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Leaf Cuticle Variations in *Amaranthus spinosus* as Indicators of Environmental Pollution

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Abstract. Investigation of the leaf epidermal characteristics of *Amaranthus spinosus* from polluted and non-polluted populations revealed that the stomatal pores of the leaves of the plants of the polluted areas were closed whereas those of the non-polluted areas were open. Mean lenght x mean width of stomatal pores on the upper leaf surface were 0.86 µm x 0.43 µm and 1.23 µm x 0.45 µm on the lower leaf surface of the non polluted microhabitats. Also, the leaves of the polluted population were smaller than those of the non-polluted population. The average leaf area of the plants of the polluted population was 7.64 cm^2 as against 12.13 cm^2 of the plants of the non-polluted areas. The results were attributed to the combined effects of air pollutant that predominated roadsides from where the samples were taken. Thus, it is inferred that this plant could serve as bio-indicator of air pollution.

Keywords: *Amaranthus spinosus,* stomatal pores, air pollution

Introduction

The use of botanicals as bio-indicator of environmental contamination has constituted an important research area in the recent times (Kayode and Otoide, 2006). The concentrations of polluting gases, or their solutions, to which plants are exposed are highly variable depending on location, wind direction, rainfall and sunlight. Study by Bonnie and Joel (2000) had revealed that gaseous pollutants such as ozone and sulfur dioxide, enter plants through natural openings, usually stomata, and react within leaf tissues to inhibit photosynthesis. Similarly, Eduardo (2002) revealed that the burning of hydrocarbons in motor vehicle engines gives rise to carbon(IV)oxide, carbon(II)oxide, sulfur(IV)oxide, nitrogen monoxide, ethylene and a variety of other hydrocarbons. Udo and Oputa (1984), Nyawuame (1992), as well as Udo and Fayemi (1999) reported that such pollutants damage the chloroplast, causing chlorosis, necrosis, glazing, etc.

Amaranthus spinosus Linn. is an annual herb which occurs as weed at road-sides, waste areas and plantations (Akobundu and Agyakwa, 1987). Thus, *Amaranthus spinonsus* is liable to be exposed constantly to the dust in its environs. The present study has been carried out with a view to understanding the role of foliar morphology of *A. spinosus* which could serve as an indicator of environmental air pollution.

Materials and Methods

Mature leaves of *A. spinosus* were collected from 3 vehicular polluted roadsides at Oluku (A_1), Agbor (A_2) and Sapele roads (A_3) and 3 microhabitats free of vehicular pollutants Ebvomodu (B_1), Ebvoneka (B_2) and Eyaen (B_3), all situated in Edo State of Nigeria.The Oluku, Agbor and Sapele roads are extremely busy due to high traffic on them, to and from Benin-City, Nigeria. Despite the importance of these roads, they lack adequate maintenance such that in several portions of the roads, the asphalt overlays had been worn out completely. Leaf samples were collected from worn out areas on these roads. Ebvomodu, Ebvoneka and Eyaen are forest communities situated at 25, 30 and 20 kilometers away from roads, respectively.

Ten leaves per population were selected and their leaf areas were determined according to Bako *et al.* (2002) as follows:

$$A = (L)\,(W)\,(0.75) \times 2$$

where:

A = leaf area/plant
L = length of leaves
W = width of leaves
0.75 = constant leaf area factor

The epidermis was gently and carefully peeled off the mesophyllic tissues of the leaves with the aid of sharp razor blade, placed on a flat surface with the outer surface facing downwards and was flooded with commercial bleaching agent (household bleach with active ingredients, sodium hypochlorite 3.5% m/v when packed).

The peels were stained with the combination of safranin and Delafield's haematoxyline and mounted temporarily on slides.

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10 slides (each of adaxial and abaxial surfaces) were prepared per population. These slides were examined under the light microscope and data were collected from 10 microscopic fields selected at random from each slide. The length and width of stomatal pores and guard cells were measured and data were collected from 25 stomata per leaf surface. This was done in 10 replications and their means and stomatal index were recorded.

Results and Discussion

The leaf area and characteristic epidermal morphology of *A. spinosus* are summarized in Table 1. The polluted population of this species had smaller leaves with mean leaf area of 7.64 cm^2 while that of the non-polluted population had an average leaf area of 12.13 cm^2. In both populations, stomata were present in upper and lower epidermis, thus, *A. spinosus* is amphistomatic. There were, however, more stomata in the lower epidermis than in the upper epidermis. The distribution of stomata in the upper and lower epidermis was observed to be 64% and 92%, respectively, in both the two populations. The walls of the subsidiary cells and other cells of the epidermis were sinuous.

The stomata in this taxon were anomocytic in both the upper and lower epidermis. Epidermal hairs were absent in this species. Closed stomatal pores were observed in the polluted population, whereas, in the non-polluted population

Table 1. Leaf size and epidermal characteristics of polluted and non-polluted populations of *Amaranthus spinous* L.

Description		Population					
		A			B		
		1	2	3	1	2	3
A. Leaf Dimension:							
Mean length (cm)		3.91	4.00	5.58	3.59	5.18	5.84
Mean width (cm)		2.28	1.94	2.61	3.15	3.37	2.82
Average leaf area (cm^2)		6.69	5.82	10.92	10.84	13.09	12.35
B. Leaf Epidermal Characteristics:							
Trait	Leaf surface						
Nature of epidermis	U	S	S	S	S	S	S
	L	S	S	S	S	S	S
Type of stomata	U	An	An	An	An	An	An
	L	An	An	An	An	An	An
Stomata index	U	64	64	64	64	64	64
	L	92	92	92	92	92	92
Type of epidermal hair	U	Ab	Ab	Ab	Ab	Ab	Ab
	L	Ab	Ab	Ab	Ab	Ab	Ab
Mean length of stomata pores (μm)	U	-	-	-	0.7	1.2	0.7
	L	-	-	-	1.5	1.5	0.7
Mean width of stomata pores (μm)	U	-	-	-	0.42	0.45	0.45
	L	-	-	-	0.46	0.45	0.45
Mean length of guard cell (μm)	U	2.2	1.5	2.4	1.8	1.9	1.9
	L	2.0	1.5	2.3	1.7	2.5	1.9
Mean width of guard cell (μm)	U	1.5	1.5	1.5	1.5	1.5	1.6
	L	1.5	1.5	1.5	1.5	1.5	1.6
Status of stomata	U	Cl	Cl	Cl	Op	Op	Op
	L	Cl	Cl	Cl	Op	Op	Op

A = vehicular polluted roadside; B = non-polluted sites; U = upper epidermis; L = lower epidermis; S = sinuous; An = anomocytic; Ab = absent; Cl = closed; Op = open pores were open with mean width of 0.44 μm and 0.45 μm in the upper and the lower epidermis, respectively.

The reduced size of leaves of *A. spinosus* growing along roadsides could be due to the combined effect of different pollutants, they derived from exhaust of automobiles. This might confer some elements of ecological disadvantage on this taxon. The small leaves might prevent this plant from capturing enough amount of light and thus, make it unable to competes effectively with other plants growing along roadsides. On the other hand, the non-polluted population of this species had large leaves. The variation in the size of the leaves could be due to the geographical location of this population, as the non-polluted population is not affected by air pollutants from automobiles. Also field observation revealed that *A. spinosus* growing in non-polluted areas appeared healthier than those found along the roadsides. All these factors tend to support the previous observation made by Kayode and Otoide (2007; 2006) on *Chromolaena odorata* and *Newbouldia laevis* as well as Otoide and Kayode (2007), on *Elaeis guineensis.*

The closed stomatal pores found in the upper and the lower epidermis of the polluted population may be a defense strategy to prevent further entrance of pollutants, which predominate in busy road side areas. This tends to confirm the previous assertion of Eduardo (2002) on stomatal closure. While the reduced sizes of leaves in the roadside species could be attributed to the impeded photosynthetic activity caused by various categories of pollutants that were deposited on the body of these species, the large leaf size observed in those from the non-polluted locations might be due to unimpeded photosynthetic and stomatal transpiration facilitated by the open pores of the stomata. Thus the species growing along roadsides are vulnerable to disease infestation because of injuries caused to them by dust and particulate pollutants. Injuries to the plant body provide entrance for pathogens into tissues of plants. Consequent to these, they showed patches of dead tissues, necrosis and perforated leaf blades. In contrast, these pathological sympotoms were not found on the leaves of the non-polluted samples.

In conclusion, leaves of *A. spinosus* of both the polluted and the unpolluted populations showed unequal reactions to the composition of air in their localities as expressed by the status of the pores and leaf size. Thus it appears that the leaves of this species could serve as marker of environmental air pollution.

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