



The reaction of *Amaranthus hybridus* to infection by *Amaranthus mosaic virus*

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ABSTRACT

This study investigated the effect of inoculating three *Amaranthus hybridus* cultivars: NHAM/114, NH84/457-IL and LOCAL GREEN cultivar with *Amaranthus* mosaic virus (AMV) consecutively for four weeks. Results indicated significant differences between the control and virus inoculated plants. Severity of infection by the virus was more pronounced in plants inoculated at early ages while the effect was very mild on plants inoculated at later stage of growth. Reduced weight values were recorded in plants with early infection compared to plants inoculated at later stages of growth. Mean values of 0.190, 0.250 and 0.218 g were obtained for fresh weights of leaves of NHAM/114, NH84/457-IL and LOCAL GREEN respectively at 2 weeks after planting (WAP) while plants that were inoculated at 5 WAP had average values of 0.516, 0.392 and 0.397 g fresh weights of leaves for the three cultivars respectively. The percentage decrease in fresh weight of stem of the LOCAL GREEN cultivar was above average (56%) and higher than those of NHAM/114 and NH84/457-IL. NHAM/114 produced the least dry weights in stem while the highest dry weights were recorded for NH84/457-IL at all the stages of inoculations. These results indicated that infection at an early age resulted in greater reduction of the parameters assessed.

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INTRODUCTION

Amaranthus hybridus L. (Smooth pigweed) is an annual plant, native to eastern North America and parts of Mexico, Central America and northern South America (Weaver and McWilliams, 1980). The genus *Amaranthus* includes species cultivated as leafy vegetables and/or for their grains (i.e. pseudocereal) in several developed and

developing countries (Harlan, 1992). *Amaranthus hybridus* is a fast growing plant producing up to six generations per year. It is eaten as a leafy-vegetable crop and is high in proteins, carbohydrates and lipids (Rawate, 1983). In Nigeria, *A. hybridus* leaves combined with condiments are used to prepare soup (Oke, 1983; Mepha et al., 2007). In Congo, their leaves are eaten as spinach or

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green vegetables (Dhellot et al., 2006). These leaves boiled and mixed with a groundnut sauce are eaten as salad in Mozambique (Oliveria and DeCarvalho, 1975) or pureed into a sauce and served over (farinaceous) vegetables in West Africa (Martin and Telek, 1979).

Amaranthus hybridus has been shown to contain large amount of squalene, a compound that has both health and industrial benefits (Rao and Newmark, 1998; Smith, 2000; He and Corke, 2003). Several studies have shown that amaranth seed or oil may be of benefit for those with hypertension and cardiovascular disease; regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters (Czerwiński et al., 2004; Gonor et al., 2006; Martirosyan et al., 2007). Amaranth appears to lower cholesterol via its content of plant stanols and squalene. The consumption of *Amaranthus* species is known to prevent ahypovitaminosis and anaemia (Reiz and Leitzmann, 1985). In addition, they have ornamental uses and contribute to the income of small scale farmers, especially during the dry season, in Nigeria (Taiwo and Owolabi, 2004a).

Virus diseases have been recognized to constitute one of the major factors limiting vegetable crop production worldwide (Grogan, 1980). In Africa, they sometimes result in significant yield losses (Ladipo, 1988). A number of viruses have been reported to infect *Amaranthus* species. Some of these include: *Cucumber mosaic virus* (Schmelzer and Molnar, 1975), *Alfalfa mosaic virus* (Rogers, 1969), *Amaranthus leaf mottle virus* (Lovisolo and Lisa, 1979), *Pigweed mosaic virus* (Singh et al., 1972) and *Amaranthus mosaic virus* (Phatak, 1975). A sap transmitted virus was isolated from the leaves of naturally infected *A. hybridus* plants. The virus induced mosaic or mottling, green vein banding and malformation of leaves of inoculated *Amaranthus* plants. It also infected *Nicotiana benthamiana* and *Chenopodium amaranticolor* but failed to infect several other plant species. The results of the

investigation indicated that the symptom observed in *A. hybridus* was incited by a mechanically transmissible virus possibly of the *Potyvirus* group named Amaranthus mosaic virus (AMV) (Taiwo et al., 1988). Amaranthus mosaic virus was efficiently transmitted by starved *Aphis craccivora* and *Myzus persicae*, transmission rates of 40% and 100% were observed with un-starved and starved aphids respectively. Starved single aphids also transmitted efficiently, suggesting a non-persistent method of transmission (Taiwo and Owolabi, 2004b). This research was conducted to examine the severity of AMV on the three *Amaranthus* cultivars and to investigate the effect of AMV on the weights of fresh and dry plants of *A. hybridus* inoculated at various ages in the greenhouse.

MATERIALS AND METHODS

Sources of viral isolate and *Amaranthus* species

The AMV isolate used for this research was the original Lagos isolate (Taiwo and Owolabi, 2004b) that had gone through single lesion culturing and was stored over calcium chloride at 4 °C. At the time of use, it was inoculated into healthy *Amaranthus* plant in the greenhouse.

Seeds of two *A. hybridus* lines, NHAM/114 and NH84/457-IL were obtained from the National Horticultural Research Institute (NIHORT), Ibadan. Seeds of the LOCAL GREEN cultivar were obtained from vegetable growers at Abule-Ado in Lagos State.

Experimental design

Plots were arranged in a completely randomized block design. Factors used as the main effects were *Amaranthus* cultivar/lines (3 levels), inoculation treatment (2 levels) and age of plants at inoculation (4 levels). Four replications were used and a total of 96 plants were used in the inoculation experiment.

Planting and greenhouse conditions

In the greenhouse, seeds of *A. hybridus* were planted in perforated plastic pots filled

with loam soil. The soil was sterilized by heating at 100 °C for 3 hrs to eliminate soil-inhabiting microorganisms and enriched with farmyard manure. A total of twenty four pots per variety of the *Amaranthus* cultivar at the rate of 3 seeds per plastic pot were used on 6th March, 2008. The seedlings were constantly watered and weeds removed. Before inoculation, seedlings were thinned out to one (1) plant per pot for better growth. All the plants were kept in a greenhouse with temperature at 25–28 °C until inoculation began.

Inoculation experiment

Wounding and inoculation: At exactly two weeks after planting (WAP), the first set of inoculations was carried out. Sap was prepared by grinding 1.0 g of virus infected leaves in 10 ml of 0.05 M K₂HPO₄ pH (7.5) buffer. Plants were dusted with carborundum before inoculation. This treatment as well as buffer (control) was rubbed onto fully expanded newest leaves of each plant in each pot. A total of 24 pots were inoculated, out of which 12 pots were inoculated with the virus and the remaining 12 inoculated with buffer. Similar procedure was repeated at 3, 4, and 5 WAP. After inoculation, the inoculated leaves were rinsed with water immediately to reduce the effects of wounding on leaves. The inoculated plants were kept in the greenhouse situated at the botanical garden of the University of Lagos. Pots were spaced (25.40 cm-35.56 cm apart) in order to prevent mechanical transmission within plants. The door of the greenhouse was always kept shut to keep away insects that could transmit viruses to the plants.

Determination of severity of infection of AMV on *A. hybridus*

The rating of severity of AMV on *A. hybridus* was done on a scale of 0-5: where 0= no symptoms; 1= slight mosaic or mild mottle on leaves; 2= mosaic or mottle on leaves; 3= blistering or chlorosis on leaves; 4= any of leaf deformation, leaf distortion, leaf curling or leaf reduction while 5= death of plants,

stunting or combination of two or more symptoms stated above (Fauquet and Fargette, 1990).

Effects of viral infection on plant growth

In order to determine the effect of viral infection on fresh and dry weights of leaf and stem per plant, plant from each treatment inoculated with AMV was harvested at 3 weeks after inoculation and the leaves were detached and weighed with a sensitive weighing balance to obtain fresh weight. Fresh stem tissue from each plant was also weighed. After obtaining the mean fresh weights, the plants were dried to constant weights in the oven after which the mean dry weights for both leaves and stems were determined. Similar data were obtained for buffer inoculated control plants. The percentage reductions in both the fresh and dry weights of leaves and stems were calculated by expressing the difference between the weights of buffer and virus inoculated plants as a percentage of the value for the weight of buffer inoculated plant.

% reduction= [(W_B-W_V) / W_B] x 100 Where
W_B = weight of buffer inoculated leaf or stem;
W_V = weight of AMV inoculated leaf or stem.

Statistical analysis

All the data were subjected to statistical analysis. The statistical package for social scientist (SPSS) version 16.0 was used for the analysis of the data obtained. Duncan's multiple range test (DMRT) was used to determine the level of significance between the virus/buffer treatments and age of plant at the time of inoculation at 5% probability level.

RESULTS

Effects of AMV inoculation on the three *Amaranthus* cultivars

All the three *Amaranthus* cultivars used in this investigation were susceptible to AMV when they were mechanically inoculated with the virus. The plant age at inoculation had profound effect on the symptom severity (Table 1). AMV did not

produce any symptoms on the NIHORT lines inoculated at the 5th week after inoculation while a very mild effect was recorded in the LOCAL GREEN cultivar one week after. Once the infected leaves have dropped, symptoms were not produced in the LOCAL GREEN cultivars during the subsequent weeks of observation at the 5th week (Table 1). However, at 2 and 3 WAP, the severity was high in the three cultivars and even increased as the weeks of observation progressed. The same pattern of symptom development was seen at 4 WAP though disease severity was not as high in these older plants (Table 1).

The result of the viral treatment on fresh and dry weights of leaves showed that there were significant differences between virus and buffer inoculated (control) plants. Generally, the values of the controls were higher than those of the virus infected plants. Moreso, early infected plants (2-3 WAP) had lower weights than late inoculated plants (4-5 WAP) (Tables 2 and 3). For the dry weights of stems, there were statistical differences between the controls and the virus infected

plants at all inoculation ages except at 4 and 5 WAP, in which virus inoculated plants of all the three cultivars had comparable values with the controls (Table 3). Similarly, the effects of the virus on the dry weight of the stem resulted in 24–53%, 18–35%, 8–9% and 5–6% reductions for plants inoculated at 2, 3, 4 and 5 weeks of ages respectively (Table 5).

Inoculations at 4 and 5 WAP resulted in significantly smaller percentage reductions in both leaf weights than early inoculations. At 2 WAP, reductions of 47%, 22% and 39% were obtained in fresh weights of leaves while 26%, 37% and 49% were recorded in dry leaf weights in the *Amaranthus* cultivars NHAM/114, NH84/457-IL and LOCAL GREEN respectively (Table 4).

Infection by AMV caused reduction in the fresh weights of stems of the three *Amaranthus* cultivars at all the inoculation stages. Reductions were 11%, 4% and 6% for plants inoculated at 5 WAP while those inoculated at 2 WAP were 49%, 27% and 56% for NHAM/114, NH84/457-IL and LOCAL GREEN cultivars respectively (Table 5).

Table 1: Severity of infection of *Amaranthus* mosaic virus on three *Amaranthus hybridus* cultivars.

PAI (Weeks)	PAO (Weeks)	NHAM/114	NH84/457-IL	LOCAL GREEN
2	3	2.0b	2.3b	2.0b
	4	2.6bc	2.6bc	3.0c
	5	4.3d	4.6e	5.0e
3	4	2.4b	2.6bc	2.0b
	5	2.6bc	2.8bc	3.6cd
	6	3.8cd	4.3d	4.6e
4	5	1.0ab	1.0ab	2.0b
	6	2.2b	2.5bc	3.0c
	7	3.0c	3.0c	3.4c
5	6	0.0a	0.0a	1.0ab
	7	0.0a	0.0a	0.0a
	8	0.0a	0.0a	0.0a
Control		0.0a	0.0a	0.0a

PAI = Plant age at the time of inoculation in weeks, PAO= Plant age at the time of observation in weeks, Values are means of four replicates. In each column, means followed the same letter are not significantly different according to Duncan's multiple range test at P=0.05. Control plants were inoculated with buffer only at the same intervals as plants were inoculated with the virus but none developed symptoms.

0= no symptoms; 1= slight mosaic or mild mottle on leaves; 2= mosaic or mottle on leaves; 3= blistering or chlorosis on leaves; 4= any of leaf deformation, leaf distortion, leaf curling or leaf reduction while 5= death of plants, stunting or combination of two or more symptoms stated above.

Table 2: Effect of buffer and Amaranthus mosaic virus inoculations on the fresh weights (g) of leaf per plant and stem of the three *Amaranthus hybridus* cultivars.

PAI (Weeks)	NHAM/114		NH84/457-IL		LOCAL GREEN	
	leaf	stem	leaf	stem	leaf	stem
2 V	0.190a	2.680a	0.250a	3.912a	0.218a	2.099a
	B	0.355b	5.301b	0.319ab	5.389b	0.359bc
3 V	0.339b	4.559ab	0.285ab	5.352b	0.267ab	5.348b
	B	0.426c	6.868b	0.352bc	6.646c	0.374bc
4 V	0.459cd	7.457c	0.374bc	7.893d	0.356bc	7.946c
	B	0.533e	8.684c	0.406c	8.352de	0.416c
5 V	0.516de	7.811c	0.392bc	9.289ef	0.397bc	9.873de
	B	0.538e	8.788c	0.414c	9.649f	0.441c
PAI = Plant age at the time of inoculation in weeks, V= virus inoculated plants, B= buffer inoculated plants. Values are means of four replicates. In each column, means followed the same letter are not significantly different according to Duncan's multiple range test at P=0.05. Cultivars were harvested at 3 weeks after inoculation.						

Table 3: Effect of buffer and Amaranthus mosaic virus inoculations on the dry weights (g) of leaf per plant and stem of the three *Amaranthus* cultivars.

PAI (Weeks)	NHAM/114		NH84/457-IL		LOCAL GREEN	
	leaf	stem	leaf	stem	leaf	stem
2 V	0.054a	0.117a	0.024a	0.443a	0.027a	0.261a
	B	0.073b	0.249b	0.038b	0.584b	0.053b
3 V	0.087c	0.202b	0.039b	0.684bc	0.036a	0.489b
	B	0.096c	0.312c	0.055c	0.834d	0.058bc
4 V	0.108d	0.649bc	0.047bc	0.792cd	0.059bc	0.765cd
	B	0.116d	0.705c	0.053c	0.862d	0.067c
5 V	0.113d	0.716c	0.048bc	0.846d	0.065bc	0.813cd
	B	0.117d	0.764c	0.052c	0.893d	0.068c
PAI = Plant age at the time of inoculation in weeks. V= virus inoculated plants, B= buffer inoculated plants. Values are means of four replicates. In each column, means followed the same letter are not significantly different according to Duncan's multiple range test at P=0.05. Cultivars were harvested at 3 weeks after inoculation.						

Table 4: Percentage reduction^a in fresh and dry weights (g) of leaf per plant of the three *Amaranthus hybridus* cultivars inoculated with Amaranthus mosaic virus.

PAI (Weeks)	NHAM/114(%)		NH84/457-IL (%)		LOCAL GREEN (%)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
2	47a	26a	22a	37a	39a	49a
3	20b	9b	19b	29b	29b	38b
4	14c	7c	8c	11c	14c	12c
5	4d	3d	5d	8d	10d	4d

^aPercentage decrease, calculated by expressing the difference between buffer and virus inoculated plants as a percentage of the value for the buffer. PAI- Plant age at the time of inoculation in weeks. Values are means of four replicates. In each column, means followed the same letter are not significantly different according to Duncan's multiple range test at P=0.05.

Table 5: Percentage reduction^a in fresh and dry weights (g) of stems of the three *Amaranthus hybridus* cultivars inoculated with Amaranthus mosaic virus.

PAI (Weeks)	NHAM/114 (%)		NH84/457-IL (%)		LOCAL GREEN (%)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
2	49a	53a	27a	24a	56a	43a
3	34b	35b	20b	18b	39b	29b
4	14c	8c	6c	8c	13c	9c
5	11d	6d	4d	5d	6d	5d

^aPercentage decrease, calculated by expressing the difference between buffer and virus inoculated plants as a percentage of the value for the buffer. PAI- Plant age at the time of inoculation in weeks. Values are means of four replicates. In each column, means followed the same letter are not significantly different according to Duncan's multiple range test at P=0.05.

DISCUSSION

The present investigation has shown that the three *Amaranthus* cultivars are susceptible to Amaranthus mosaic virus (AMV). There have been reports of viruses infecting *Amaranthus* species in the tropics. This supports the findings of Brunt et al. (1994) who asserted AMV as one of the viruses of tropical plants. Moreso, the research of Taiwo and Owolabi (2004b) which compared Amaranthus leaf mottle and Amaranthus mosaic virus revealed that ALMV and AMV had fairly similar host ranges that included some species of Chenopodiaceae, Solanaceae and Amaranthaceae. The prevalence of AMV in *A. hybridus* cultivars was confirmed in this study from the results obtained on severity and plant biomass. This result is also in support of Taiwo and Owolabi (2004b) which stated that AMV is highly prevalent in commercially cultivated *A. hybridus* in Lagos, Nigeria and it causes considerable economic losses.

This study revealed that age of plant at the time of inoculation influenced the severity of infection in plants because early infection of *A. hybridus* resulted in more severe symptoms and also reduced both the fresh and dry weights of plants. These findings are in agreement with the report of Agrios et al. (1985) and Langham et al. (2005) which stated that the younger the plants at the time

of viral infection the greater the severity of disease symptoms.

Furthermore, it was discovered that the younger the age of plants at inoculation the more the effect of AMV on all the parameters investigated in this study. This indicated a relationship between age at time of inoculation and virus effect. These results are in conformity with the observation made by Agrios et al. (1985) when they conducted a study on the effect of successive weekly inoculation of cucumber mosaic virus on the growth of pepper (*Capsicum annuum*) plants. The results showed that the growth of the plants was drastically affected at the earlier stages compared to the later stages of inoculation.

In addition, significantly higher fresh and dry weight values were obtained for plants at later stage of growth than at an early stage. This agrees with the previous report of Owolabi and Taiwo (2001) who opined that the attainment of maturity by plants before inoculation could result in significantly higher fresh and dry weight values for plants. Similarly, the research of Kareem and Taiwo (2007) observed progressively reduced effects of viruses on cowpea cultivars infected by Cowpea mosaic virus (CABMV), Cowpea mottle virus (CMeV) and Bean southern mosaic virus (SBMV) when inoculated at later stages of growth. When *A. hybridus* was

mechanically inoculated with AMV, reductions in plant biomass were recorded in all the inoculated plants including those inoculated at later stages of growth. However, these plants that received late inoculation did not show any symptoms of infection. The non appearance of symptoms may be as a result of latent infection usually exhibited by matured plants. Latent infections in plants have been reported by many authors. Garcia-Ruiz and Murphy (2001) demonstrated the occurrence of mature plant resistance in *Capsicum annuum* (Early Calwonder) to Cucumber mosaic virus (CMV) under greenhouse conditions and they concluded that late inoculated plants were asymptomatic while early inoculated plant developed systemic symptoms. Odedara et al. (2009) affirmed the presence of latent infections by viruses in asymptomatic cowpea varieties by subjecting them to serological indexing using both antigen-coated plate enzyme-linked immunosorbent assay (ACP ELISA) and Protein A sandwich (PAS) ELISA.

Mechanical inoculation of the three *A. hybridus* cultivars with AMV revealed that all the cultivars were susceptible to the virus. This implies that mechanical transmission is one of the means by which the virus can be transmitted in this plant. Therefore, spread of AMV by this method should be avoided by restricting the movement of personnel and equipment through field-grown *A. hybridus* plants. Nonetheless, insect transmission of AMV on the field was the commonest method of transmission and this was reported by Taiwo and Owolabi (2004b) in which non-persistent transmission of AMV by *Aphis craccivora* and *Myzus persicae* was demonstrated in the greenhouse. Planting of *A. hybridus* when insect vectors are out of season and use of integrated pest control management were suggested as means of controlling the insect vectors of AMV.

This study therefore concluded that

AMV had drastic effects on biomass and symptom induction at early age of infection while mild effects were recorded at later stage of inoculation of the three *A. hybridus* cultivars.

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