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LEAF EPIDERMAL AND GROSS MORPHOLOGICAL ADAPTATIONS IN *SALIX NIGRA* MARSH. (SALICACEAE) IN RELATION TO ENVIRONMENTAL POLLUTION

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ABSTRACT--Eleven populations of *Salix nigra* Marsh. (black willow) in the mid-southern part of the United States were analyzed to determine the relationship between environmental contamination and variation in leaf epidermal and leaf morphological patterns. Plant populations of polluted habitats exhibited a decrease in leaf length and leaf width. Furthermore, stomatal frequency values, size of the largest stoma, and the epidermal wall undulations were reduced in these plant populations. Subsidiary cell complex remained unaffected by environmental pollution.

Various studies (Feder, 1970; Mathis and Tomlinson, 1972; Sharma and Butler, 1972) have demonstrated morphological relationships between plants and environmental pollution. Detrimental effects of fluorides, sulfur dioxide, ozone, and a host of other pollutants on plants have been documented (Solberg and Adams, 1956; Bennett et al., 1974). Chamberlain (1934) suggested that industrial pollutants of a large mid-western city in the United States were damaging to the coniferous flora of the area. He singled out *Pinus banksiana* Lamb. for its susceptibility to industrial pollution. Hill and Thomas (1933) pointed out that alfalfa yield showed a decrease after exposure to sulfur dioxide. Pyatt's (1970) studies on the lichen flora showed that thallus size decreased and the lichen flora decreased in the number of species with increasing proximity of the pollution source in a steel-producing town in Wales. Relatively few studies, however, deal with the relation between environmental pollution and the leaf cuticular dynamics of plants. Earlier work on the subject (Walker and Dunn, 1967; Au, 1969; Sharma, 1972; Sharma and Tyree, 1973; Sharma and Edwards, 1985) reveals the existence of such a correlation. To understand further these relationships and explore their usefulness as indicators of environmental pollution, *Salix nigra* Marsh. (black willow) was selected because of its economic significance in the area.

Salix nigra is a woody dicotyledonous plant of common occurrence in the southeastern United States. It is the largest and tallest of any native species of willow in the United States. It has its best growth in moist, swampy habitats. Used as mattresses along the larger rivers for reinforcing levees and protecting them from washing, it helps control erosion of river banks (Steyermark, 1981). The bark is known to be used as an ingredient for blood medicines during spring (Lewis and Elvin-Lewis, 1976). The wood is used for a wide variety of construction purposes in the southern United States.

MATERIALS AND METHODS

Seven population groups (A to G) representing a total of 11 populations (Fig. 1) of *S. nigra* representing varied levels of environmental pollution were selected from similar microhabitats in the mid-southern parts of the United States (Table 1). Populations 1, 2, and 3 were collected in the relatively unpolluted, uninhabited habitats of Land Between The Lakes (LBL) in western Kentucky and Tennessee. This area of 68,800 ha represents a fairly rural, unpolluted habitat with no industry on its premises. However, there is some indigenous pollution because of vehicular traffic especially during the summer months. Populations of *S. nigra* on LBL provide an environment in sharp contrast to the metropolitan area of St. Louis, Missouri, or the industrial complex of Calvert City, Kentucky. Populations 4, 5, and 6 were collected in the Calvert City area. Calvert City is a small town in northern Marshall County of western Kentucky in close proximity to the LBL area. In sharp contrast to the clean surroundings of LBL, it has a vast complex of industrial units (e.g., petroleum, smelters, plastics) emitting a wide variety of pollutants and, therefore, represents a highly contaminated habitat. Population 9 from nearby Smithland, Kentucky, was also affected by the gaseous pollution generated by the industrial complex of Calvert City. Populations 7 and 8 represented two widely separated habitats in St. Louis, Missouri, one of the largest industrial cities in the United States. While population 7 was located in the wooded area in the outskirts, population 8 was collected from the heavily polluted downtown section of St. Louis. Population 10 from Paducah, Kentucky, 35 km north of LBL, was located in an area exposed to air, water, and soil pollution generated by automobiles, a power plant, and other nearby industrial units. Population 11, characterized by the absence of industry and excessive vehicular traffic, was collected from the rural and relatively unpolluted environment of Reelfoot Lake in western Tennessee.

Each sample consisted of 10 leaves collected at random from the lower portions of stems of five plants growing in the area. The leaf samples were collected in late summer to ensure their maturity at the time of sampling. Macroscopic data were tabulated from the leaves. Leaf length, leaf width, and petiole length were recorded on each of the 10 leaves from each sample. Mean values and standard deviation of the vegetative data are shown in Table 2. Macroscopic leaf damage such as chlorosis, necrosis, and other anomalies were noted.

Cuticular impressions of upper and lower leaf surfaces were prepared by applying Duco cement to washed and dried leaves (Williams, 1973). A small portion from the central area of each leaf cuticular imprint was used to make cuticular slides for adaxial and abaxial leaf surfaces of each population. Microscopic data on stomatal frequency,

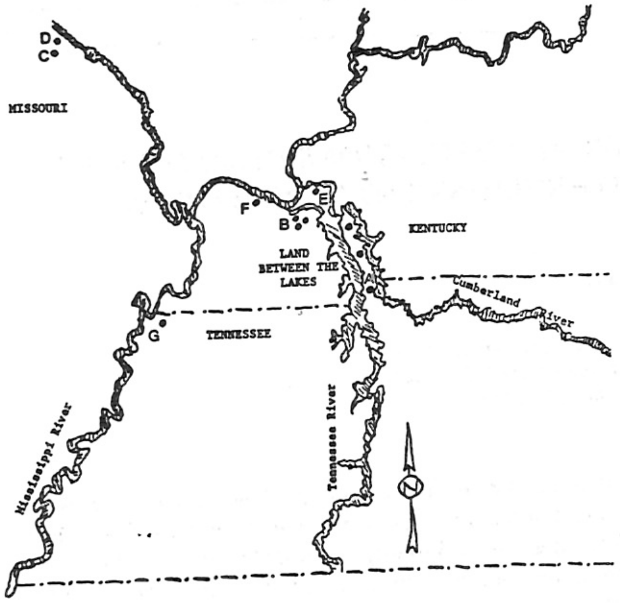

FIG. 1. Map showing localities of populations of *Salix nigra* from which leaves were sampled.

smallest and largest stomatal size, epidermal cell wall undulations, and subsidiary cell complex were recorded by randomly selecting 20 fields ($n = 20$) from each cuticular slide. The microscopic analysis was made at 400x magnification. Mean values and standard deviation of the cuticular data are shown in Table 3. Furthermore, mean values and standard deviation of the vegetative and cuticular data are summarized in Table 4.

RESULTS

For ease of comparison the population samples were organized into seven population groups (A to G) identifying locale or proximity (Table 1). Vegetative characteristics are shown in Table 2. Statistical analysis of the data shows that leaf length within populations of group A (LBL) showed little variation, the statistical mean ranging from 10.9 cm to 11.1 cm. Similar readings (9.1 cm to 9.3 cm) were found in population group B representing the polluted habitat of Calvert City. Mean values for leaf length ranged from 8.6 cm in one of the most polluted population groups (group D) to 11.5 cm in group G representing the rural environs of Reelfoot Lake. Leaf width ranged from 0.4 cm in Smithland to 1.4 cm in the Reelfoot Lake area. Reelfoot Lake area, obviously one of the least polluted areas, had a mean leaf length value of 11.5 cm and leaf width value of 1.4 cm for all populations combined. Petiole length varied from 0.4 cm in Groups D and F to 0.6 cm in the population groups of Reelfoot Lake, LBL, and St. Louis outskirts.

Cuticular characteristics are shown in Table 3. Stomatal frequency on the upper leaf surface ranged from 20.9 in group D (St. Louis downtown) to 49.6 in group G of Reelfoot Lake. On the lower leaf surface, stomatal frequency means ranged from 40.2 in group D (St. Louis downtown) to 72.4 in group A (LBL). Largest stomatal size on the upper leaf surface ranged from 13.8µ in group B (Calvert City) to 27.6µ in group G (Reelfoot Lake). On the lower surface, the mean size variation for the largest stoma was from 16.8µ in group B (Calvert City) to 29.5µ in group G (Reelfoot Lake). Smallest stoma on the upper leaf surface had a range of 8.7µ (group G, Reelfoot Lake) to 14.0µ (group C, St. Louis outskirts). On the lower surface, it ranged from 9.0µ (group A) to 16.1µ (group C).

Number of epidermal wall undulations on the upper leaf surface had the highest statistical mean value of 10.1 in group A (LBL), and the lowest mean value of 4.9 in group C (St. Louis outskirts). For the lower leaf surface, the highest mean value (10.9) was in group A, while the lowest mean value (5.1) was found in the polluted group B (Calvert City). Table 4 shows a summary of vegetative and cuticular characteristics.

Subsidiary cell complex consisting of several ordinary epidermal cells irregularly surrounding the stoma–the "anomocytic" type (Metcalfe and Chalk, 1950) remained the same in all the 11 populations. The taxonomic significance of this feature should be investigated for this taxon.

DISCUSSION

The study points out modifications in the leaf epidermal and morphological features of *S. nigra* possibly in response to environmental contamination. The data suggest a general decrease in leaf length and leaf width with an increase in the degree of pollution in the environment. The reverse was true in the relatively clean habitats of groups A, C , and G. Clearly, environmental pollution seems to have a detrimental effect on plant growth and hence its photosynthetic productivity.

Stomatal frequency on both the abaxial and adaxial leaf surfaces decreased with an increase in environmental pollution. Reelfoot Lake and LBL plant population groups from the least polluted environs exhibited the highest stomatal frequency. Epidermal wall undulations had the highest mean values again in group A and group G, while the lowest mean values were associated with contaminated sites (groups B

TABLE 1. Distribution and habitat features of populations of *Salix nigra*.

Population group	Populations	Locality	Relative degree of pollution[1]	Source of pollution
A	1, 2, 3	Land Between The Lakes	+	None
B	4, 5, 6	Calvert City, Kentucky	+++++	Industry
C	7	St. Louis--outskirts	+	Vehicular traffic
D	8	St. Louis--downtown	+++++	Industry, vehicular traffic
E	9	Smithland, Kentucky	++++	Industry
F	10	Paducah, Kentucky	+++	Vehicular traffic
G	11	Reelfoot Lake, Tennessee	+	None

[1] +++++ = highest level; + = lowest level.

TABLE 2. Vegetative characteristics[1] of population groups of *Salix nigra*.

Group	Population	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)
A	1	11.1 ± 0.3	1.2 ± 0.6	0.7 ± 0.1
	2	10.9 ± 0.1	1.2 ± 0.8	0.6 ± 0.1
	3	11.1 ± 0.3	1.2 ± 0.1	0.7 ± 0.1
B	4	9.1 ± 0.2	0.9 ± 0.1	0.5 ± 0.1
	5	9.3 ± 0.1	0.9 ± 0.1	0.5 ± 0.1
	6	9.3 ± 0.1	1.0 ± 0.1	0.5 ± 0.1
C	7	11.3 ± 0.4	1.1 ± 0.7	0.6 ± 0.1
D	8	8.6 ± 0.4	0.7 ± 0.1	0.4 ± 0.1
E	9	9.1 ± 0.3	0.4 ± 0.1	0.5 ± 0.1
F	10	9.5 ± 0.2	1.0 ± 0.9	0.4 ± 0.1
G	11	11.5 ± 0.6	1.4 ± 0.9	0.6 ± 0.1

[1] The values represent means of 10 measurements ±1 *SD*.

through F), suggesting that this cuticular feature may have a very distinct modification in polluted habitats. Size of the largest stoma decreased with an increase in pollution level. However, the size of the smallest stoma seemed to exhibit no distinct trend in the populations of *S. nigra* growing in habitats of varying degrees of environmental pollution. Subsidiary cell complex remained unaffected by environmental pollution and hence must be regarded as a useful species character owing to its constancy.

The comparisons made in this study suggest that leaf length and leaf width in *S. nigra* were adversely affected by environmental pollution. A decrease in stomatal frequency in *S. nigra* leaves from polluted areas suggests an adaptive modification that may serve to keep out gaseous pollutants that otherwise may enter the leaf and destroy the plant tissues. A decrease in the epidermal wall undulations suggests another adaptive feature that may reduce the surface area exposed to environmental pollution. A decrease in the size of the largest stoma in polluted habitat populations suggests an adaptation to keep out excessive gaseous pollutants.

The leaf morphological and cuticular modifications exhibited by *S. nigra* populations in this study suggest that these modifications may be of adaptive value in polluted environments. Bennett et al. (1974) suggested that plants do adapt to low levels of pollution. It is, therefore, safe to hypothesize that the patterns found in the study may be important bioindicators of environmental pollution. However, additional species are under investigation to confirm the findings for a wider spectrum of the plant kingdom.

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TABLE 3. Cuticular characteristics[1] of population groups of *Salix nigra*.. Subsidiary cell complex (for upper and lower surfaces) was anomocytic for all populations.

Group	Population	Stomatal frequency[2]		Largest stoma (μ)		Smallest stoma (μ)		Epidermal wall undulations	
		U	L	U	L	U	L	U	L
A	1	47.8 ± 4.3	72.4 ± 3.1	25.3 ± 1.6	25.0 ± 1.6	9.1 ± 1.9	9.8 ± 1.7	10.1 ± 0.8	10.4 ± 0.8
	2	47.2 ± 3.1	72.4 ± 3.0	24.6 ± 3.0	25.8 ± 1.3	9.1 ± 1.6	10.2 ± 1.6	9.6 ± 0.5	10.9 ± 0.9
	3	47.0 ± 4.8	71.1 ± 3.6	24.5 ± 2.0	28.3 ± 1.6	9.5 ± 1.5	9.0 ± 1.4	9.8 ± 0.6	9.7 ± 0.6
B	4	41.3 ± 2.6	52.5 ± 3.6	14.8 ± 1.8	20.0 ± 1.6	11.0 ± 1.2	10.8 ± 1.2	4.9 ± 0.7	5.1 ± 0.7
	5	42.7 ± 3.4	51.6 ± 2.7	14.3 ± 1.7	19.8 ± 1.7	11.2 ± 1.5	11.0 ± 1.2	5.5 ± 0.5	5.6 ± 0.4
	6	43.3 ± 4.0	57.4 ± 5.5	13.8 ± 1.7	16.8 ± 1.9	12.1 ± 1.8	11.7 ± 1.8	5.5 ± 0.5	5.6 ± 0.5
C	7	43.1 ± 3.5	55.6 ± 3.8	22.1 ± 2.0	27.5 ± 2.1	14.0 ± 1.2	16.1 ± 1.2	4.9 ± 0.7	6.3 ± 0.7
D	8	20.9 ± 2.8	40.2 ± 4.0	17.3 ± 1.8	19.2 ± 1.6	9.5 ± 1.5	9.6 ± 1.6	5.0 ± 0.7	6.1 ± 0.7
E	9	32.3 ± 3.6	52.5 ± 3.6	15.2 ± 1.9	19.8 ± 1.5	10.0 ± 1.6	11.8 ± 2.1	5.7 ± 0.4	5.7 ± 0.3
F	10	24.1 ± 4.3	42.9 ± 3.9	15.6 ± 1.5	23.0 ± 2.0	10.8 ± 1.2	11.0 ± 1.2	5.4 ± 0.6	6.4 ± 0.6
G	11	49.6 ± 4.6	70.6 ± 3.3	27.6 ± 2.0	29.5 ± 2.6	8.7 ± 1.2	11.1 ± 1.2	9.7 ± 0.6	10.7 ± 0.8

[1] The values represent means of 20 measurements ±1 *SD* (except mean values for subsidiary cell complex.

[2] Means stomatal frequency = stomata of the leaf surface observed through a 40x objective and 10x ocular (field area = 0.152 mm^2). U = upper surface of leaf; L = lower surface of leaf.

TABLE 4. Summary of vegetative and cuticular characteristics (mean ±1 *SD*) of population groups of *Salix nigra*. Subsidiary cell complex (for upper and lower surfaces) was anomocytic for all population groups.

Trait	Population group						
	A	B	C	D	E	F	G
Leaf length (cm)	11.1 ± 0.1	9.2 ± 0.1	11.3 ± 0.4	8.6 ± 0.4	9.1 ± 0.3	9.5 ± 0.2	11.5 ± 0.6
Leaf width (cm)	1.2 ± 0.0	0.9 ± 0.1	1.1 ± 0.7	0.7 ± 0.1	0.4 ± 0.1	1.0 ± 0.9	1.4 ± 0.9
Petiole length (cm)	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
Stomatal frequency							
Upper surface	47.3 ± 0.4	42.4 ± 1.0	43.1 ± 3.5	20.9 ± 2.8	32.3 ± 3.6	24.1 ± 4.3	49.6 ± 4.6
Lower surface	71.9 ± 0.7	53.8 ± 3.1	55.6 ± 3.8	40.2 ± 4.0	52.5 ± 3.6	42.9 ± 3.9	70.6 ± 3.3
Largest stoma (μ)							
Upper surface	24.8 ± 0.4	14.3 ± 0.5	22.1 ± 2.0	17.3 ± 1.8	15.2 ± 1.9	15.6 ± 1.5	27.6 ± 2.0
Lower surface	26.3 ± 1.7	18.8 ± 1.7	27.5 ± 2.1	19.2 ± 1.6	19.8 ± 1.5	23.0 ± 2.0	29.5 ± 2.6
Smallest stoma (μ)							
Upper surface	9.3 ± 0.2	11.4 ± 0.5	14.0 ± 1.2	9.5 ± 1.5	10.0 ± 1.6	10.8 ± 1.2	8.7 ± 1.2
Lower surface	9.6 ± 0.6	11.1 ± 0.4	16.1 ± 1.2	9.6 ± 1.6	11.8 ± 2.1	11.0 ± 1.2	11.1 ± 1.2
Epidermal wall undulations							
Upper surface	9.8 ± 0.2	5.3 ± 0.3	4.9 ± 0.7	5.0 ± 0.7	5.7 ± 0.4	5.4 ± 0.6	9.7 ± 0.6
Lower surface	10.7 ± 0.3	5.4 ± 0.2	6.3 ± 0.7	6.1 ± 0.7	5.7 ± 0.3	6.4 ± 0.6	10.7 ± 0.8

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