

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

ABSTRACT

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. The purpose was to identify potential biological control agents for this plant invading natural and protected areas in Florida, USA. The survey covered five regions based on herbarium records from three academic institutions in Ghana. A total of 40 phytophagous insect species in 9 orders were encountered on the plant with 24 species attacking *D. bulbifera* in two feeding guilds foliage and bulbil in Ghana. 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 *D. alata* and *D. bulbifera*. Even though the damage it caused to aerial yam was relatively small, it exhibited 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. They however exhibited a wide host range attacking other *Dioscorea* species and therefore do not appear to be good biological control candidates.

Keywords: *invasive, aerial yam, evaluation, Estigmene, 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 tropical Africa and Australia [1, 2, 3]. Whereas [4] believe that it is native to Asia and was subsequently introduced into Africa.

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 [5; 6]. 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 [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 [7, 8, 9,]. *D. bulbifera* was introduced to the Americas from Africa during the slave trade [2] and specifically into Florida in 1905 for scientific study and now constitutes one of the most aggressive weeds ever introduced to Florida and currently a widespread weed throughout many southern states [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).

Because of its underground tuber and abundant bulbils (aerial tubers), it is difficult to eradicate [12] and according to Duxbury *et al.* [13], 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 [7]. 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 [7] 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.* [14], 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 [1]. Ayensu and Coursey [15] 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 [16]. 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 [16]. 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 as stated by [1] as one of the native ranges in Africa . 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 wild and cultivated aerial yams are found in Ghana from May 2004 to August 2004. Wild types were predominantly found 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. Farms of respondents were visited in daytime and night with LED lanterns. In each farm 5 plants were visually assessed and where insects were available collections were made. Additionally, the coordinates of locations where *D. bulbifera* were sampled were 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. 6° 42N; Long. 1° 40W; 262m above sea level). It was repeated 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 as control and no protection treatments in three replications for each. 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. 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 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.

2.3 Data collection of exclusion experiment

Data were taken on five plants in systematic sampling. On each sampled plant, all healthy as well as damaged leaves were counted. 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 Vernier caliper. Percent defoliation was scored at the upper, middle and lower portions of each plant on a scale of 1 – 4, where a score of 1 indicates 25% of total leaves perforated or damaged by insects, 2 represents 50% damaged leaves, 3 represents 75% of leaves damaged and 4 represents all leaves showing perforations and damage by insects. Number of bulbils damaged was also recorded. Plant biomass (dry weight of vines) was assessed at harvest. Performance of the plants under unprotected and protected (control) treatments were then compared.

2.4 Catalogue of insect herbivores

All invertebrate herbivores that visited the plants were recorded 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 LED lantern L1000 6 W. 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 noted by observing part of plant mostly attacked by insects. 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. Collected live samples were kept in Kilner jars and fed with bulbils and detached leaves of *D. bulbifera* and other *Dioscorea* species in laboratory investigation.

2.5 Data Analysis

Data were analyzed 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 [18].

3. RESULTS

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

Communities in all the five regions visited during the survey are shown in Table 1.

Out of the five regions visited, only the Central region recorded wild types of aerial yams. In the Kakum forest of 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.

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 mostly found along the edges of 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 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, Mantodea, 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 did not show any significant differences ($P \leq 0.05$). Damage to leaves was significantly greater in the unprotected plots than the protected plots (Figure 3). 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. Number of damaged bulbils from 11 to 21 weeks showed significant differences for both years of cultivation (Figure 4).

Two members of the family Scarabaeidae, *Anomala* sp. and *Adoretus pulus* that caused damage to the leaves of *D. bulbifera*, previously reported in earlier studies by CRI scientists [16] were encountered. The insecticide exclusion protected the plants from their damage (Plate 1). On the contrary, the unprotected plots experienced feeding damage by the scarabs (Plate 2). They fed on *D. bulbifera* and *D. alata*, however, there was no infestation on leaves of *D. rotundata*. Their selective feeding on *D. bulbifera* leaves in the mist *D. rotundata* yams is an indication of their preference for *D. bulbifera* (Plate 3). 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 5).

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*. 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 at 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 [12]. Similarly in Malaysia Lepidoptera larvae were found feeding on aerial yam.] Results gathered from the exploratory survey suggested that several factors could account for the non pestiferous nature of the aerial yam in Ghana. Among these factors are insects as well as rodents which might consume fallen bulbils (Abubakari Lansah personal comm.).

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 [19]. 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. and *Adoretus pullus* were observed to have caused damage to the leaves of *D. bulbifera* and *D. alata*, however, there was no defoliation on leaves of *D. rotundata*. Laboratory investigations I carried out as part of the study confirmed the results obtained from the field as regards the non-pestiferous nature of the beetles on the *D.*

rotundata yams (Figure 5). 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; 20].

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 need 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 [21; 22]. The larval host range in the laboratory was broader than the field host range for both Lepidoptera species. This situation according to Harris [23] arises when the field host range is determined by adult habit and host finding requirements. According to Thompson *et al.* [24], 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 [23]. 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 [23]. 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 (Adama et al Unpub.).** Larvae of both Lepidoptera species completely devoured all the *Dioscorea* species provided them and successfully completed their development on leaves and bulbils. *Estigmene* and *Diacrisia* species even though exhibited high consumption preference for bulbils, the main source of propagation and spread do not appear to be good biological control candidates.

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	Presence/ Absence of <i>D. bulbifera</i>	Other <i>Dioscorea</i> species found	insect feeding on <i>D. bulbifera</i>	Part of plant attacked
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	-
	Hiayeaninguase*	Present	Yes	Yes	Leaf
Eastern	Buokukruwa*	Present	No	No	-
	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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388 Table 2. Invertebrate herbivores collected and other attributes from *D. bulbifera* in Ghana during the study
 389 period of 2004 – 2006

390 Insects Order	391 Family	392 Species	393 Stage	394 Part damaged	395 Specificity	396 Remarks
397 Coleoptera	398 Scarabaeidae	399 <i>Anomala</i> sp.	400 Adult	401 foliage	402 y Agent 1	
	403 Scarabaeidae	404 <i>Adoretus pullus</i>	405 Adult	406 foliage	407 y Agent 2	
	408 Scarabaeidae	409 <i>Pachnoda cordat</i>	410 Adult	411 bulbils	412 x	
	413 Bruchidae	414 <i>Callosobruchus maculatus</i>	415 Adult	416 unknown	417 b	
	418 Chrysomelidae	419 <i>Ootheca mutabilis</i>	420 Adult	421 foliage	422 b	
	423 Chrysomelidae	424 <i>Podagrica uniformis</i>	425 Adult	426 foliage	427 b	
	428 Coccinellidae	429 <i>Coccinella septempunctata</i>	430 Adult	431 beneficial	432 c	
	433 Lagriidae	434 <i>Lagria villosa</i>	435 Adult	436 unknown	437 b	
	438 Lagriidae	439 <i>Lagria cuspidata</i>	440 Adult	441 unknown	442 b	
	443 Unidentified		444 Adult	445 unknown	446 b	
	447 Hemiptera	448 Pentatomidae	449 <i>Nezara viridula</i>	450 Adult	451 unknown	452 b
	453 Alydidae	454 <i>Riptortus dentipes</i>	455 Adult	456 unknown	457 b	
	458 Pyrrhocoridae	459 <i>Dysdercus superstitionis</i>	460 Adult	461 unknown	462 b	
	463 Alydidae	464 <i>Anoplocnemis curvipes</i>	465 Adult/Nymph	466 unknown	467 b	
468 Hemiptera	469 Aphididae	470 <i>Aphis craccivora</i>	471 Adult	472 foliage	473 x	
	474 Aphididae	475 <i>Brevicoryne brassicae</i>	476 Adult	477 foliage	478 x	
	479 Aleyrodidae	480 <i>Bemisia tabaci</i>	481 Adult	482 foliage	483 x	
	484 Aleyrodidae	485 <i>Aleurodicus dispersus</i>	486 Adult	487 foliage	488 x	
	489 Cicadellidae	490 <i>Empoasca fabae</i>	491 Adult	492 unknown	493 b	
	494 Pseudococcidae	495 <i>Phenacoccus manihoti</i>	496 Adult	497 foliage	498 x	
	499 Dermaptera	500 Forficulidae	501 <i>Forficula auricularia</i>	502 Adult	503 unknown	504 b
	505 Mantodea	506 Mantidae	507 <i>Sphodromantis viridis</i>	508 Adult & Nymph	509 beneficial	510 c
	511 Mantidae	512 <i>Mantis religiosa</i>	513 Adult & Nymph	514 beneficial	515 c	
	516 Diptera	517 Muscidae	518 <i>Musca domestica</i>	519 Adult	520 unknown	521 b
522 Orthoptera	523 Pyrgomorphidae	524 <i>Zonocerus variegatus</i>	525 Adult	526 foliage	527 x	
	528 Acrididae	529 Not identified	530 Adult	531 unknown	532 b	
	533 Tettigidae	534 Not identified	535 Adult	536 unknown	537 b	
	538 Tettigonidae	539 Not identified	540 Adult	541 foliage	542 x	

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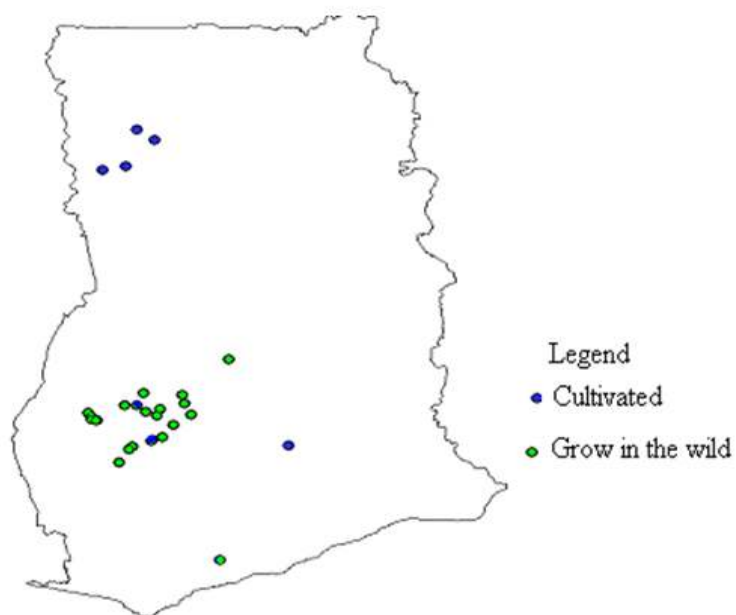


Figure 1: Distribution of *Dioscorea bulbifera* sampled areas in Ghana



Plate 1 Insecticide treated leaves of *D. bulbifera*

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449 Plate2 Untreated, leaves damaged by Scarabs

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456 Plate3 Selective damage of *D. bulbifera* leaves

457 within *D. rotundata* leaves on a common stake.

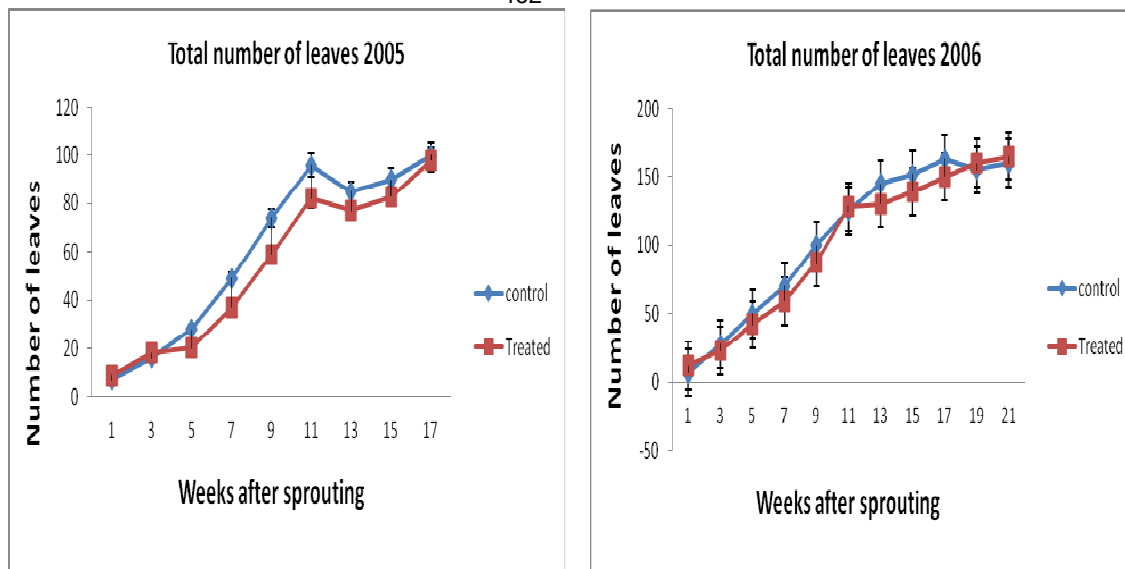
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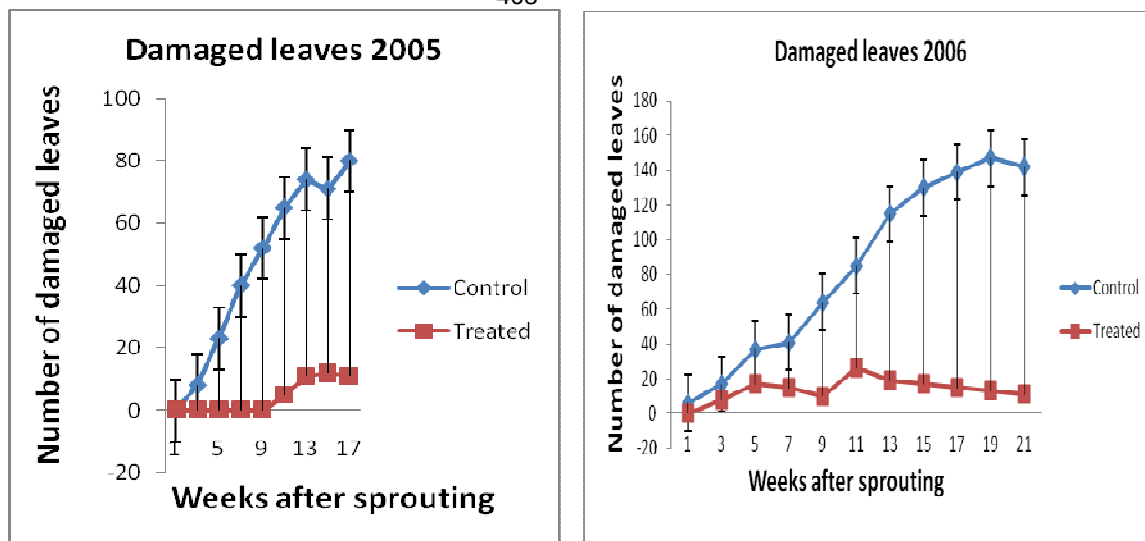
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464 Figure 2. Effect of Cymethoate Super and water on mean number of insect-damaged leaves of *D. bulbifera* in
 465t the field at Kwadaso, Kumasi, Ghana
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471 Figure 3. Effect of Cymethoate Super and water on mean number of insect-damaged leaves of *D. bulbifera* in
 472 the field, Kwadaso, Kumasi, Ghana.
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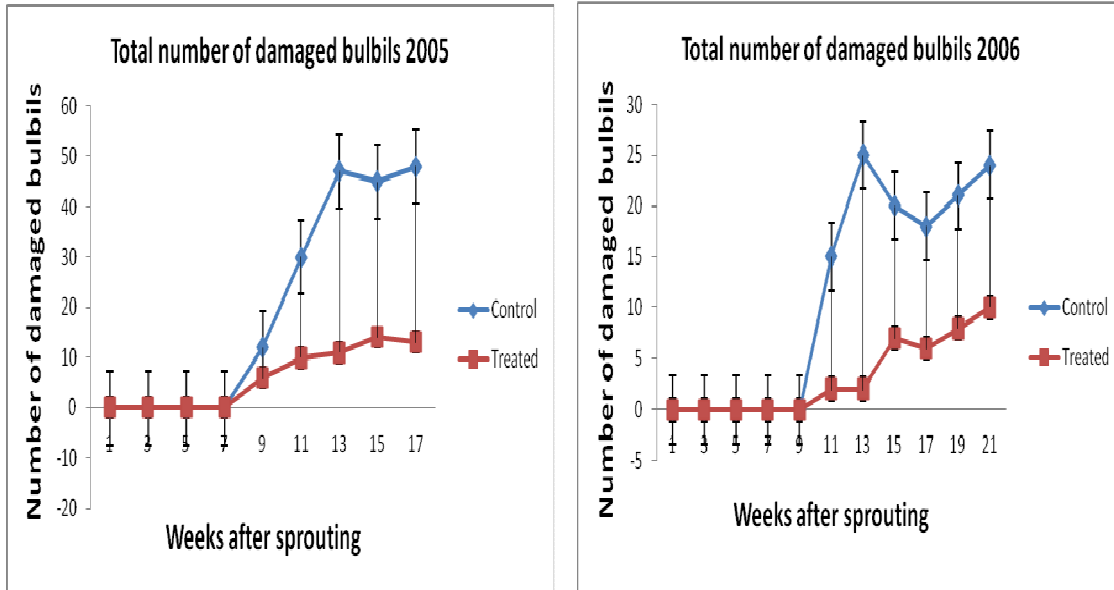


Figure 4. Effect of Cymethoate Super and water on mean number of damaged bulbils of *D. bulbifera* in the field, Kwadaso, Kumasi, Ghana.

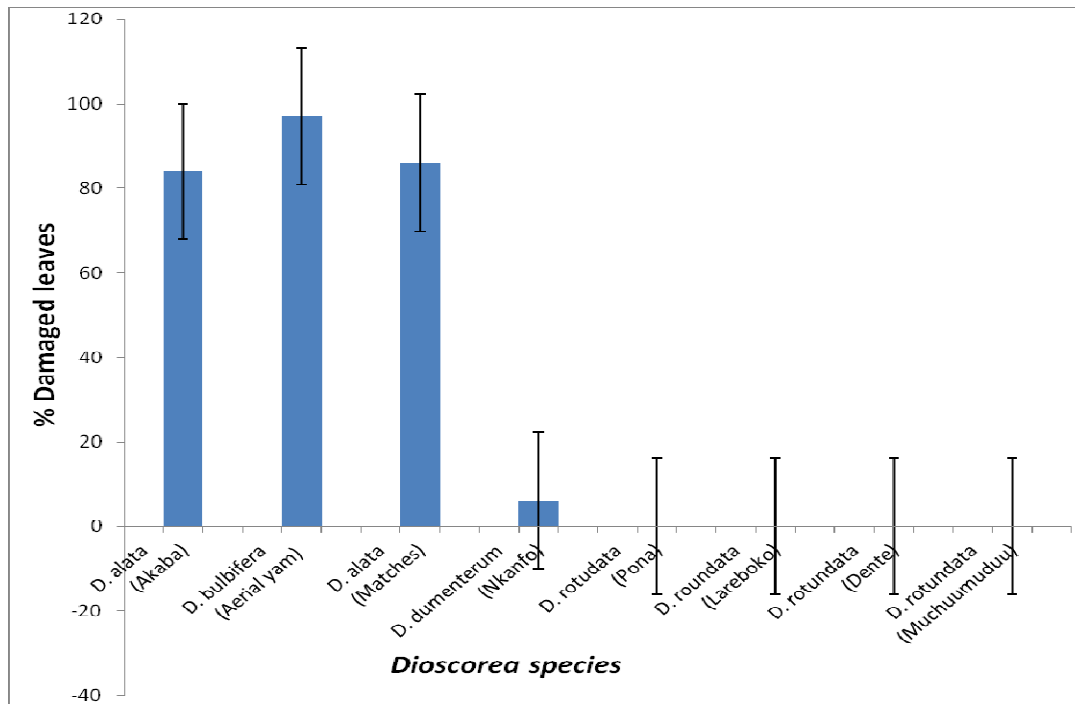


Figure 5. Host preference of *Anomala* sp. within the Dioscoreaceae

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