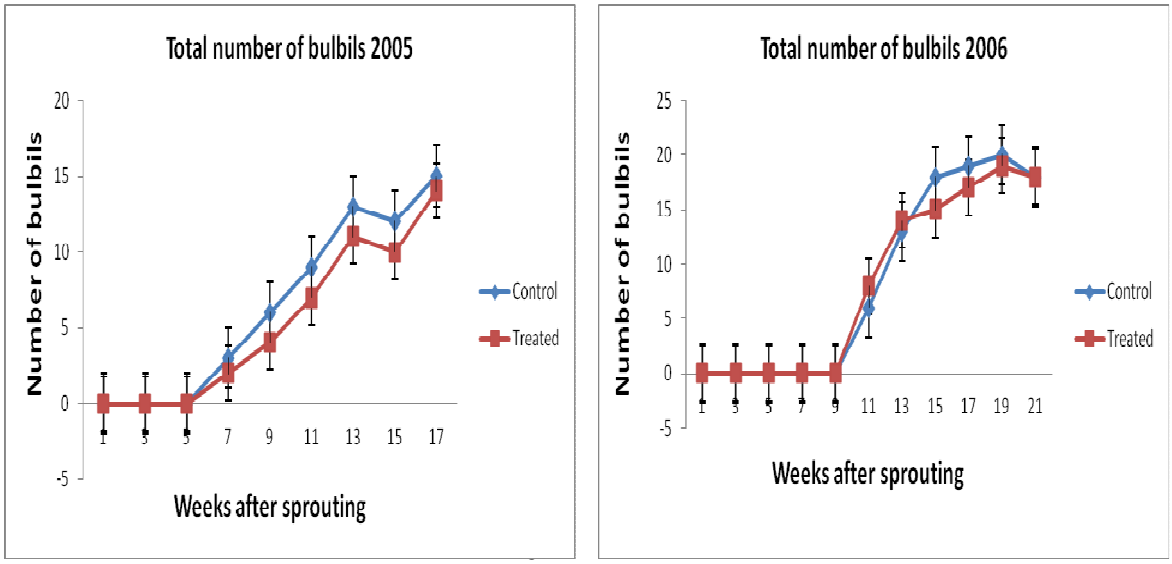


Figure 6. Effect of Cymethoate Super and water on mean number of bulbils of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana

UNDER PEER REVIEW

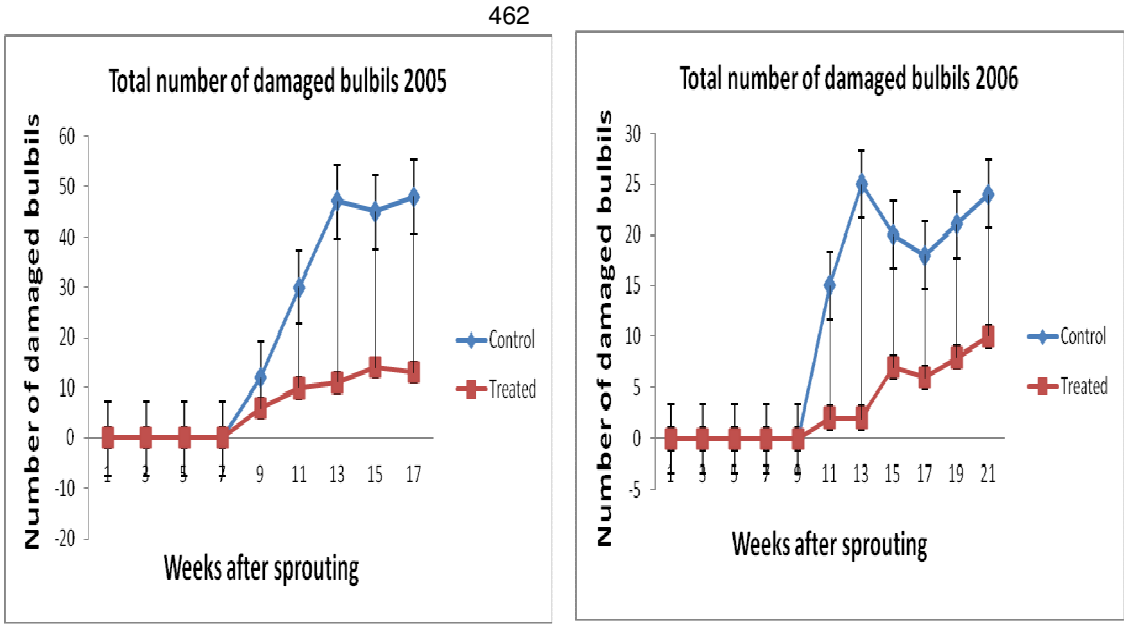


Figure 7. Effect of Cymethoate Super and water on mean number of damaged bulbils of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana.

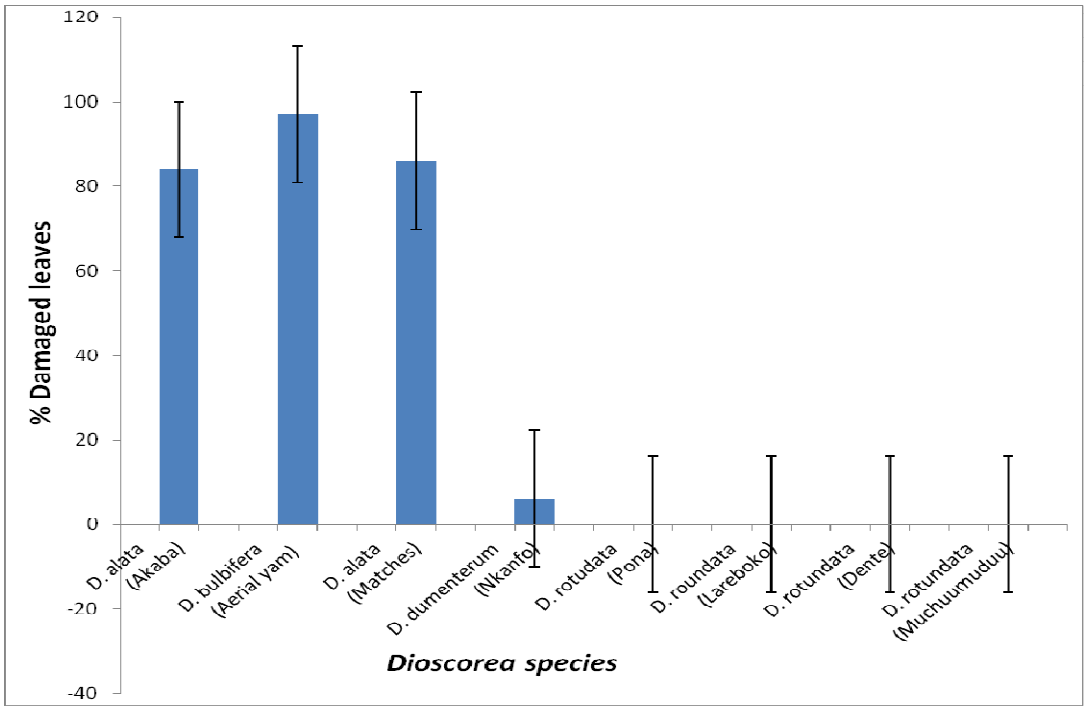
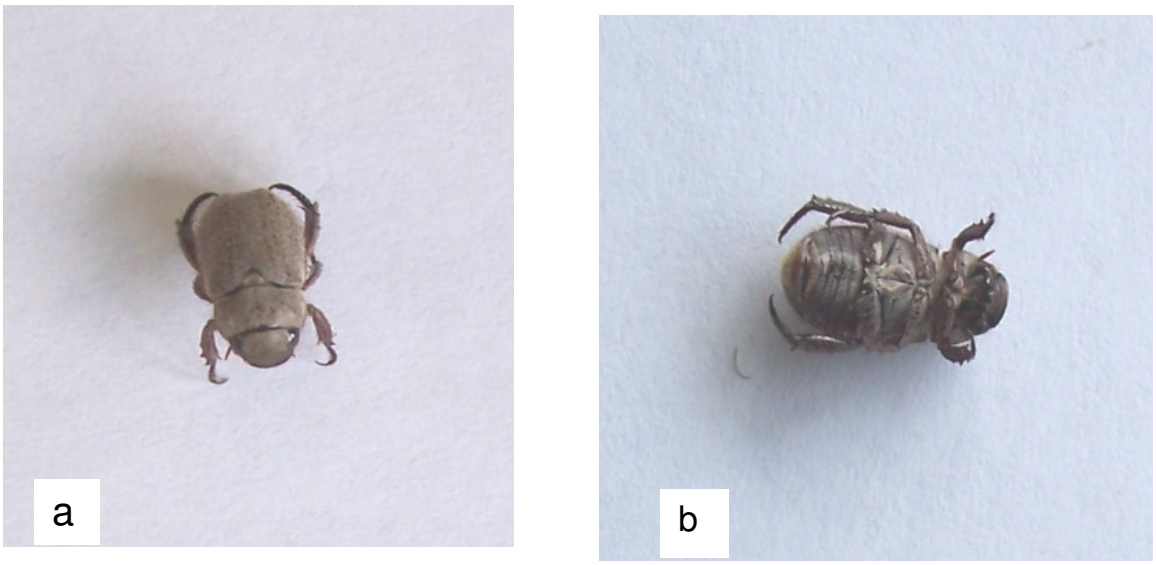


Figure 8. Host preference of *Anomala* sp. within the Dioscoreaceae

UNDER PEER REVIEW

Plate 1 (a). Field for insecticide exclusion studies Plate 2 (b). (Close up photograph)



UNDER PEER REVIEW

Plate 2. Dorsal view (a) and ventral view (b) of *Anomala* sp. observed on leaves of *D. bulbifera* and *D. alata* at Kwadaso, Kumasi, Ghana..

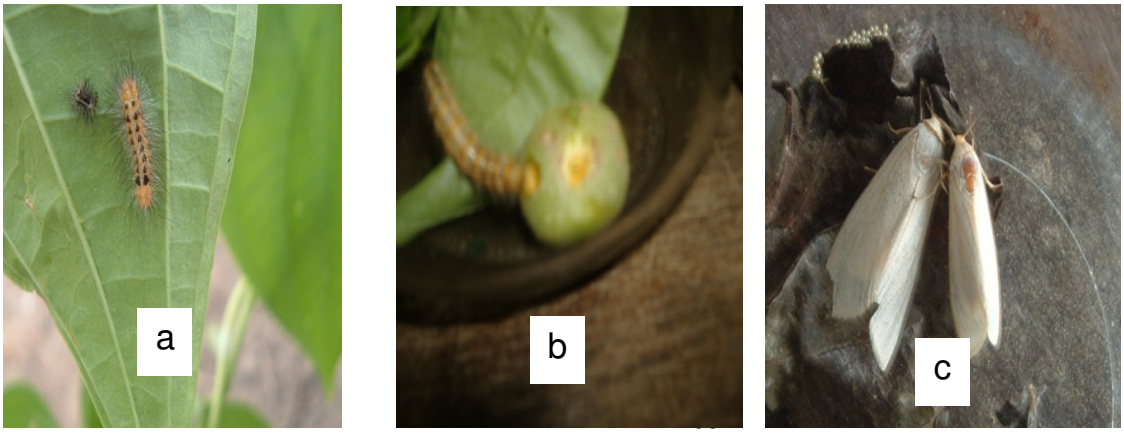


Plate 4. Larva of *Estigmene* sp. feeding on (a) leaves, on (b) bulbils of *D. bulbifera* and (c) male (right) and female (left) adults.

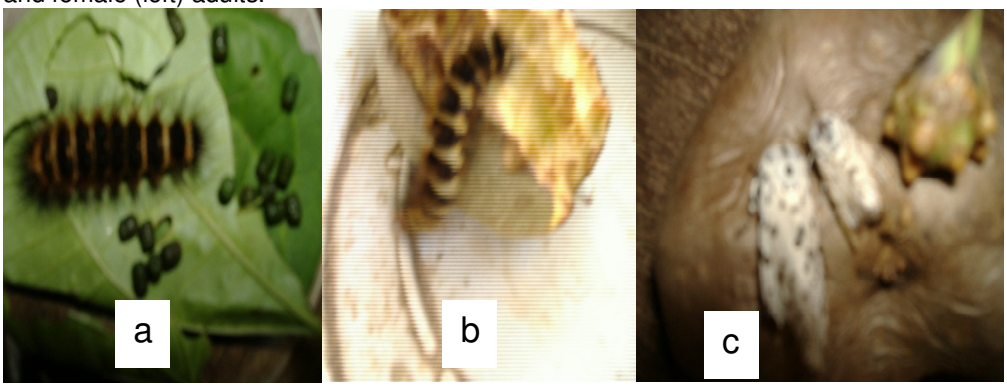


Plate 5. Larva of *Diacrisia* sp. feeding on (a) leaves, on (b) bulbils of *D. bulbifera* and (c) male (right) and female (left) adults