sidering such an approach as described herein. A single course in no way constitutes a comprehensive environmental education program. Rather, it can serve as a foundation for the development of a total program or a method of broadening the scope of an existing program.

No city or community, large or small, is immune to the problems associated with environmental degradation. A prime opportunity exists to involve students, particularly those of high school age, in the study of environmental problems at the local level. A course, such as the one described here, is a good starting point in equipping the student with the techniques and skills needed to realistically evaluate and solve problems of the environment that will be with them throughout their lives.

Finally, it must be realized that an endeavor of this experimental nature does not provide a ready-made panacea for the many curricular problems and conflicts within the field of environmental education. However, it does provide a foundation upon which others may build. It is from this cyclic process of design, development, trial, evaluation, and revision that the urgently needed unique approaches to environmental education will be forthcoming.

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LEAF CUTICULAR AND MORPHOLOGICAL VARIATIONS IN *PLANTAGO LANCEOLATA* AS INDICATORS OF ENVIRONMENTAL POLLUTION

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Abstract

Samples of *Plantago lanceolata* were studied to determine the relationship between environmental pollution and variation in leaf cuticular and gross morphological features. Stomatal frequency on the upper surface of leaves from the polluted area was higher than that from the non-polluted area. Trichome density and trichome length may be correlated with assumed high environmental pollution. Leaf size decreased in polluted areas. Leaf length—width ratio was highly variable. Stomatal size range and number of subsidiary cells remained the same in all populations.

Introduction

Environmental effects upon plants have been widely studied. Differences in anatomical leaf structure among populations of *Oxyria digyna* in diverse arctic and alpine environments were found by Au (1968). Sharma and Dunn (1969) found that under controlled experimental conditions gross morphological characteristics conventionally used in taxonomy (height, leaf length, and leaf width) were less reliable than several microscopic characters. In *Verbena canadensis* the morphological and epidermal characters which were not affected by environmental variations were leaf length/width ratio, stomatal size ranges, the subsidiary cell pattern, and the absence of trichomes on the upper surface (Sharma, 1972).

Plant responses to pollution have also been studied. As pointed out by Haagen-Smit, et al. (1952), sublethal concentrations of toxic agents had a depressing effect on plant growth. Their investigation demonstrated the utility of plants in analyzing air pollutants. They showed for the first time that hydrocarbons, normally harmless air pollutants of organic nature, can cause severe damage through their reaction with substances known to be in the air. Solberg and Adams (1956) found it was difficult to ascertain whether the upper epidermis is injured by pollutant accumulation from inside the leaf of a dicotyledonous plant or through exposure to the pollutant on the outside. They concluded that the

spongy mesophyll and lower epidermis first collapsed, and the upper epidermis finally distorted and collapsed last.

Ozone sensitivity in tobacco leaves was inversely related to leaf maturity as determined by rate of leaf expansion. Recently mature leaves were more ozone-sensitive than older or rapidly expanding leaves (Menser et, al. 1963). In addition, it was found that the physiological age of bean plants and not the degree of stomatal opening determines the susceptibility to the oxidants ozone and peroxyacetyl nitrate (Dugger, et al. 1962).

In a series of experiments of an exploratory nature on the developing first leaf of barley seedlings (Stebbins, et al., 1967), the following were applied: hot water shock at 35°C; extreme lowering of pH by HCl and raising it by NaOH and NaCl; indole acetic acid, gibberellic acid, and kinetin at various concentrations; weak herbicides such as maleic hydrazide and isopropyl-phenyl-carbonate; and colchicine. All of these agents produced some depression or abnormalities of growth and many of them partially inhibited subsidiary cell formation. Only colchicine produced a small percentage of reorientations of the mitotic spindle of stomatal guard cell divisions (Stebbins, et al., 1967).

Mishra and Pradhan (1972) found the most important outcome of their investigation is the possibility of using growth retardants for decreasing transpiration without affecting yield in tomato plants. Sharma and Butler (1973) found that stomatal frequency in *Trifolium repens* L. seemed to decrease with increasing amounts of pollution. A correlation between high trichome density and assumed high pollution factors was found. Sharma and Tyree (1973) found reduced leaf size in *Liquidambar styraciflua* L. in polluted environments which not only indicate retarded growth, but also a reduction in surface area of the leaf to be exposed to the pollutant.

Although significant attention has been given to the anatomical study of cuticular features for taxonomic purposes, little attention has been given to the importance of cuticular features as indicators of environmental pollution. The objective of this study was to determine the relationship between environmental pollution and variation in leaf cuticular and gross morphological features in populations of *Plantago lanceolata*.

METHODS

Twenty populations comprising ten samples each of *Plantago lanceolata*, which was chosen for its relative sensitivity to air pollution, were collected from different locations. Ten populations were studied from the Nashville, Tennessee, area along with ten populations from the Center Hill area in DeKalb and Putnam Counties in Tennessee. The overall variations in cuticular features were minimized by collecting the samples from similar microhabitats.

Macroscopic data were tabulated from each plant in the twenty populations representing a total of two hundred plants. Plant height, leaf size, petiole length, inflorescence length, and leaf length/width ratio were recorded from each plant. From each of the twenty populations one plant was randomly chosen for microscopic study. The leaves to be examined were taken from an intermediate area between the innermost and outermost leaves around the basal ring. Cuticular imprints were made of the leaves using a method developed by Sinclair and Dunn (1961). To minimize cuticular variations the leaves were consistently examined at the area halfway between the tip and base and between the midvein and margin. Microscopic data on stomatal frequency and size range, trichome density and lengths, and subsidiary cell number and type were collected from twenty-five fields counted at random from each slide. Although actual pollution levels were not monitored, there were relatively high pollution levels in the Nashville area as compared to those areas of Center Hill.

RESULTS

The samples were organized into two major groups according to locale representing relative high and low air pollution levels (Tables 1 and 2). Ten different sample populations taken from similar microhabitats comprise each major group. A comparison of the statistical means of group A and B indicate that much variation occurred. In group A (Table 3) the mean range of plant height varied from 32.6 cm in a sample from a grass lawn to 58.8 cm from a country roadside in the same locality. Group B (Table 4) showed plant height variation from 20.8 cm in a sample from a lawn approximately one hundred yards from a chemical plant to 49.4 cm for a lawn near a city street within the same locality. Leaf width in group A ranged from 1.2 cm to 2.7 cm. In group B leaf width ranged from 0.9 cm to 1.6 cm. The significant difference in leaf

TABLE 1: *Characteristics of Group 'A'.*

Sample Group	Number of Samples	Locality in Tennessee	Relative Degree* of Pollution	Source of Pollution
1	10	DeKalb Co.	+	Automobile
2	10	DeKalb Co.	+	Automobile
3	10	DeKalb Co.	+	Automobile
4	10	DeKalb Co.	+	Automobile
5	10	DeKalb Co.	++	Automobile
6	10	DeKalb Co.	+	Automobile
7	10	DeKalb Co.	+	Automobile
8	10	DeKalb Co.	++	Automobile
9	10	Putnam Co.	++	Automobile
10	10	Putnam Co.	+	Automobile

* A continuum; + indicating the lowest level of pollution, +++ + indicating the highest level.

TABLE 2: *Characteristics of Group 'B'.*

Sample Group	Number of Samples	Locality in Tennessee	Relative Degree* of Pollution	Source of Pollution
1	10	Old Hickory	++++	Industry, Automobile
2	10	Old Hickory	++++	Industry, Automobile
3	10	Old Hickory	++++	Industry, Automobile
4	10	Madison	+++	Industry, Automobile
5	10	Nashville	++++	Industry, Automobile
6	10	Nashville	++	Automobile
7	10	Nashville	+++	Industry, Automobile
8	10	Nashville	+++	Industry, Automobile
9	10	Nashville	+++	Industry, Automobile
10	10	Nashville	++	Automobile

* A continuum; + indicating the lowest level of pollution, +++ + indicating the highest level.

width range between the two groups is probably due to differences in microhabitats. Of the two groups leaf length showed no significant variation. Leaf length in group A ranged from 10.7 cm to 20.6 cm. In group B leaf length varied from 9.9 cm to 20.0 cm. Petiole length and inflorescence length differences correlated to plant height variations. Group A plants had significantly longer petioles and inflorescences. The mean petiole length in group A ranged from 27.1 cm to 54.4 cm, while the mean inflorescence length varied from 1.9 cm to 4.4 cm. In group B the mean petiole length ranged from 19. cm to 46.9 cm. The mean inflorescence length of group B varied from 1.2 cm to 2.6 cm. These variations are probably related to microhabitat differences.

Stomata were present on both upper and lower leaf surfaces. The stomata were arranged in parallel rows which is the common distribution in leaves with parallel venation (Fahn, 1967). In group A (Table 5) the stomatal frequency on the upper surface varied from 17.0 to 33.5 with a mean of 26.4, while group B (Table 6) on the same surface varied from 23.8 to 38.2 with a mean of 30.6. At the ninety percent level of probability the stomatal frequency on the upper surface of leaves from the polluted area was higher than that from the non-polluted area. On the lower leaf surfaces of groups A and B there is no significant difference in stomatal frequency. In group A the stomatal frequency of the lower epidermis varied from 24.8 to 42.8, while group B ranged from 22.8 to 44.0. In group A the stomatal frequency of the lower leaf surfaces is significantly greater than that of the upper leaf surfaces. However, group B indicates only a slight increase in stomatal frequency on the lower surfaces as compared to the upper leaf surfaces. There was a trend of increased stomatal frequency on the upper surface in areas of high environmental pollution; however, this could not be documented on the lower leaf surface.

Stomatal size range also varied between the two population groups. Group A showed a 11μ to 21μ variation on the upper leaf surfaces. Group B ranged from 10μ to 18μ on the upper epidermis. On the lower leaf surface the stomatal size range of group A varied from 8μ to 19μ, while group B ranged from 10μ to 18μ.

TABLE 3: *Vegetative characteristics of Group 'A'.*

Sample Number	Plant Height $x + b$ (cm)	Leaf Width $x + b$ (cm)	Leaf Length $x + b$ (cm)	Leaf Length/ Width	Petiole Length $x + b$ (cm)	Inflorescence Length $x + b$ (cm)
1	45.5± 5.9	1.6±0.5	12.9± 2.7	7.7	43.4± 3.6	2.3±0.7
2	54.5±10.9	1.9±1.2	16.3± 3.1	8.6	51.3± 9.8	3.3±1.4
3	45.7± 5.5	1.9±0.2	14.7± 2.3	7.7	42.4± 3.9	3.3±0.6
4	42.9± 4.6	1.8±2.8	17.9 ±14.3	10.2	39.5±12.9	3.4±1.8
5	57.4± 8.9	2.7±0.8	20.6± 3.7	7.9	53.0± 8.5	4.4±1.3
6	32.6± 4.0	1.5±1.2	12.6± 6.5	9.9	30.7±11.6	1.9±1.4
7	28.9± 3.1	1.3±0.8	10.7± 1.7	7.6	27.1± 2.0	1.9±0.5
8	49.2± 4.0	1.9±0.8	17.6± 2.7	9.2	45.7± 4.7	3.5±1.4
9	58.8± 8.0	1.6±0.3	17.8± 4.3	12.3	54.4± 7.6	4.4±0.9
10	40.4± 5.4	1.2±0.6	14.1± 2.3	11.1	37.5± 5.2	2.96±1.6

x = mean
b = standard deviation

TABLE 4: *Vegetative characteristics of Group 'B'.*

Sample Number	Plant Height x + b (cm)	Leaf Width x + b (cm)	Leaf Length x + b (cm)	Leaf Length/ Width	Petiole Length x + b (cm)	Inflorescence Length x + b (cm)
1	37.9± 5.0	1.3±0.2	14.7 ±1.9	11.2	35.3±4.4	2.6±1.0
2	39.5± 6.4	1.6±0.2	16.0 ±2.9	10.3	37.2±5.6	2.3±1.0
3	35.5± 9.0	1.5±0.3	13.8 ±2.6	8.9	32.9±8.4	2.6±1.1
4	22.2± 5.2	0.9±0.5	10.1 ±2.3	10.1	20.9±4.9	1.2±0.6
5	20.8± 4.3	1.2±0.4	9.9 ±3.9	8.9	19.2±3.9	1.5±0.5
6	36.6± 5.9	1.2±0.3	15.2 ±3.1	13.6	34.6±5.4	1.9±0.7
7	25.6± 3.2	1.1±0.2	10.5 ±0.9	9.8	23.7±2.9	1.9±0.4
8	49.4±10.3	1.6±0.5	20.0 ±6.9	12.9	46.9±9.9	2.5±0.7
9	37.2± 6.4	1.3±0.3	15.5 ±2.1	12.7	35.4±6.2	1.8±0.4
10	25.3± 6.4	1.1±0.2	10.4 ±3.4	10.1	23.5±6.3	1.7±0.5

x = mean
b = standard deviation

TABLE 5: *Cuticular characteristics of Group 'A'.*

Sample Number	Stomatal Frequency* x + b upper	Stomatal Frequency* x + b lower	Stomatal Size (μ) upper	Stomatal Size (μ) lower	Trichome Density cm² upper	Trichome Density cm² lower	Trichome Length x (μ) upper	Trichome Length x (μ) lower
1	31.9±6.3	36.2±5.1	11-18	12-17	188	286	675	738
2	25.8±3.6	36.6±3.6	16-21	14-18	4	4	500	800
3	26.2±3.1	37.5±4.3	13-18	8-14	0	4	0	900
4	17.0±1.6	42.8±4.4	17-21	11-19	0	4	0	1190
5	22.4±2.0	26.8±4.9	18-21	14-18	48	18	686	1113
6	33.5±5.3	32.8±3.0	13-18	13-17	0	4	0	600
7	24.3±3.4	31.5±2.5	13-18	13-17	22	4	1630	1150
8	27.4±2.2	24.8±5.4	12-17	12-16	0	4	0	650
9	28.8±3.4	35.4±3.7	15-19	12-17	0	0	0	0
10	26.3±2.4	24.4±2.5	13-19	15-18	0	0	0	0

x = mean
b = standard deviation
* = mean stomatal frequency — stomata observed through 43× objective and 10× oculars

TABLE 6: *Cuticular characteristics of Group 'B'.*

Sample Number	Stomatal Frequency* x + b upper	Stomatal Frequency* x + b lower	Stomatal Size (μ) upper	Stomatal Size (μ) lower	Trichome Density cm² upper	Trichome Density cm² lower	Trichome Length x (μ) upper	Trichome Length x (μ) lower
1	37.3±5.1	33.9±3.8	11-18	11-17	198	158	1505	1147
2	34.2±6.4	34.4±4.3	11-16	—	154	110	941	929
3	26.6±2.9	36.5±4.8	13-17	14-18	18	9	1250	1200
4	31.0±4.4	44.0±4.6	13-18	11-16	0	0	0	0
5	28.2±3.1	40.9±3.1	12-14	12-15	26	31	1750	1471
6	24.2±2.8	38.4±2.8	13-18	13-17	22	9	1700	1225
7	33.3±3.9	36.8±4.5	13-17	13-17	0	0	0	0
8	23.8±2.2	22.8±3.2	13-18	13-18	13	13	466	1350
9	38.2±4.2	33.3±4.2	13-18	12-16	57	9	1523	925
10	29.6±4.6	27.2±3.1	10-13	10-13	22	52	750	1145

x = mean
b = standard deviation
* = mean stomatal frequency — stomata observed through a 43× objective and 10× oculars

Trichome density and stomatal frequency were not correlated with leaf size. Instead, they were related to environmental variations. Group A had a trichome density range of 0 to 188/cm² on the upper leaf surface. The mean trichome density of the upper epidermis of this group was 26/cm². The lower surface trichome density mean was 33/cm². Group B had an upper surface trichome density range of 0 to 198/cm². The mean trichome density for this surface was 51/cm². The lower epidermis had a trichome density mean of 39/cm².

Trichome lengths indicate a significant difference between the two groups. In group A trichome length ranged from 675μ to 1,630μ on the upper epidermis and from 600μ to 1,190μ on the lower leaf surface. The mean trichome length for group A was 349μ for the upper epidermis and 714μ for the lower surface. In group B trichome length varied from 466μ to 1,750μ with a mean length of 988μ on the upper epidermis. On the lower leaf surface the range was from 925μ to 1,471μ with a mean trichome length of 939μ. In some instances the extreme trichome length may have been due to the hair cells being forked and dovetailed with one another to form very long trichomes in *Plantago lanceolata* (Metcalfe and Chalk, 1950). Increased trichome lengths of group B may be correlated with assumed high pollution factors. A pubescent leaf may act as an insulator in a polluted environment by shading the leaf cells and thus reducing the temperature of the leaf tissues (Sharma and Tyree, 1973).

The subsidiary cell complex remained constant with the stoma enclosed by a pair of subsidiary cells whose common wall is at right angles to the guard cells. This type is termed the "caryophyllaceous" or diacytic type (Metcalfe and Chalk, 1950). The consistency of the subsidiary cell complex in all the populations indicates it is taxonomically significant.

Discussion

This study points out the variations in the leaf cuticular and morphological features in *Plantago lanceolata,* as indicated by modifications in plant height, leaf length, leaf width, petiole length, stomatal frequency, stomatal size range, trichome density, and tricrome length in the sample groups. Leaf length-width ratio was highly variable. However, in a similar study the leaf length-width ratio remained the same in all populations of *Liquidambar styraciflua* L. (Sharma and Tyree, 1973). The subsidiary cell complex of the diacytic type remained constant in both groups. The subsidiary cell complex has been found to be consistent and is considered as a taxonomically reliable trait (Sharma and Dunn, 1969; Sharma, 1972; Sharma and Butler, 1973; and Sharma and Tyree, 1973).

Stomatal frequency was variable. The stomatal frequency on the upper surfaces of leaves from the polluted area was higher than that from the non-polluted area. Sharma and Tyree (1973) found a tendency for stomatal frequency in *Liquidambar styraciflua* L. to be high in areas of assumed high pollution, although a trend for low stomatal frequency has been observed in *Trifolium repens* L. (Sharma and Butler, 1973).

In this study trichome density and trichome length may be correlated with assumed high environmental pollution. Other studies also indicate increased trichome density and trichome length associated with high environmental pollution. (Sharma and Butler, 1973; and Sharma and Tyree, 1973).

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