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Arthropods Associated with *Amaranthus hybridus* in Southwestern Nigeria and Aggregation Patterns of *Gasteroclisus rhomboidalis*, *Hypolixus nubilosus* (Coleoptera: Curculionidae) and Brown Marmorated Stink Bug, *Halyomorpha halys* (Hemiptera: Pentatomidae) in Relation to Host's Morphology

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors YIU and MOAO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To generate data on arthropod communities associated with grain amaranth, *Amaranthus hybridus* (Caryophyllales: Amaranthaceae) within a single organic cropping system in Southwestern Nigeria and to study arthropod-host interactions which can be potentially exploited in cultural management.

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Place and Duration of the Study: The study was conducted from September to November, 2016 and repeated in December, 2016 to February 2017 during the dry season in Southwestern Nigeria.
Methodology: The farm area was divided into twenty plots containing four beds each. The total area of the field was approximately 180 m^2. Survey of arthropods on amaranth was commenced at six days after sowing and continued until maturity. Scheduled daily visits to the field was done at 6:00-9:00 am, 12:00 noon-3:00 pm and 6:00 pm-8:00 pm to scout for insects. At 40-45 days, when the flowering heads have developed,the height of 24 plants per plot were measured and ranked randomly into three height ranges (Tall, >100 cm; Intermediate, 50-100 cm; Short, <3 cm), using a marked meter stick. Another 24 plants per plot were classified into three stem girth ranges (Large, > 6 cm; Intermediate, 3-6 cm; Thin, <3 cm), using a calliper and the plants were tagged. Density of *Halyomorpha halys* on the inflorescence of the plants in the different height groups were sampled. Activities of borers, *Hypolixus nubilosus* and *Gasteroclisus rhomboidalis* mixed infestation on plants in the different stem girth ranges were assessed, using stem damage characteristics.
Results: Approximately, 41.7% of the total arthropods occurred during the vegetative stage while the remaining 58.3% occurred during flowering and fruiting. Beneficial insects, (25% of the total arthropod community) were predatory and they occurred during fruiting and flowering stages. The beneficial insects, *Dolichurus spp*., *Scelifron spirifex* and *Lasius niger* belong to the Order Hymenoptera. Other beneficial insects were *Dysnactus trigorus* (Diptera), *Cora marina* (Odonata) and *Mantis religiosa* (mantodea). *H. halys* was responsible for damage to the flowering heads, while the stems were attacked by *H. nubilosus* and *G. rhomboidalis*.
Conclusion: The borers showed behavioural preference in relation to stem girth of amaranth plants while *H. halys* preferred tall plants. The observed host preference behaviours can be useful in cultural management.

Keywords: Beneficial insects; host preference; Halyomorpha halys; mixed infestation; grain amaranth.

1. INTRODUCTION

Amaranthus hybridus is an important member of the family Amaranthaceae, cultivated as a leafy vegetable or grain amaranth in different parts of the world. It is a fast growing species that has a considerable tolerance for water stress, making it a choice of green vegetable in agro-ecological areas with low or medium rainfall distribution [1] In Nigeria, *A. hybridus* cultivated as a leafy vegetable is very crucial to food security in Nigeria and it is a source of income for low-income farmers because of its relatively short cycle of production. The small-scale farmers that are involved in its cultivation often leave a few stands in the field to fruit and produce seeds for the next production cycle. As far as we know, intensive cultivation of *A. hybridus* or other amaranth species as grain amaranth is yet to be adopted in Nigeria despite that results of studies on yield and quality performance of grain amaranth varieties in the Southwestern Nigeria [2] were promising. Also, it is known that entomological pests limit productivity of vegetables, but the effect of insect interactions on grain amaranth production have not been reported in Southwestern Nigeria.

Grain amaranth pilot farm was cultivated at the Teaching and Research Farms, Ekiti State University Ado-Ekiti to monitor arthropods that are associated with the crop. Successful grain amaranth production is expected to increase the range of horticultural crops that can be cultivated in the Southwest, as alternative crop to support food security. However, economic damage occurred due to severe pest invasions. The crops were ravaged by borers that attacked stems and caused snapping or abortion of fruiting. The flowering heads were also attacked heavily by stink bugs.

Management of the borers using insecticides could be a challenge after the stems had been invaded and unacceptable pesticide residues that are often left in fresh vegetables after intensive use of inorganic chemicals [3,4] was also a serious concern. Presently, non-chemical management option for the major pests of grain amaranth in Nigeria is yet to be established.

Insect pests that are potential threats to leaf amaranth production in Nigeria have been reported in several studies [5,6] but information on effects of insect pests in grain amaranth production is rare, except for the report on agro-ecological variations in growth and development of grain amaranth in Nigeria [7], which covered some aspects of pest occurrence. The conventional cultural management of vegetable pests by farm sanitation (destruction of crop left overs that serve as breeding grounds) may be ineffective when applied to grain amaranth, because of diverse alternative weed-hosts that are capable of relaying pests into new production cycles [8] In addition, vegetable farmers are often reluctant to use chemical insecticides, considering human and environmental safety and the cost of production. Grain amaranth stay longer in the field (50-70 days), thereby exposing the crop to a wider range of pests during the production cycle compared with leaf amaranth (14-21 days).

It has been observed that the vegetative, flowering and fruiting stages of amaranth are attacked by different pests in a succession, but this has not been scientifically documented within agro-ecological regions of Nigeria. Most studies on amaranth pests had focused on occurrence, abundance and control, without paying attention to important aspects of bioecology of pests, innate behaviours, habits and interactions with the host. Data of stage-by-stage pest occurrence could be a useful primary information for developing sustainable, environmentally supportive cultural management plans and decision making on pest thresholds, at which initiation of a control action would be justified. Lack of accurate information on habits of pests, basic biology and ecology may have serious consequences in the development of Integrated Pest Management protocols. The objectives of this report therefore, were to (a) present the data of stage-by-stage occurrence of arthropods on *A. hybridus* during vegetative, flowering and fruiting stages (b) document major pests that could be potential targets of control programmes in grain amaranth and beneficial insects species, which can be conserved or exploited for inundative biological control (c) to report pest-host interactions and habits of some of the major pests identified at the flowering and fruiting stages. These kind of data could be useful for developing novel cultural control within grain amaranth cropping systems.

2. MATERIALS AND METHODS

2.1 Description of Experimental Site

The study was carried out at the Teaching Research Farms, Ekiti State University Ado-Ekiti, Nigeria (7.6124° N and 5.2731° E), from September to November, 2016 and repeated between December, 2016andFebruary 2017 during the dry season, under irrigation. The study area has an average temperature of 27°C and there are wide fluctuations between day and night. The wet season is usually from April – October, with bimodal rainfall pattern which peaks in June and October, while the dry season is from November to March.

2.2 Experimental Design

The land was cleared and plant debris were packed before preparation of beds measuring 2 x 2 m^2 with inter-bed spacing of one meter. The farm area was divided into twenty blocks containing four beds Each. The total farm area was approximately 180 m^2.

2.3 Calculation of Seed Sowing Rate

Crop Density, CD (= number of seeds to be sown per square meter) was determined from equivalent weight in relation to pre-estimated number of seeds in a gram, using the proposed formula for standardizing the seed rate of amaranth, Uwaidem and Borisade (2017), here summarized: $E\ (g) = \frac{W}{NS} x \frac{R}{1}$, where E = Equivalent weight (g), W=Weight of 1 g amaranth seed, NS = Counted number of amaranth seed g^{-1}, R= Required number of plants per bed. One seed of the amaranth used in the current study weighed 0.000441 g. Thus, considering an average crop density of 200 stands m^{-2}, approximately 0.08882 g of the amaranth seeds were sown per bed.

2.4 Method of Sowing

Dry sand was passed through 0.5 mm mesh and 100 g of the fine sand was mixed with the seed to ensure even seed distribution during sowing. A plastic container with a tight fitting lid (100 mL) was modified for sowing the seeds by creating pin-sized perforations (~ 1 mm) within a pre-marked 3.0 cm^2 circle on the lid. The sand-seed mixture was poured into the plastic and used manually to distribute the seeds along1-5 cm deep rows made on the beds and covered lightly with soil. The beds were irrigated as required using water hose pipe and shower-head during afternoon periods until fruiting.

2.5 Classification of Plants into Similar Stem Girth and Height Ranges

At 40-45 days, when the flowering heads had developed, the plants were ranked into three height ranges, using a marked one-meter stick for measurement and three stem girth ranges, using a caliper and the plants were tagged (Table 1). Different ranked groups consisted of twenty four randomly selected plants from each plot. Aggregation of insects on the inflorescence in relation to height of the plants and severity of activities of stem borers in relation to stem girth were evaluated.

2.6 Assessment of Pest Profile

Visual survey of insect pests on the amaranth was commenced at six days after sowing and continued until maturity. Scheduled daily visits to the field was done in the morning (6:00-9:00 am), afternoon (12:00 noon-3:00 pm) and evening (6:00 pm-8:00 pm), to scout for insect pests. Insect samples were collected and brought into the Agricultural Entomology Laboratory of the Crop Protection Unit, Faculty of Agricultural Sciences, Ekiti State University Nigeria, for identification. Thereafter, the pests were sorted into different Orders and families and the periods of occurrence were classified into vegetative, fruiting and flowering. The insects which were responsible for damage to stem and flowering heads were identified. Pest counts on plants within the same height range were recorded at intervals of 2 days, starting from 45-60 post planting. Sixty days old plants within the same stem girth range were uprooted and perforations (entry and exit holes of borers) were counted along the entire stem, using a hand lens and a marker to avoid error of double counting. The number of holes on the stem was used for assessment of stem damage. The nature of internal damage was assessed by cutting a longitudinal section through the stem to reveal insect galleries and feeding activities of larvae. Snap-shots of the insect activities within the stem were processed into JPEG files and presented as photographic data.

2.7 Statistical Analysis

The number of individual arthropods which occurred at the vegetative, flowering and fruiting stages were expressed as percentages of the total number of arthropods recorded on the amaranth through the entire production cycle of sixty to seventy days. Relative numbers of arthropod pests and beneficial insects from different Orders were expressed in percentage and presented using bar graphs. The data of pest count on plants which were grouped into different height ranges and the data of damage to stem (counted holes on stems within different stem girth ranges) were analysed for compliance with the requirements of a parametric statistical procedure and thereafter subjected to analysis of variance (ANOVA). Where significant differences were found, means were separated using Tukey's Honestly Significant Difference (HSD) ($P<0.05$). All the data were analysed using IBM SPSS Statistical Package 23® and graphs were drawn using Microsoft Excel 2013.

Table 1. Ranking of *A. hybridus* into similar stem girth and plant height ranges

Stem girth	**Range (cm)**
Large	>6
Intermediate	3-6
Thin	<3
Plant height	**Range (cm)**
Tall	>100
Intermediate	50-100
Short	<50

4. RESULTS

4.1 Pest Profile and Nature of Damage by Stink Bugs and Stem Borers

Table 2. shows the pest profile of *A. hybridus* recorded from seven days after sowing up till the end of the fruiting period. Twenty three insects and one species of red mite, *Tetranycus* sp occurred on the amaranth. Approximately, 41.7% of the total arthropods were recorded at the vegetative stage while the remaining 58.3% occurred during flowering and fruiting. Beneficial insects, which constituted 25% of the arthropods were predatory and they occurred during fruiting and flowering stages. Three of the beneficial insects, *Dolichurus spp*., *Scelifron spirifex* and *Lasius niger* belong to the Order Hymenoptera. Other beneficial insects were *Dysnactus trigorus* (Diptera) *Cora marina* (Odonata) and *Mantis religiosa* (Mantodea).

The brown marmorated stink bug, *Halyomorpha halys* was responsible for the damage to the flowering heads of the amaranth, while stems were attacked by the pig weed weevil, *Hypolixus nubilosus* and amaranth stem borer, *Gasteroclisus rhomboidalis* in a mixed infestation. The activities of stem borer larvae (Fig. 1) caused damage to the transport system of the plants and paved way for microbial infection of stem tissues and eventual wilting or snapping, depending on the severity.

Table 2. Occurrence of arthropods on *A. hybridus* cultivated as grain amaranth in Southwestern Nigeria

S/N	Common name	Scientific name	Order	Family	Activity	Period of occurrence/percentage of total arthropods recorded
1	Angle-Wing Katydid	*Microcentrum rhombifolium*	Orthoptera	Tettigoniidae	Foliage feeder	Vegetative stage
2	Beet webworm moth	*Spoladea recurvalis*	Lepidoptera	Crambidae	Foliage feeder/ web spinner	(41.7%)
3	Green stripped grasshopper	*Chortophaga viridifasciata*	Orthoptera	Acrididae	Foliage feeder	
4	Katydids	*Tettigonia viridissima*	Orthoptera	Tettigoniidae	Foliage feeder	
5	Leaf miner fly	*Liriomyza sp.*	Diptera	Agromyzidae	Foliage feeder	
6	Moth	*Psara basalis*	Lepidoptera	Crambidae	Foliage feeder	
7	Reddish-orange ladybird beetle	*Epilachna elaterii*	Coleoptera	Coccinellidae	Foliage feeder	
8	Silverleaf whitefly	*Bemisia tabaci*	Hemiptera	Aleyrodidae	Sap sucker	
9	Slant faced grasshopper	*Orphulella speciosa*	Orthoptera	Acrididae	Foliage feeder	
10	Variegated grasshopper	*Zonocerus variagatus*	Orthoptera	Pyrgomorphidae	Foliage feeder	
1	Amaranth stem borer	*Gasteroclisus rhomboidalis*	Coleoptera	Curculionidae	Stem borer	Flowering/fruiting stages
2	Pig weed weevil	*Hypolixus nubilosus*	Coleoptera	Curculionidae	Stem borer	(33.3%)
3	Red mite	*Tetranycus sp.*	Trombidiformis	Tetranichidae	Sapsucker/web spinner	
4	Beet moth larvae	*Scrobipalpa ocellatella*	Lepidoptera	Gelechiidae	Borer/web spinner	
5	Brown mammorated stink bug	*Halymorpha halys*	Hemiptera	Pentatomidae	Sap sucker	
6	Crickets	*Velarifictorus micado*	Orthoptera	Gryllidae	Root feeder	
7	Darkling beetle	*Lagria villosa*	Coleoptera	Lagriidae	Foliage feeder	
8	Green stink Bugs	*Nezara viridula*	Hemiptera	Pentatomidae	Sap sucker	
	Beneficial insects					
1	Black cockroach-wasp	*Dolichurus spp*	Hymenoptera	Ampulicidae	Predatory	Flowering/fruiting Stages
2	Sphecid wasp (Mud dauber)	*Scelifron spirifex*	Hymenoptera	Sphecidae	Parasitoid	(25%)
3	Common black ants	*Lasius niger*	Hymenoptera	Formicidae	Predatory	**[Beneficial insects]**
4	Robber fly	*Dysnactus trigorus*	Diptera	Asilidae	Predatory	
5	Dragon fly	*Cora marina*	Odonata	Polythoridae	Predatory	
6	Praying mantids	*Mantis religiosa*	Mantodea	Mantoididae	Predatory	

4.2` Relative Abundance of Arthropods from Different Orders

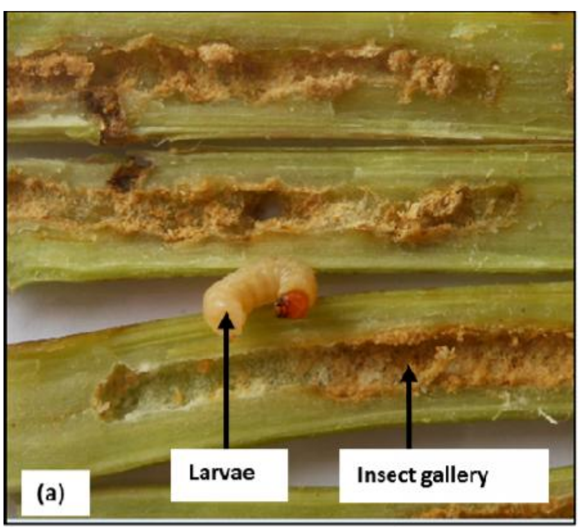
The most abundant pests were the Orthopterans and the Coleopterans, which represented 33% and 22% respectively, of the entire number of pests recorded in the field during the grain amaranth production cycle (Fig. 2). The relative abundance of Hemipterans and Lepidopterans were 17% each while 6% were from the Order Diptera and the mite, Order Trombidiformis was also 6%. Fifty percent of the beneficial insects were Hymenopterans while beneficial Dipterans, Odonata and Mantodea represented the remaining 50% in equal splits of approximately 17%.

4.3 Plant Height-modulated Pattern of Aggregation of Mammorated Stink Bugs

Plant height modulated aggregation pattern of mammorated stink bugs on *A. hybridus* inflorescence is shown in Fig. 3. Plant height significantly influenced the distribution and density of the stink bugs [F (2, 485.59) =7.312, P=0.016], such that taller plants harboured greater numbers. The trend followed a consistent pattern, where the highest number of bugs plant^{-1} were recorded on the tall plants (> 1 m height) (Fig. 3a), followed by the plant group with intermediate height (0.5-1 m). The lowest number of stink bugs were recorded on the short plants (<0.5 m). The mean density of the stink bugs decreased as the plants approached maturity. For example, the mean number of stink bugs on the tall plant group at the first sampling (plant age=45 days) was 8.7 bugs plant^{-1} but decreased to 1.7 bugs plant^{-1} when the fruits were fully ripe for harvest at 65 days. The mean percentage bug density from the pooled data of the first and the second pest assessment studies (Fig. 3b), showed that 66.5% of the brown marmorated stink bug populations aggregated on the tall plant groups in the field, while 28.2% and 5.3% occurred on the intermediate and the short plant groups respectively.

4.4 Stem Girth-modulated Activities of Borers

There were significant variabilities in the activities of stem borers, indicated by the number of holes created by ovipositing females and the number of adult exit holes, in relation to stem thickness, F(2, 33)= 22.103, P=0.0001. Longitudinal sections of the stems showed that the galleries created by the internally feeding larvae were more in the large stems (Stem girth> 6 cm) compared to the intermediate (Stem girth, 3-6 cm) and the thin stems, Stem girth <3 cm (Data not shown). A mixed infestation of two stem borer species, *G. rhomboidalis* and *H. nubilosus* were responsible for the damage to the amaranth stems. The recorded data on activities of stem borers during the first and the second experimental trials were comparable, therefore, the two data sets were pooled. The average number of holes on the large stems (Fig. 4), was approximately, 21 holes plant^{-1}, intermediate stems (16 holes plant^{-1}) and the thin stems (11 holes plant^{-1}).



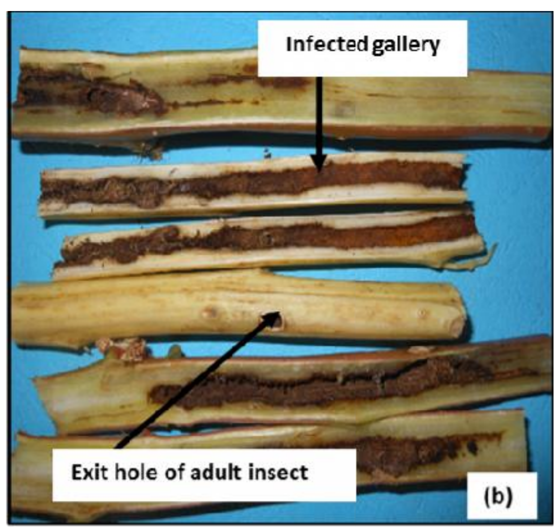


Fig. 1. Damage to amaranth stem showing (a) stem borer larvae and burrowing activities (b) decaying transport tissues and adult exit hole

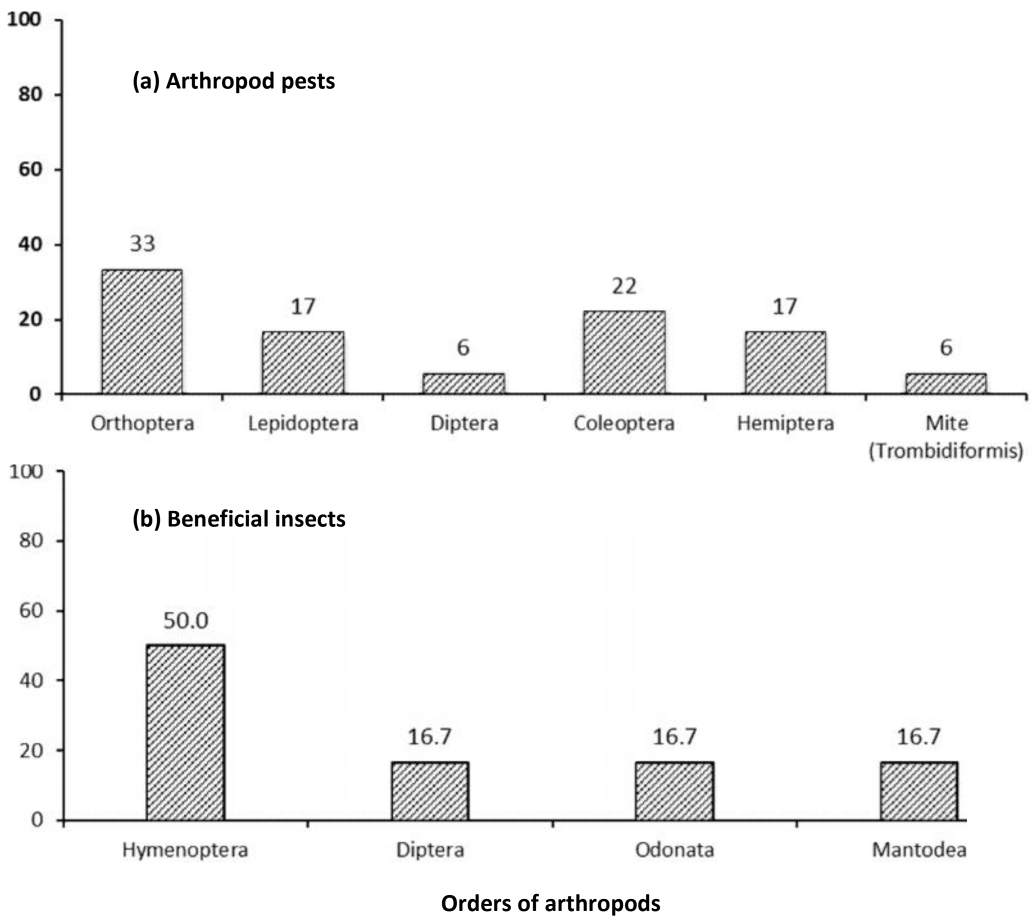


Fig. 2. Relative abundance of arthropods from different Orders (a) arthropod pests (b) beneficial insects

5. DISCUSSION

This preliminary study reported arthropod communities associated with *A. hybridus,* cultivated as grain amaranth in the Southwestern Nigeria, and described host-pest interactions, which can be potentially exploited in the design of a sustainable cultural component of an Integrated Pest Management (IPM) for some of the major pests in grain amaranth. The work focused on Orders and Families of arthropods that invaded the amaranth from early leaf stage to the end of fruiting within a single organic agro-ecosystem in the Southwestern Nigeria. The recorded pests during the vegetative stage constituted approximately 42% of the total arthropods and they were foliage feeders, except the silverleaf whitefly (*Bemisia tabaci*)-Hemipteran pest and sap-sucker. Fifty eight percent of the arthropod communities were associated with the flowering and fruiting stages, among which 25% were beneficial insects. The beneficial insects were predatory species and mainly Hymenopterans. The sphecid wasp (*Scelifron spirifex*) which is also a Hymenopteran was the only parasitoid among the beneficial insect groups.

The pest profile of amaranth reported in this study is similar to earlier records in other parts of Nigeria [9] and West Africa [10] that share resemblances in climate, but there is no data to compare the period of occurrence of the individual pest species and the beneficial insects during the Vegetative, flowering and fruiting stages. The stem borer complex, *Gasteroclisus rhomboidalis* and *Hypolixus nubilosus,* causing economic damage to grain amaranth in a mixed infestation is being reported for the first time in Nigeria. However, *H. nubilosus* has been reported as a major pest of amaranth in many parts of Southwestern Nigeria [11] and West

Africa [10]. Damage to stem and leaves by *H. nubilosus* resulting in up to 27% yield loss had been reported in many species of amaranth [12], but there is no information on assessment of damage to grain amaranth due to *G. rhomboidalis* attack.

Data of arthropod communities and the timing of appearance of individual pest species associated with amaranth within the agro-ecosystem being reported, could be useful in the design of local pest response plans. The information on the pest status of the different arthropods could also be useful in identification of those which could be perfect targets of control programs. The major pest groups, capable of causing economic damage to commercial-scale grain amaranth production within the agro-ecological area are the stem borers, *G. rhomboidalis* and *H. nubilosus* and the brown marmorated stink bugs.

The burrowing activities of the stem borer larvae caused damaged to the transport system of the plants and allowed fungal colonization of the tissues, which resulted into wilting and snapping. Adults borers were cited on stems, with their elytra covered with frass, indicating they were newly emerged adults that bred within the farm and they occurred in all the plots in large numbers. Longitudinal sections through the stems also revealed the presence of the stem borer larvae.

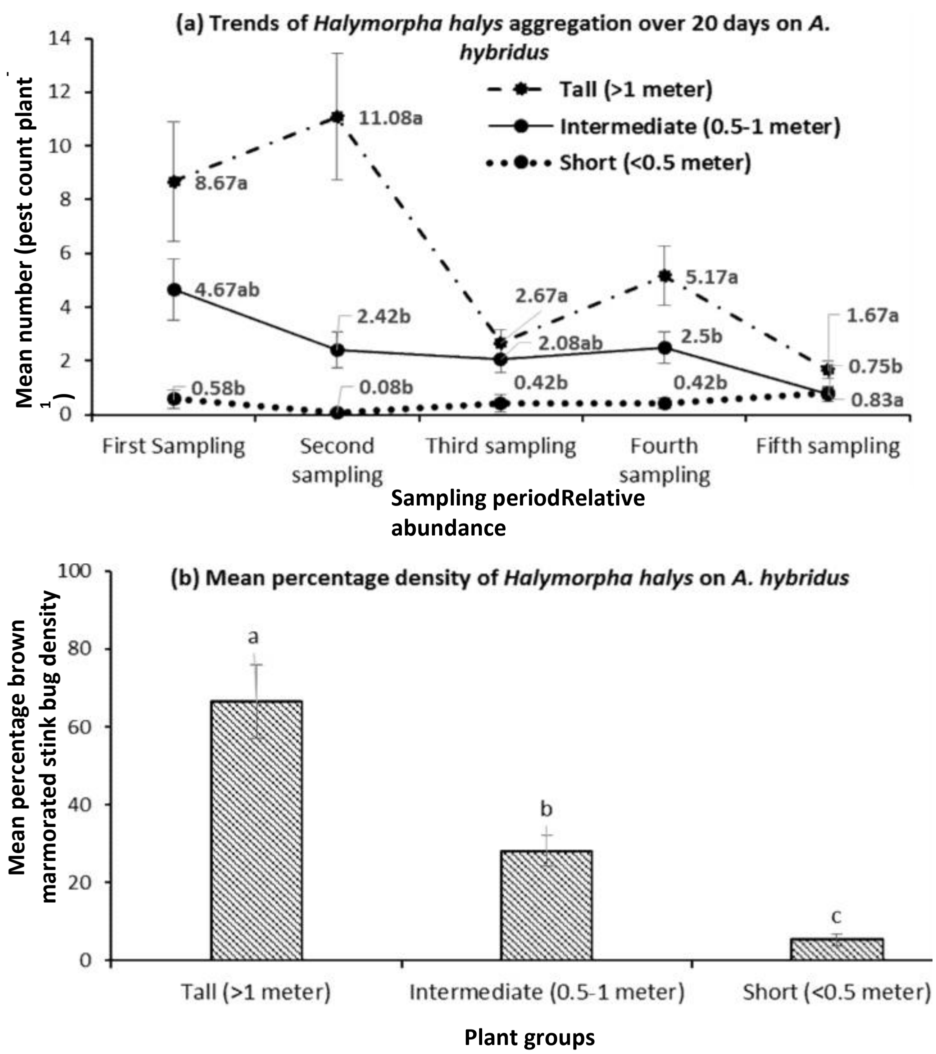


Fig. 3. Plant height-modulated pattern of distribution of mammorated stink bugs on *A. hybridus*

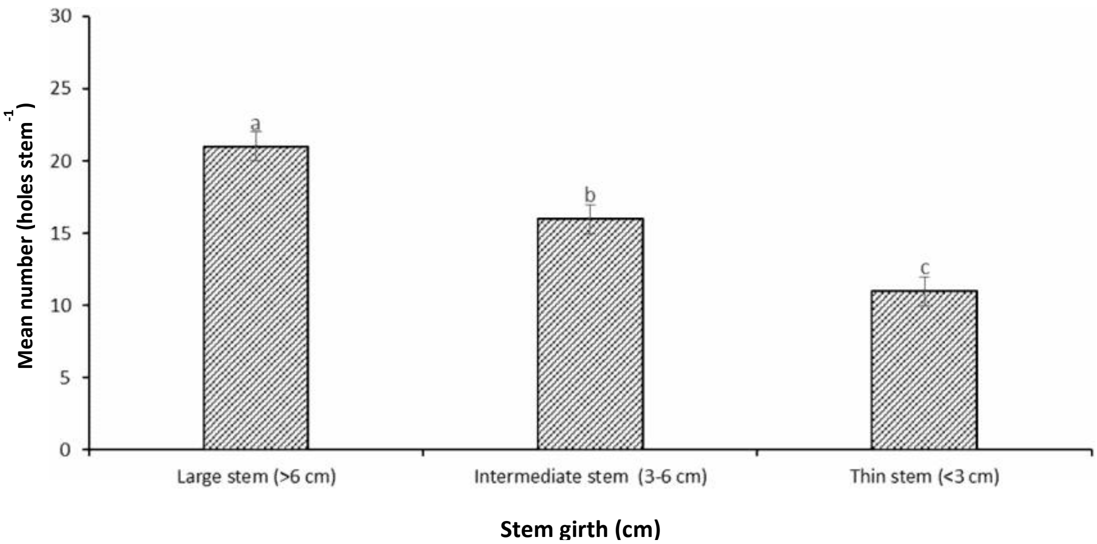


Fig. 4. Stem girth-modulated pattern of distribution of stem borers on *A. hybridus*

Adults of the borers resting on stems can be relatively easy to control using insecticide sprays, where plants are cultivated in rows and carefully spaced to allow insecticide mists to reach below the plant canopy area. However, control of the larvae that cause most damage while developing within the stem may pose a serious challenge. Control of adult amaranth stem borers using insecticides has not been widely studied and reports on the effect of foliar sprays or aerial applications of insecticides on development of larvae populations within the stem *in situ* are unavailable. Thus, the level of success which can be achieved by the use of existing pest control options (crop sanitation and the use of chemicals) in the management of amaranth borers in Nigeria is unknown.

The brown marmorated stink bugs, *H. halys* were found causing damage to the flowering heads. The brown marmorated stink bug has been reported as a major pest of vegetable crops in many parts of the world [13-15] and it is especially attracted to amaranth. It has been described as an emerging polyphagous insect of global concern [15,16] and capable of becoming a threat to vegetable production in sub-sahara Africa by its ability to exploit the climatic limits of the agro-ecological zones. Its propensity to undermine commercial-scale grain amaranth production is being reported, based on the sudden appearance and overwhelming populations on the amaranth within the flowering and fruiting periods. The brown marmorated stink bug can be classified as an invasive species, considering the short window of time between the initial appearance and subsequent population build-up.

The pattern of aggregation of the brown marmorated stink bugs and the stem borers showed they exhibit preference behaviours in relation to the height and the stem girth of the host respectively. The marmorated stink bugs preferred the tall plant group (> 1 meter) or the intermediate (0.5-1 meter) to the dwarf plant groups (<0.5 m). The trend showed that the density of the bugs (mean pest count plant^{-1}) were consistently and significantly higher on the tall plant group compared to the intermediate or on the intermediate compared to the short plant groups at five equally spaced, consecutive sampling periods within twenty five days. However, the density of the bugs generally reduced on the different plant groups as the fruits approached maturity. The activities of the borers on the thick stem (> 6 cm stem girth) was significantly the highest, followed by the intermediate stem (3-6 cm stem girth) and the thin stem group (< 3 cm), considering the number of entry and exit holes on the stem.

Behavioural ecology of the pests can be exploited in developing novel cultural management options, which are capable of keeping pest numbers below economically important thresholds [17] in a crop. For example, a cultural management system for the brown marmorated stink bug in grain amaranth may be developed using tall-short amaranth species intercrop or tall edge crop in a lure-and-spray system to reduce aggregation on the short species (the main crop). After the bugs might have aggregated on the tall species, chemical sprays may be targeted at the tall plant heads, which has served as the lure plant.

It was also observed that highest bug numbers occurred in the morning and during the evening periods, when the weather was relatively cool. Pesticide spraying regimes may be scheduled to target these periods, in order to expose significant pest populations to insecticides. However, where an intercrop would be applied, it could be necessary to plan the planting periods of the two species, such that there would be an over-lap of flowering and fruiting. A careful timing of destruction of the lure crops or application of chemical sprays could prevent potential spread of pests to the main plant. Thick-stem amaranth species may also be intercropped or used as a lure, boarder crop for the management of stem borers, but this would require further evaluations. Properly scheduled timing of insecticide sprays may enhance efficacy and reduce exposure rates of non-target insects. The use of intercrop and lure crops for the management of pest populations have been demonstrated in several studies [18,19]

6. CONCLUSIONS

The timing of peak abundances of the beneficial insects was during the flowering and the fruiting periods, probably the pest species to which they were attracted occurred at the period. Phenological relations between the interacting species can be studied and exploited in modulating the populations of the beneficial insects in addition to the use of the cropping systems earlier discussed. We proposed further studies to evaluate intercrop between tall/short- and thin/thick-stemmed amaranth species in relation to the activities of the brown marmorated stink bugs and the stem borers respectively. The current results are useful primary information in the design of further *invitro* and field studies to understand how host preference behaviours of the major pests can be applied in an IPM system.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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