EFFECTS OF VEHICULAR EMISSION ON MORPHOLOGICAL CHARACTERISTICS OF YOUNG AND MATURE LEAVES OF SUNFLOWER (Tithonia diversifolia) AND NAPIER GRASS (Pennisetum purpureum)

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ABSTRACT The research was conducted at two study sites, at Crop Techno Station, Puguis, La Trinidad, Benguet, designated as the control site and along Bokawkan Road, Baguio City, designated as the polluted site. Conducted from June to October 2008, the study sought to ascertain leaf responses to vehicular emission in terms of gross morphological and epidermal microscopic changes between young and mature leaves of a dicotyledonous plant, sunflower (Tithonia diversifolia) and a monocotyledonous one, napier grass (Pennisetum purpureum) in indicating the presence of vehicular air pollutants in the environment.

Results revealed that leaf gross morphological changes such as yellowing and browning, deformity in shape, spotting, drying of leaf margins and less hairy features were more observed in plants from the more polluted site than in the control site. The stomatal size was significantly affected by the interaction of plant site and growth stage. Young plants from the polluted site significantly showed larger stomatal sizes than those in the control site. The stomatal index was significantly affected by the interaction of plant sites and growth stages. The young monocots in the polluted site have significantly higher stomatal means than those in the control area. As to the interaction between plant site and growth stage, young plants in Bokawkan (polluted) had significantly higher stomatal index than those in Puguis (control). Plant site, plant type and growth stage significantly affected the trichome length. Mature monocots from the control site exhibited the longest trichome length while the shortest were exhibited by young monocots from the polluted site. Trichome length of both monocots and dicots was significantly affected by the plant sites. The monocot samples from Puguis (control) were longer in trichome length than those in Bokawkan (polluted) while the dicot samples in Bokawkan (polluted) were longer than those in Puguis (control). The effect of the interactions of plant sites, plant types and growth stages on trichome density was not significantly different. The result obtained between the interaction of trichome density and plant types was also the same. Trichome density in young leaves from the control site was significantly higher than those in the polluted site. The two factors namely plant site and plant type significantly affected the chlorophyll content of the leaves. The dicots from the control site have significantly higher chlorophyll contents than those in the polluted site. Plants can capture significant quantities of dust particles from the atmosphere with the potential to improve local air quality. Epidermal leaf surface features, including stomates, trichomes and chlorophyll content in plants growing along roadsides are changed due to the stress of automobile exhaust emission with high traffic density in urban areas. These modifications can be considered as indicators of environmental stress. Among the plant species studied, seemingly monocot types of the young stage considering trichome length and stomatal index are potential species in indicating the symptoms of pollutant assimilation.

INTRODUCTION

Two of the major contributors to air pollution occur primarily in high-density urban areas: these are smoke belching from vehicles, and industrial emissions. Baguio City ranks first in terms of total suspended particulates (TSP) of 119-532 µg/m³ from 1991-2001 among key urban areas as Iloilo City, Davao City and Cebu City outside Manila. The leaf epidermis is the first target of air pollution as the pollutant first passes through the stomata where most of the gas
exchange takes place through these small pores on the exposed surfaces. The two common plants found along roadsides are sunflower and napier grass. The sunflower (*Tithonia diversifolia*) which grows abundantly in the mountains and along roadsides, is a rich source of nitrogen (N) fertilizer for rice. Napier grass (*Pennisetum purpureum*) which belongs to the Poaceae family, is incredibly large. Grasses are treated as horticultural crops when used as turf for lawn, and agronomic when used for pastures (Bautista, 1994).

The study was conducted to look into the effects of air pollution on the dicot and monocot representatives, specifically on selected leaf anatomical and histological features. More specifically, it sought to:

1. Determine the morphological changes in gross leaf features in terms of color, shape and texture;
2. Examine the difference in the epidermal microscopic features in terms of:
   a. Stomatal size and index;
   b. Trichome length and density;
3. Determine changes in chlorophyll content, in plants exposed to vehicular emissions.

**METHODOLOGY**

The specific site in Bokawkan Road, Baguio City, where many vehicles pass by, was designated as the polluted site in this study. It is also an area where heavy traffic often happens and where vegetation is exposed to vehicular fumes everyday. The other site, which is in Crop Techno Station, Puguis, La Trinidad, Benguet, is the control site, where plants are grown in nurseries, is adjacent to a forested area and where vehicular emissions are assumed to be a lot lower.

**Gross leaf morphological changes.** Leaf epidermal leaf abnormalities of both young and mature plants were observed on-site, with the following criteria: change in color (chlorosis, browning, yellowing, spotting or change in the leaf’s normal pigment), shape (normal shape or deformed/modified) and texture (drying and hairiness).

**Stomatal size.** Leaves were wiped with tissue paper and a drop of nail varnish was spread on the marked area of the leaf surface and imprinted to make a film. The film was allowed to dry for a minimum of 15 minutes. The dried film was detached from the leaf using a needle and then mounted on a glass slide making a paradermal leaf epidermal imprint (Gardner *et al.*, 1995). Samples were subjected to microscopic observation. The area of stomatal aperture was taken using the formula to compute the area of a triangle $A = \frac{1}{2}bh$ where:

- $A =$ area
- $b =$ is the width of the stomatal aperture
- $h =$ is $\frac{1}{2}$ the length of the stomatal aperture

**Stomatal index.** This is the ratio of the number of stomata in a given area divided by the total number of epidermal cells in the area (Salisbury, 1927). This is given by the formula $I = \frac{S}{(E+S)} \times 100$ where:

- $S =$ is the number of stomata per unit area, and
- $E =$ is the number of epidermal cells per same unit area.

**Trichome density.** Trichome densities were counted under a light microscope using the low power objective (LPO) with a magnification of 4x. The number of trichomes were counted in a transect of one millimeter scale of the ocular eyepiece with five replicates per young and mature species.
Trichome length. For measurements of trichome length, a calibrated ocular micrometer was used. Ten trichomes were measured per young and mature leaf with three replicates.

Chlorophyll content measurements. A 0.25 g sample of the newly collected leaves were weighed and cut into pieces then placed in a clean mortar. To this was added 20 ml of 80% (v/v) acetone then ground into a fine slurry. Extract was taken by transferring the slurry to a Buchner funnel lined with a pad of Whatman Number 42 filter paper. The grinding procedure was repeated with a fresh 10 ml aliquot of acetone making a second extract to be added to the first one. For the third time, the tissue was grounded with fresh 10 ml aliquot of acetone then filtered into the flask containing the other filtrates making a volume of filtrate to 40 ml.

Three replicates were prepared per treatment. Absorbance was read in a spectronic 20+ spectrophotometer at wavelength 645 and 663 nm and read against an 80% acetone solvent blank. The total chlorophyll concentration was obtained using the equation (Arnon, 1949):

\[ \text{mg total chlorophyll / g} = 20.2(\text{D645}) + 8.02(\text{D663}) \times \frac{\text{V}}{1000(\text{w})} \]

Where:
- \( \text{D} \) is the optical density reading of the chlorophyll extract at the indicated wavelength
- \( \text{v} \) is the final volume of 80% acetone chlorophyll extract
- \( \text{w} \) is the fresh weight in grams of the tissue extracted

**Data Treatment**

Three Factor ANOVA with three replicates was used in the analysis of the study.

**RESULTS AND DISCUSSIONS**

Gross Leaf Morphological Changes

Table 1 shows the gross morphological changes of the different leaves in terms of color, shape and texture. Plant leaves found in Puguis (the control site) are greener and more robust since the surrounding area is forested and few vehicles usually pass by.

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Color</th>
<th>Shape</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawan (polluted)</td>
<td>Monocot</td>
<td>Young</td>
<td>Yellowish</td>
<td>Deformed</td>
<td>Spotted Hairy</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Mature</td>
<td>Exhibited Browning</td>
<td>Deformed</td>
<td>Dried Margin Less Hairy</td>
</tr>
<tr>
<td>Puguis (control)</td>
<td>Monocot</td>
<td>Young</td>
<td>Light Green</td>
<td>Typical</td>
<td>Robust More Hairy</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Mature</td>
<td>Dark Green</td>
<td>Typical</td>
<td>Robust Hairy</td>
</tr>
</tbody>
</table>

These conditions might have produced such observations. Also, browning and yellowing of leaves, as well as spotting were less observed as compared to those found in the polluted site (Bokawan).

Leaves found in Bokawan on the other hand were observed to have yellow spots on the surface, marginal necrosis, tip burn and a deformed leaf shape. Brown and yellowish colors were also commonly observed. However, hairiness were more apparent in young leaves than in mature ones for both site.
The severe pollutant emission from jeepneys and trucks in Bokawkan may have contributed to the abnormal changes in gross morphology of the leaves. Daubenmire (1974) stated that the exhaust from gasoline engines contains hydrocarbons (especially \( \text{CH}_4 \)), oxidation of N (nitrogen) and some \( \text{SO}_2 \) (sulfur dioxide).

**Microscopic Epidermal Features**

**Stomatal size.** Table 2 shows the mean stomatal size of the different plant types and growth stages exposed to the two different sites. There was no significant difference on the mean stomatal size of the different growth stages of the plants collected from the two sampling sites.

**Table 2.** Stomatal size of monocots and dicots (\( \mu \text{m}^2 \)) at different growth stages exposed to the two different sites

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawkan</td>
<td>Monocot</td>
<td>Young</td>
<td>0.088a</td>
</tr>
<tr>
<td>(polluted)</td>
<td></td>
<td>Mature</td>
<td>0.047a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>0.055a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>0.030a</td>
</tr>
<tr>
<td>Puguis</td>
<td>Monocot</td>
<td>Young</td>
<td>0.026a</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td>Mature</td>
<td>0.027a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>0.021a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>0.020a</td>
</tr>
</tbody>
</table>

Means with a common letter are not significantly different at 5% level by DMRT

F-value = 0.47ns

However, the analysis of variance showed that the plant site alone significantly affected the mean stomatal size of plants. The highest mean of 0.088 \( \mu \text{m}^2 \) was exhibited by young monocot plants in Bokawkan while young monocot plants in Puguis had a mean stomatal size of 0.026 \( \mu \text{m}^2 \).

**Interaction effects.** As to the mean stomatal size of monocots and dicots exposed to two different sites, polluted and control, there is no significant difference.

As to the mean stomatal size affected by the interaction effect between plant site and growth stage, there is a significant interaction. The mean stomatal size of 0.071 \( \mu \text{m}^2 \) of young plants and 0.038\( \mu \text{m}^2 \) of the mature plants in the polluted site were relatively larger compared with the smaller mean stomatal size of 0.024 \( \mu \text{m}^2 \) in young plants and 0.023 \( \mu \text{m}^2 \) in mature plants in the control site. The toxic effect of pollutants present in Bokawkan may have contributed to the larger stomatal sizes exhibited by the young and mature plants found in it.

**Stomatal index.** Table 3 shows the mean stomatal index of the interaction between plant site, plant type, and growth stages. There was a significant effect on the mean stomatal indices of the interaction between plant types, plant sites, and growth stages. Higher mean stomatal index were exhibited by young monocot plants with a mean stomatal index of 30.48 % in Bokawkan compared to those of the young monocot plants in Puguis with a mean stomatal index of 13.89 %. Although young and mature dicot plants from both sites showed no statistical difference, higher stomatal index means were exhibited from the polluted site.
Table 3. Stomatal index of monocots and dicots (%) at different growth stages exposed to the two different sites

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawkan (polluted)</td>
<td>Monocot</td>
<td>Young</td>
<td>30.48a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>20.63b</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>17.20b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>17.37b</td>
</tr>
<tr>
<td>Puguis (control)</td>
<td>Monocot</td>
<td>Young</td>
<td>13.89b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>31.67a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>16.00b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>12.73b</td>
</tr>
</tbody>
</table>

Means with a common letter are not significantly different at 5% level by DMRT
F-value = 14.57** (highly significant)

Interaction effects. As to the mean stomatal index of monocots and dicots exposed to the two different sites with differential air quality conditions, no significant interactions were noted. Based on the single factor ANOVA of plant types alone, irrespective of exposure and stage of maturity, this shows that stomatal index may vary between plant types, and in this case, the napier grass recorded more stomates as compared to the sunflower. This could be concluded as something genetic in difference.

As to the mean stomatal index between plant site and growth stage, a significant difference was noted. Young plants collected from the polluted site have a higher mean stomatal index of 23.83 % as contrasted with those in the control site having a mean stomatal index of 14.95 %. Mature plants in both sites showed no statistical difference in that plants collected from the polluted site have a mean stomatal index of 19.00 % and 22.20 % in the control site. This observation on the apparent difference of the mean stomatal index of young plants against mature ones may be attributed to the plants’ ability to combat stressors or pollutants in relation to its growth stage.

Trichome length. Table 4 shows the mean trichome length as affected by the different interactions between plant site, plant type, and growth stage. There is a significant interaction of the plant types and growth stages as exposed to the two different sites.

Table 4. Trichome length of monocots and dicots (µm) at different growth stages exposed to the two different sites

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawkan (polluted)</td>
<td>Monocot</td>
<td>Young</td>
<td>0.390e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>1.017b</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>0.857c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>0.807c</td>
</tr>
<tr>
<td>Puguis (control)</td>
<td>Monocot</td>
<td>Young</td>
<td>0.747c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>1.700a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>1.043b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>0.513d</td>
</tr>
</tbody>
</table>

Means with a common letter are not significantly different at 5% level by DMRT
F-value = 63.66** (highly significant)

The longest trichome length was apparently observed in mature monocot plants in Puguis with a mean trichome length of 1.700 µm and followed by those found in Bokawkan with a mean trichome length of 1.017 µm. Nevertheless, mature dicot plants found in Bokawkan had significantly longer mean trichome length of 0.807 µm than that in Puguis with a mean trichome length of 0.513 µm. Apparently, longer trichomes may be found in the monocot sample than in the dicot sample. The exhaust particles, deposited on leaves could have reduced growth of the trichomes. In polluted areas, in some cases, trichomes/hairs lose their structure due to abrasive
action of exhaust particle deposition. Monn et al. (1995) reported that metals such as lead induce chromosomal abnormalities and also decrease the rate of cell division.

**Interaction effects.** As to the mean trichome length affected by the interaction between plant site and plant type, there is a significant effect. Longer mean trichome length was exhibited by monocot plants found in Puguis with a mean trichome length of 1.223 µm as compared to monocot plants found in Bokawkan with a mean trichome length of 0.703 µm. On the other hand, mature dicots exhibited longer mean trichome length of 0.832 µm in the polluted site than in the control site whose mean trichome length was 0.778 µm. Various trichome lengths may be exhibited by plants of different types. However, there is a significant difference in the mean trichome length of dicots in the polluted site which was longer than in the control site. Statistics revealed that plant sites did not significantly affect the trichome length of young and mature plants.

**Trichome density.** Table 5 presents the mean trichome density as affected by the interaction of the three factors, namely plant site, plant type and growth stages. No significant differences in trichome density were detected among the plant sites, plant type growth stages of plants.

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawkan</td>
<td>Monocot</td>
<td>Young</td>
<td>4.67a</td>
</tr>
<tr>
<td>(polluted)</td>
<td></td>
<td>Mature</td>
<td>2.67a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>3.33a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>4.00a</td>
</tr>
<tr>
<td>Puguis</td>
<td>Monocot</td>
<td>Young</td>
<td>5.67a</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td>Mature</td>
<td>3.00a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>6.33a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>4.67a</td>
</tr>
</tbody>
</table>

Means with a common letter are not significantly different at 5% level by DMRT
F-value = 4.16ns

Trichomes, or plant hairs, are found on vegetative and reproductive structures in all higher plant families. Several factors, including light, temperature, moisture availability and soil conditions affect the development and expression of trichomes. In a number of plants, they have evolved a defensive function (Jasrai and Arya, 2003).

**Interaction effects.** As to the trichome density of monocots and dicots exposed to the two different sites, no significant interactions were observed. Plant species differ significantly in their ability to mitigate traffic pollution due to differences in their leaf surface characteristics such as epicuticular wax, cuticle, epidermis, stomata and trichomes (Kulshreshtha et al., 2005).

As to the trichome density of young and mature plants exposed to two different sites, there is a significant difference. Young leaves exhibited greater mean trichome density of 4.00 per mm in Bokawkan and 6.00 per mm in Puguis as compared with the mature ones with trichome density means of 3.33 per mm and 3.83 per mm, respectively. Changes in the number of trichomes and in composition and concentrations of their exudates throughout leaf development may have important consequences for plant adaptation to abiotic and biotic factors (Valkama et al., 2003). Such factors may include chemical pollutants (abiotic) and insect associations (biotic).

**Chlorophyll content.** Table 6 presents the mean chlorophyll content as affected by the interaction of the three factors, namely plant site, plant type and growth stages. There is no significant effect of the interaction between plant sites, plant type and growth stage on the chlorophyll content of the different leaves.
Table 6. Chlorophyll content of monocots and dicots (mg/g) at different growth stages exposed in the two different sites

<table>
<thead>
<tr>
<th>Plant Site</th>
<th>Plant Type</th>
<th>Growth Stage</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bokawkan</td>
<td>Monocot</td>
<td>Young</td>
<td>1.71a</td>
</tr>
<tr>
<td>(polluted)</td>
<td></td>
<td>Mature</td>
<td>3.18a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>3.24a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>4.44a</td>
</tr>
<tr>
<td>Puguis</td>
<td>Monocot</td>
<td>Young</td>
<td>2.02a</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td>Mature</td>
<td>3.86a</td>
</tr>
<tr>
<td></td>
<td>Dicot</td>
<td>Young</td>
<td>5.38a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>6.12a</td>
</tr>
</tbody>
</table>

Means with a common letter are not significantly different at 5% level by DMRT
F-value = 1.12ns

In instances where chlorophyll content has considerably decreased, pollutant effects may have passed through the inner thylakoid membrane of the chloroplast. The precise proteins affected would rationally vary with the pollutant, but enzymes essential to carbon dioxide fixation appear to be especially sensitive.

Interaction effects. As to the mean chlorophyll content affected by the interaction between plant site and plant type, there is a significant difference. Plant site significantly affected the chlorophyll content of monocots and dicots. The dicot representative had significantly higher mean chlorophyll content of 5.75 mg/g in the control site than the representative monocot plants with mean chlorophyll content of 2.44 and 2.94 mg/g in both sites, respectively. In some monocot plants, the leaf sheath has few organelles and rather small chloroplasts so that at low magnifications the cells appear empty and clear in striking contrast to the chloroplast-rich mesophyll (Laetsch, 1974).

As to the mean chlorophyll content affected by the interaction between plant site and plant type, the chlorophyll content in the following growth stages are comparable.

CONCLUSIONS

Based from these findings, the following conclusions are offered as regards the effect of vehicular emissions:

1. It causes gross leaf morphological changes for both young and mature monocots and dicots.
2. It seemed to affect stomatal size of young leaves through an apparent increase.
3. Vehicular emissions apparently affected stomatal index of young leaves; as to the interaction between plant site, plant type, and growth stages, monocots were more indicative of this response.
4. The emissions seemed to shorten the trichome length of monocots; as to the interaction between plant site, plant type, and growth stages, the shortest trichome lengths were observed in young monocots found in the polluted site. Distinctly otherwise well is the mature dicots where longer trichome lengths were observed in the polluted site than in the control site. Although it may have a protective role as the stomates, severe damage may happen to these epidermal extensions particularly that of monocots due to pollutants in the environment.
5. It apparently decreased trichome density of young plants.
6. It apparently decreased the chlorophyll content of dicot plants.
7. Specifically, monocot types of the young stage along roadsides play a vital role as indicators of automobile pollution considering trichome length and stomatal index. They are
significant sinks for trapping and absorbing many gaseous, particulate, air-borne pollutants indicated by its changes in its leaf morphology and epidermal features.

RECOMMENDATIONS

Further studies on other plants having sensitive response in leaf morphology and epidermal leaf surface features in their stomates, trichomes and chlorophyll content in high traffic density areas must be considered for the early detection of automobile pollution. Aside from the morphological characteristics used and the epidermal leaf features, internal structure of a foliage leaf including the palisade and spongy tissue of the mesophyll could be tested to assess the impact of pollutants released from vehicular exhaust. Levels of chemical pollutants emitted by vehicles on different leaf types should be measured and compared to indicate the plant’s ability to sequester pollutants. More plants of monocot types of the young stage are encouraged to be planted along roadsides as potential species since they apparently indicate the symptoms of pollutant intake in terms of stomatal index and trichome length to improve local air quality.

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