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Observations on the polluted and non-polluted leaf epidermal surfaces of some plants

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Abstract

The epidermis of *Polyalthia longifolia*, *Digitaria gayana* and *Trianthema portulacastrum* growing within the radii of 0.1-0.25m to exhaust-pipes of power generators(Polluted populations) and their individual counterparts growing at distances of 100-110m far away from power generators(Non-polluted populations) in the human settlements of Ado-Ekiti, Ekiti State, were investigated with the aid of light microscope. Damages such as eroded epicuticular wax, epidermal cell alterations, plugged stomatal apertures, blurred epidermal surfaces, polluted cells, ruptured stomatal ledges and eroded cell walls were observed in the polluted populations of these species. These kinds of damages were, however, not observed in the counterparts of these species which were not polluted by exhausts. The sizes of leaves of the polluted populations were smaller in area than those of the non-polluted populations. The average leaf areas of polluted populations of *Polyalthia longifolia*, *Digitaria gayana* and *Trianthema portulacastrum* were 9.94± 0.8, 0.31± 0.2 and 4.78± 0.4 respectively. On the other hand, the average leaf areas of the non-polluted populations of these same species were, for *Polyalthia longifolia*, *Digitaria gayana* and *Trianthema portulacastrum* 31.6± 0.2, 1.92± 0.1 and 10.97± 0.5 respectively. The non-polluted populations, therefore, had higher leaf areas probably because they were not affected by exhaust-pollutants. It was suggested that the species investigated in this study could serve as bioindicators of environmental air pollution.

Key words : Epidermal leaf surfaces, *Polialthia longifolia, Digitaria gayana, Trianthema portulacastrum*

INTRODUCTION

According to LENNTECH [1] ,Air pollution means the presence of one or more substances in air, which has negative effects on humans, animals and plants and on the air quality. Substances that change the composition of air negatively and substances that cause nuisance are called air pollutants.

The main air pollutants are Sulphur oxides, Nitrogen oxides, Volatile organic compounds, Carbon monoxides, small dust particles and Ozone particulates [2,3,4].

Agricultural crops can be damaged when exposed to high concentrations of various air pollutants. Injury ranging from visible markings on the foliage to reduced growth and yield, as well as premature death of plants had been reported [5,6,7]. The development and severity of injury depends not only on the concentration of the particular pollutant but also on a number of other factors. These include the length of exposure of the pollutant, the plant species and its stage of development as well as the environmental factors conducive to a build-up of the pollutant and to the preconditioning of the plant which make it either susceptible or resistant to injury.

Air pollution injury to plants can be evident in several ways. Injury to foliage may be visible in a short time and appear as necrotic leisons (dead tissue), or it can develop slowly as a yellowing or chlorosis of the leaf. There may be a reduction in growth of various portions of a plant. Plant may be killed outright, but they usually do not succumb until they have suffered recurrent injury [8]. Furthermore, researchers such as Bobrov [9], Chakrabarty and Gupta[10], Gupta[11], Singh and Trivedi[12], Pal *et al.,* [13] and Luzimar *et al.,*[14] reported collapse of lower epidermal cells, dehydrated substomatal chambers and formation of cork-like layer on the lower leaf surface of *Beta vulgaris* (Table beet) as a result of response to smog. In the same vein, Chakrabarty and Gupta [10] and Gupta [11] and Singh and Trivedi[15] carried out studies on Morpho-histology and epidermal trait of *Boerhavia diffusa* together with other herbaceous species. They reported that pollution affected the histomorphology, epidermal traits, chlorophyll and protein contents *of Boerhavia diffusa*. Their results indicated the incidence of air pollution [16]. Similarly, Pal *et. al.,* [13] investigated the effects of auto-exhaust pollution on the leaves of some plants growing along roadsides in low and high traffic density areas. They reported changes in structure of the leaf surfaces such as eroded epicuticular wax, irregularly fused cell boundaries and collapsed epidermal cells in the species studied.

MATERIALS AND METHODS

Collection of Plant Samples

Matured leaf samples of species of *Polialthia longifolia Digitaria gayana, Trianthema portulacastrum* were collected from within 0.1- 0.25m radii of the exhaustpipes of power generators used for domestic purposes especially by the elites in Ado-Ekiti, Ekiti State. Another collection of same species were made in the same environment, but at distances of 100-110m away from the power generators [17]

The first set of collections represented the polluted populations, while the second set represented the non-polluted populations which served as control for this study.

Leaf Dimension

Ten (10) leaves per species from the polluted and non-polluted areas were randomly selected, their sizes were measured with the aid of plastic ruler and the data were recorded.

The leaf Area (LA) of each leaf was then determined according to Otoide and Kayode [17] as:

LA: L x W x 0.75

Where, L = Length of Leaf

W = Width of Leaf

0.75 = Constant

Preparation of Slides

The epidermal peels of each leaf sample were obtained using the methods of Metcalfe and Chalk[18] and Olowokudejo [19]. The leaves were placed, with the outer surface facing downward, on a flat surface and flooded with 8% sodium hypochlorite solution (NaOCl). An area of about 1cm square was removed from a central / standard position, always midway between the base and the apex of the leaves. The peels were mounted temporarily on slides. 10 slides (each of adaxial and abaxial surfaces) were prepared per population.

Measurement of pores, guard cells and collection of data:

The slides were examined under the light microscope using x20 and x40 objectives. 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 using ocular micrometer. Data were collected from 25 stomata per leaf surface. This was done in 10 replications.

The data obtained were subjected to relevant statistics using mean, standard deviation and ANOVA. Significant differences were determined at $p \leq 0.05$.

RESULTS

Polyalthia longifolia (Sonn.) Thw.

Microscopic observation of the leaves of this species revealed the absence of stomata at the adaxial epidermis. *Polyalthia longifolia* is therefore, hypostomatic. Stomata apertures were plugged by soot particles. Also, there were deposits of soot particles in the epidermal cells, guard cells and cell walls in the polluted population. In portions of the leaves where there was large deposit of soot the epidermal cells consequently appeared to be obscured by the soot (black carbon) particles. In contrast, opened stomata apertures were observed in the non-polluted populations of this species. In addition, there was no trace of deposit of soot particles at both epidermis.

Hexacytic type of stomata was observed in both populations. At the lower epidermis, the mean length of pores was 1.47±0.12µm while the mean width of pores was 0.59±0.49µm. The mean length of guard cells was 0.59±0.49µm. The mean length of guard cells was 2.76±0.01µm in the polluted population while in the non-polluted population, the mean length was 2.39±0.01µm. Similarly, the mean width of guard cells in the polluted population was 2.88±0.42µm while in the non-polluted population, the mean width of guard cells was 1.49±0.61µm.In the polluted population, stomata index and stomata density were 44.44% and 87 respectively. While in the non-polluted population, stomata index and stomata density were 38.46% and 110 respectively.

Slightly zig-zag nature of epidermal cell wall were observed at the adaxial and abaxial epidermis in the polluted and non-polluted populations of this species.

***Digitaria gayana* (Kunth) Stapf ex**

Plugged stomatal apertures were observed on the upper and lower epidermis in the polluted populations of this species. Conversely, the pores were opened at the upper and lower epidermis in the non-polluted populations of this plant.

The pores, being opened, measured 0.41±0.09µm in mean length and 0.29±0.21µm in mean width at the upper epidermis while at the lower epidermis the mean length and mean width were 0.62±0.94µm and 0.35±0.14µm respectively.

Guard cells in both populations were measured. The mean lengths and mean width of guard cells at the upper epidermal surfaces in the polluted population were 2.88±0.60µm and 1.95±0.12µm respectively. While at the lower epidermis, the mean length and mean width of guard cells were 2.46±0.05µm and 1.47±0.22µm respectively. In the same vein, at the upper epidermis in the non-polluted populations, the mean length and mean width of guard cells were 3.09±0.04µm and 2.01±0.51µm respectively, while at the lower epidermis, the mean length and mean width of guard cells were 2.95±0.05µm and 1.99±0.50µm respectively.

The stomata index and stomata density in the polluted populations of this taxon were at the upper epidermis, 18.18% and 40 respectively, while at the lower epidermis, the stomata index and stomata density were 46.66% and 86 respectively. On the other hand, the non-polluted populations of this plant had stomata index of 25.41% and stomata density of 50 at the upper epidermis

Table 1 : Average leaf area (cm2) of plants from polluted and non-polluted populations.

S/N	SPECIES	POPULATIONS	
		Polluted	Non-polluted
1	*Polyalthia longifolia* (Sonn.)Thw.	9.94±0.8a	31.6±0.2b
2	*Digitaria gayana* (Kunth) Stapf ex.	0.31±0.2a	1.92±0.1b
3	*Trianthema portulacastrum* Linn.	4.78±0.4a	10.97±0.5b

Means with different letters in each row are significantly different at 0.05.

Table 2 : Leaf epidermal characteristics of the plant samples from polluted and non-polluted populations.

DESCRIPTIONS	SURFACE	*Polyalthia longifolia* A	*Polyalthia longifolia* B	*Digitaria gayana* A	*Digitaria gayana* B	*Trianthema portulacastrum* A	*Trianthema portulacastrum* B
Mean Length of Pore (µm)	U	-	-	Plugged	0.41±0.09	Plugged	1.51±0.50
	L	Plugged	1.47±0.12	Plugged	0.62±0.94	Plugged	1.39±0.36
Mean Width of Pore (µm)	U	-	-	Plugged	0.29±0.21	Plugged	0.45±0.01
	L	Plugged	0.59±0.49	Plugged	0.35±0.14	Plugged	0.69±0.40
Mean Length of guard cell (µm)	U	-	-	2.88±0.60	3.09±0.04	2.85±0.41	3.38±0.11
	L	2.76±0.01	2.39±0.01	2.46±0.05	2.95±0.05	Not clear	3.38±0.11
Mean Width of guard cell (µm)	U	-	-	1.95±0.12	2.01±0.51	1.47±0.34	1.99±0.41
	L	2.88±0.42	1.49±0.61	1.47±0.22	1.99±0.50	Not clear	2.84±0.58
Stomata index (%)	U	-	-	18.18	25.41	20.00	27.27
	L	44.44	38.46	46.66	61.52	Not clear	22.22
Stomata type	U	-	-	Anomocytic	Anomocytic	Anisocytic and Anomocytic	Anomocyitc and Anisocytic
	L	Hexacytic	Hexacytic	Anomocytic	Anomocytic	Not clear	Anomocytic and Anisocytic
Stomata Density Per (mm^2)	U	-	-	40	50	36	56
	L	87	110	86	104	Not clear	62
Nature of epidermal cell wall	U	Slightly Zig-zag	Slightly Zig-zag	Straight	Straight and Slightly sinuous	Slightly sinuous	Slightly sinuous
	L	Slightly Zig-zag	Slightly Zig-zag	Straight	Straight and Slightly sinuous	Not clear	Slightly sinuous

KEY: A= Polluted Population B= Non-polluted Population
U= Upper epidermis L= Lower epidermis

while at the lower epidermis, the stomata index and stomata density were 61.52% and 104 respectively. Anomocytic stomata were observed in the upper and lower leaf surfaces in the polluted and non-polluted populations of this taxon.

The epidermal cell walls were straight at the upper and lower epidermis in the polluted populations whereas, these were found to be straight but slightly sinuous nearer the veins in both the upper and lower epidermis in the non-polluted populations. Field observation of leaves in the polluted populations showed glued leaf surfaces. This was as a result of the black soot particles that were deposited on the leaves. Hence, deposits of black carbon were observed in the epidermal peels, where they plugged stomates and obscured most of the surface cells and tissues as well as damage some of them. These aberrations were not observed in the epidermis in the non-polluted populations of this taxon.

Trianthema portulacastrum **Linn**

Microscopic observation of leaves of this species showed that the stomates at the adaxial and abaxial epidermis in the polluted populations were plugged by deposits of soot particles. So their length and width could not be determined. However, the stomatal guard cells at the adaxial epidermis in this population were measured for lengths and widths. At the adaxial epidermis, the mean length and width of guard cells were 2.85±0.41µm and 1.47±0.34µm respectively.

The opened stomates at the epidermal surfaces in the non-polluted populations were measured. At the adaxial epidermis, the mean length and mean width of pores were 1.51±0.50µm and

0.45±0.01µm respectively, while at the abaxial epidermis, the mean length and mean width of pores were 1.39±0.36µm and 0.69±0.40µm respectively. Similarly, the mean length and mean width of guard cells at the adaxial epidermis in the non-polluted populations were 3.38±0.11µm and 1.99±0.41µm respectively, while at the abaxial epidermis, the mean length and mean width of guard cells were 3.38±0.11µm and 2.84±0.58µm respectively.

Combinations of anisocytic and anomocytic types of stomata were observed at the adaxial epidermis in the polluted populations. The epidermal surface features of the abaxial epidermis in this population have been tremendously obscured by large dose of black carbon. This rendered the data collection on this surface to be unattainable. There were negligible amount of black carbon deposits on the adaxial epidermis of this polluted population. Therefore, stomata index of 20.00% and stomata density of 36 were calculated at the adaxial epidermis in the polluted population. The epidermal cell walls were slightly sinuous. Similarly, at the adaxial epidermis in the non-polluted population, the stomata index and stomata density were 27.27% and 56 respectively while at the abaxial epidermis, the stomata index and stomata density were 22.22% and 62 respectively.

Combinations of anomocytic and anisocytic stomata types were observed at the adaxial and abaxial epidermis. The cell walls of the epidermis were slightly sinuous in both surfaces. Both adaxial and abaxial epidermis of the non-polluted populations were devoid of particulate pollutants, since there was no evidences of soot deposits on the field.

DISCUSSIONS

While collecting the leaves that constituted the polluted populations, it was observed that they were weakly attached to their stems because they were easy to pluck when compared with leaves of the non-polluted populations. This could be attributed to the abrasive effects of the exhausts from the power generator to which the plants were exposed. The effects of the heat as part of the exhausts cannot be underrated in the events. Furthermore, field observation of the polluted leaves belonging to the three plants revealed presence of black soot particles firmly glued to the surfaces of the leaves in an uneven pattern. Some drops of oil which was believed to have leaked out of the exhaust-pipe were also observed on the leaf surfaces of the polluted populations. Thus, the affected portions appeared black. These “foreign” and extraneous materials produced by the power generator were not found on the leaves of the non-polluted populations since they were not exposed to pollution by power generators. Hence, the surfaces of their leaves were clear and devoid of particulates.

Microscopic study of the leaves in the polluted populations revealed damages such as epidermal cell alterations, plugged stomatal apertures, blurred epidermal surfaces, polluted cells, ruptured stomatal hedges and eroded cell walls (Table2). These damages could be attributed to the activities of the gaseous pollutants and the soot particles (black carbon) from the incomplete combustion of fossil fuel which the plants species were continuously exposed to. Conversely, no forms of damages were observed in leaves of the non-polluted populations. Their cells, stomata and cell walls showed clear and normal appearances under the light microscope. These observations tend to confirm the previous assertion by Edwardo [20] that air pollutants bind to plasma membranes, alter metabolism, destroy stomata apertures, damage chloroplast thylakoid membranes, degrade rubisco, erode epicuticular wax, destroy epidermal cells and inhibit photosynthesis. It further confirmed the recent reports of damages in leaves of some groups of plants exposed to exhausts from power generators by Otoide and Kayode [16].

It is genuine to suspect that the reduction in sizes of leaves of the polluted populations might be part of the consequences of the damages they suffered by virtue of their closeness to the exhaust-pipe of the power generator since the black soot particulates and oil drops could screen out solar energy (sunlight) and prevent carbon dioxide assimilation in them. The leaves of the non-polluted populations were, however, not reduced. They were large and well developed (Table1). These observations further support the previous assertions of Adela [21] that air pollutants injure plants by damaging their foliage and impairing the process of photosynthesis (food making).

It is further suspected that the deposits of soot particulates and gaseous toxins discovered in the polluted population could initiate chemical reactions with moisture to produce complex acids. The polluted leaves, in this condition, might suffer some elements of ecological and physiological disadvantages which could make them unable to compete with their counterparts of the non-polluted population. Other disadvantages might also be suffered by these group of plants in the field of ethnomedicine, in that, the polluted leaves may not be useful in herbal preparations as the soot particulates on the outer and in the inner surfaces are contaminants.

CONCLUSION

Results obtained from this study is a pointer to what is being experienced in residents where power generators continuously serve as sources of electricity supply. Human beings and animals, being components of the areas covered by this study might be affected as well.

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