Comparison of Pollen Abortiveness in Four Weed Species Treated with Mercuric Chloride

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ABSTRACT

Pollen grain abortiveness due to mercury exposure was investigated in four species of weeds, namely *Cleome rutidosperma* Mart., *Commelina diffusa* Burm.f., *Ludwigia micrantha* (L.) Hara, and *Stachytarpheta jamaicensis* (L.) Vahl. All four species tested showed mean pollen grain abortion rates significantly higher than those of their unexposed cohorts. Pollen grain abortion was manifested by reduced size and staining deficiencies. Scheffe's test for variability indicated that higher mercury concentrations are required to effect changes in pollen grain abortiveness. The weed species tested can possibly be used as bioindicators of mercury pollution. Because of the plant's ability to absorb mercury, these species can also be considered as possible bioremediators.

Key Words: Pollen abortiveness, Mercury, Bioindicator, *Cleome rutidosperma*, *Commelina diffusa*, *Stachytarpheta jamaicensis, Ludwigia micrantha*

INTRODUCTION

Weeds are sturdy plants that are able to survive in a variety of adverse conditions such as abandoned patch, wasteland, roadside, footpaths, and trails. In the Philippines, weeds abound in a variety of places both rural and urban, including agricultural and industrial areas, cemeteries, and waste dumps. They are known to possess growth rates higher than the crops with which they compete. Even in mining areas contaminated with mercury, weeds have been found to thrive (Regis, 1999). In the studies of Murin (1995) and Micieta & Murin (1996), the weeds were included as indicators of genotoxicity of polluted environment because of their cosmopolitan nature and because their microspores respond to heavy metal pollution by aborting. Of the forty one (41) species tested for genotoxicity, the researchers found thirty (30) that showed significant pollen grain abortion.

Polluted environments in the Philippines include gold mining areas. When the area is favorable to acid mine drainage, heavy metals can come into solution (Jackson & Jackson, 1996). Mercury, a heavy metal used during the amalgamation process in alluvial gold extraction, can be introduced in the mining areas (Kim & Kim, 1996; Jackson & Jackson, 1996). The process of recovering gold from the amalgam is by heating it in an open pan, causing mercury to vaporize. Consequently, the areas within the vicinity of the gold mines become contaminated not only through water runoff and mine tailings but also through dry deposition. Thus, there is a

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need to monitor this kind of environmental problem to safeguard the health of people and other living organisms.

Monitoring pollution may be done through pollen studies (Murin, 1995; Micieta & Murin, 1996). For instance, high pollen abortive tendency above the normal level of 5% (Murrin, 1995) can be used as indicator of mercury pollution of contaminated environments.

The studies of Murin (1995) and Micieta & Murin (1996) suggest that the abortive tendencies exhibited by the pollen grains may be due to genetic and physiological damages. These damages were manifested as (a) larger than normal size, (b) altered form, and (c) staining deficiency. Chapman & Mulcahy (1997) reported that pollen grain abortion is due, at least in part, to non-disjunctional meiotic events because of nucleocytoplasmic incompatibility in experimented hybrids. Other researchers provided additional observation on pollen grain abortion. Larson (1965) for instance observed cytoplasmic death that occured in aborting pollen grains. The cytoplasm became compartmentalized, and this compartmentalization may be due to a modified endoplasmic reticulum (ER). This occurrence was also observed in injured maize root tip cells. The event was followed by organelle degeneration.

In this study, the degree of pollen grain abortiveness as a result of mercury exposure was studied in four species of weeds, namely, *Cleome rutidosperma* Mart., *Commelina diffusa* Burm.f., *Ludwigia micrantha* (L.) Hara, and *Stachytarpheta jamaicensis* (L.) Vahl, to identify possible bioindicators of mercury pollution. Possible bioindicators will be especially useful in remote areas where testing facilities and services are inaccessible and/or expensive. Furthermore, results of this study can help identify promising phytoremediators for mercury pollution.

MATERIALS AND METHODS

Preliminary investigation

A preliminary investigation of at least twenty four weeds growing in comparable sites in Camarines Norte and in Naga City was conducted to be able to select the target weed species for the experiment. The two sites were initially compared as to their weed composition. Based on the vegetational analysis conducted, Sorensen's Coefficient of Similarity (Brower, Zar, & Von Ende, 1990) was computed at 66.67% during the dry season and 70.69% during the rainy season. In the first site in Labo, Camarines Norte, gold mining activities were mostly illicit and miners used mercury for amalgamation. Chemical analyses of soil taken from this site showed an average of 0.747 mg/kg mercury content (Regis, 1999). In the second site, a rural area in Naga City, there was no known history of gold mining activities conducted and chemical analyses of soil recorded only an average of 0.047 mg/kg mercury content. Of the twenty four weeds examined, four showed pollen grain abortion above the normal 5% abortiveness (Murin, 1995). Other characteristics deemed convenient for the selection of possible bioindicator species were: (a) anther can be easily distinguished in the flower, (b) pollen grains were large enough to be seen with ease under 100x magnification of the compound microscope, (c) are present in the two comparable sites chosen during the preliminary investigation, and (d) could be easily grown in hydrophonics set-up.

Hydrophonic set-up

The experiment was conducted inside a greenhouse measuring 75 m^2 located within the school campus of Ateneo de Naga University. Plants were raised in liquid media using the hydrophonic method described by Dushenkov et al. (1995) and Hershey (1995). A modified Hoagland solution was used in the preparation of the liquid media (Hershey, 1995).

For each species studied, sixty hydrophonics bottle were filled with 1 liter nutrient solution each. Ten of these served as control – no $HgCl_2$ was added. Appropriate amounts of $HgCl_2$ were added to the nutrient solution in the other bottles to make the following concentrations: 0.1, 0.3, 0.9, 2.7, and 8.1 ppm. Ten bottles per species were used for each of these concentrations. Full strength Hoagland solution was used in growing *C. rutidosperma* and *S. jamaicensis* while half strength solution was used for *L. micrantha* and *C. diffusa* because these two latter species became vegetative and rarely flowered in full strength Hoagland solution during the first trial.

Two days prior to the preparation of the hydrophonics bottle, young plants of the species for study were collected from the rural area of Naga City (second site mentioned in the preliminary investigation). These plants were given preliminary treatment before being transferred to purely liquid media. They were first planted in clean sand placed in make-shift clean plastic containers and watered with distilled water. After two days, the plants were transferred into the hydrophonics bottles.

The hydrophonics bottles were arranged on six experimental tables. Only one type of treatment was placed in each table. Each bottle was covered with black plastic to prevent light from entering the lower part of the plant. The bottle was also fitted with a hose which was attached to an aerator. Once the young plant was securely set in the bottle, another black plastic covered the set-up to the point where the stem was inserted through a hole in the bottle cap. In addition, each table was provided with a transparent plastic cover to reduce contamination from other sources, such as dust carried by strong winds and splashes from the rain. The cover was also provided with slit openings to allow interchange of air. Every day, the aerators were switched on for 10 hours, from 7:00 AM to 5:00 PM.

For plants raised in full-strength nutrient solution, the various preparations (control and with pollutant) were continually added as soon as the level of solutions fell below the designated 1-liter level. For those raised in half-strength nutrient solution, the exposure to the pollutant was only done once a week and only the nutrient solution was added daily to each set-up when the solution inside the bottles fell below the designated 1-liter level. Every two weeks, the solutions were replaced with new stock.

As soon as the plants bore flowers, flower buds just before anthesis were collected (usually in the early morning or late afternoon). The buds were fixed in ethanol-acetic acid (3:1 v/v). After 24 hours, the solution was replaced with 70% ethanol as preservative, following the methods of Murin (1995). After having collected enough flower buds (about 50 samples), the plants were removed from the hydrophonics bottles, washed, and air-dried. During times of high humidity or rainy days, drying of the samples was done inside a drying oven maintained at 40°-50°C for 12 hours or for several days until the tissues were dried crisp. The dried samples were then pulverized. Two grams of each sample were brought to the Natural Science Research Institute, University of the Philippines (UP NSRI) for chemical analysis of total mercury content using Atomic Absorption Spectrometer or AAS (Bouchard, 1973).

Pollen grain analysis

Thirty flower buds were selected at random from the preserved samples. They were washed in water, placed on a glass slide, and with the aid of a stereomicroscope, one or two anthers, depending upon the species, were separated from the flower. Then, the anther was treated with a drop of stain (1% aniline blue in Lactophenol), cut, and crushed carefully using two fine-point needles. A minute drop of HCl was added to the preparation of *C. rutidosperma* and *L. micrantha* in order to enhance the stain while H_2SO_4 was used for *S. jamaicensis* to remove the outer thin cover and enhance the stained protoplasm. No acid was added to *C. diffusa* because of the tendency of its pollen to burst. A coverslip was then placed over the glass slide and after a few hours (6 hours at the least), the pollen sample was examined under the microscope.

The counting of normal and abortive pollen grains followed the method of Micieta & Murin (1996). This was done with the aid of a compound microscope, a mechanical counter, and a grid measuring 0.5 in. x 0.5 in. (0.25 sq. in). Ten grids were used for both *C. rutidosperma* and *C. diffusa* while 20 grids were used for *L. micrantha*. A total count was made for *S. jamaicensis* because there are only two anthers per flower, and the number of pollen grains of this species is small. According to Murin (1995), normal pollen abortiveness is 5%. Above this value, the abortive tendency may be attributed to other factors such as mercury contamination, which was introduced in the experiment.

RESULTS AND DISCUSSION

Based on the results of the chemical analyses by UP NSRI, the total mercury content of all the

experimented plants (composite of leaves, stems, roots, flowers) treated with 0.3 ppm $HgCl_2$ were as follows: *C. rutidosperma* - 7.6 ppm, *L. micrantha* - 17 ppm, and *Stachytarpheta jamaicensis* - 18 ppm. For *C. diffusa*, mercury accumulated was 56.6 ppm at 0.9 ppm treatment.

Pollen grain abortion

Normal and aborted pollen grains are shown in Figs. 1, 1a, 2, 2a, 3, 3a, and 4. The figures reveal that aborted pollen grains exhibit two types of pollen abnormalities – staining deficiencies and reduction in pollen sizes. Staining deficiencies are manifested as: (a) protoplasm seemed absent due to the pollen's inability to absorb stain, and (b) the protoplasm seemed undeveloped because only part of it is distinct. Figs. 1a, 2a, and 3a present the structures of the aborted pollen under oil immersion objective (1000x). Aborted pollen grains of *S. jamaicensis* are large, so that they are easily distinguishable even at low magnification (Fig. 4). Moreover, none exhibited larger sizes than normal although Murin (1995) and Micieta & Murin (1996) have reported this type of abnormality in some species growing in areas contaminated with several kinds of heavy metals.

The results of pollen grain analyses are presented in Table 1. The mean average pollen abortion in all the four species shows abortive tendencies above the controls. ANOVA analysis under the SAS system suggests a significant difference between the control plants and those raised under various concentrations of $HgCl_2$. Scheffe's test for variability also indicated a reduction in groupings involving concentrations that elicited pollen abortion, but the results did not differ much from their immediate neighbors. For instance, results obtained for *Commelina diffusa* showed that there is no significant difference in pollen abortiveness at concentrations of 0.1 ppm, 0.3 ppm, and 0.9 ppm $HgCl_2$ (Table 1). Also, results obtained for the concentration of 2.7 ppm are not significantly different from 8.1 ppm. Thus, instead of six groups being significantly different from each other (including the control), only 3 groupings were found to be significant: (a) control; (b) 0.1, 0.3,

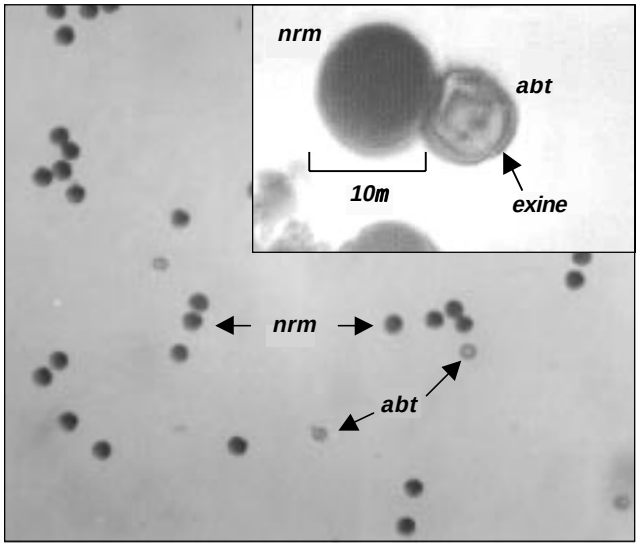

Fig. 1. Pollen grains of *Cleome rutidosperma* Mart. viewed at 100x magnification of the compound microscope. Aborted pollen grains do not stain dark. Normal (nrm) pollen grains measure 10m to 12m in diameter whereas aborted (abt) pollen measures 8m or less.

Fig. 1a (inset). Normal (nrm) and aborted (abt) pollen grains of *Cleome rutidosperma* Mart. viewed under the oil immersion objective at 1000x magnification of the compound microscope. The thick exine is distinct.

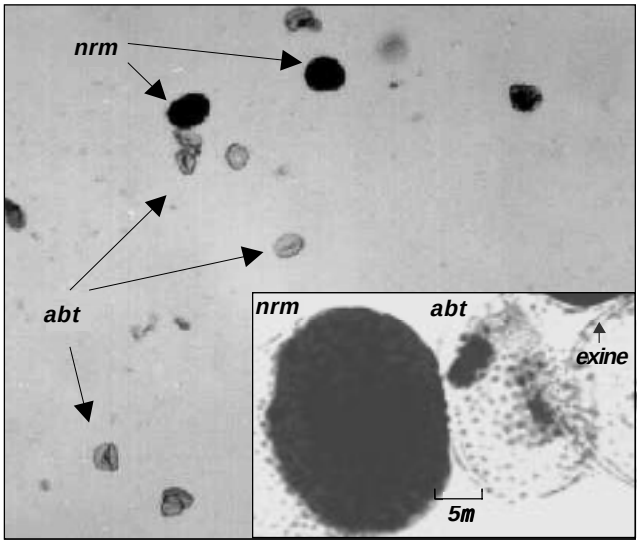

Fig. 2. Normal (nrm) and aborted (abt) pollen grains of *Commelina diffusa* Burm. f. viewed at 100x magnification of the compound microscope. Size ranges of normal pollen is between 20µ to 30µ and aborted pollen, 15µ to 20µ long.

Fig. 2a (inset). Normal (nrm) and aborted (abt) pollen grains of *Commelina diffusa* Burm. f. viewed under the oil immersion objective at 1000x magnification. The undeveloped protoplasm can be seen in the aborted pollen.

Table 1. Mean % pollen grain abortion in flowers from plants under various treatments of $HgCl_2$ (in ppm)

	$HgCl_2$ treatment (ppm)+					
Species	**Control+**	0.1	0.3	0.9	2.7	8.1
Cleome rutidosperma	2.47^{b}	9.74^{a}	8.54^{a}	10.27^{a}	9.50^{a}	11.44^{a}
Commelina diffusa	2.22^{d}	5.21^{c}	6.59^{bc}	6.83^{abc}	9.57^{a}	9.30^{ab}
Ludwigia micrantha	3.51^{c}	9.83^{cb}	*	9.89^{cb}	12.27^{b}	11.84^{b}
Stachytarpheta jamaicensis	2.89^{c}	11.73^{cb}	17.61^{b}	19.29^{b}	21.53^{b}	41.71^{a}

* Results were not included due to observed microbial infection during the experiment.
+ Scheffe's test for variable: Means with the same letter (shown as superscript) are not significantly different from each other; n = 30.

and 0.9 ppm cluster; and (c) 2.7 and 8.1 ppm cluster. Thus, it can be concluded that changes in pollen abortiveness require higher increases in the concentration of pollutants to elicit a response.

For *Cleome rutidosperma*, all the treatments were not significantly different from each other in terms of pollen abortiveness. Only the control was significantly different from the rest of the treatments. It can be concluded, therefore, that any concentrations above the natural concentration of pollutants in plant tissues will elicit abortion of pollen grains. Scheffe's test for *Ludwigia micrantha* on the other hand, revealed that results obtained for control plants are not significantly different from those treated with 0.1 ppm and 0.9 ppm. These were, however, significantly different from those obtained at 2.7 ppm and 8.1 ppm (Table 1). For this species, pollen abortiveness is more distinct when

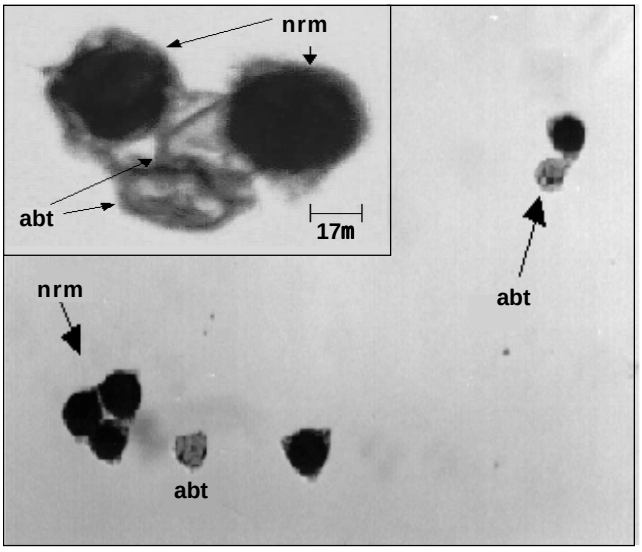

Fig. 3. Normal (nrm) and aborted (abt) pollen grains of *Ludwigia micrantha* (L.) Hara viewed at 100x magnification of the compound microscope. The size of normal pollen is about 36µ while aborted ones measure 20µ in diameter.

Fig. 3a (inset). Normal (nrm) and aborted (abt) pollen grains of *Ludwigia micrantha* (L.) Hara viewed under the oil immersion objective at 1000x magnification of the compound microscope.

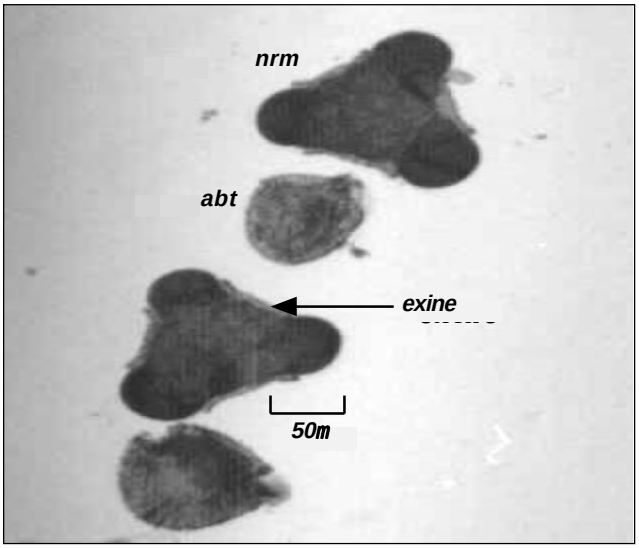

Fig. 4. Normal (nrm) and aborted (abt) pollen grains of *Stachytarpheta jamaicensis* (L.) Vahl. viewed at 100x magnification of the compound microscope. The pollens were treated with small amount of acid to make the protoplasm distinct from the exine.

the pollutant is at higher concentrations, which are 2.7 ppm and above.

Stachytarpheta jamaicensis exhibited 3 groups in the Scheffe's test for variable. Group A at 8.1 ppm corresponds to the highest concentration used in the experiment. Group B, which includes 0.3 ppm, 0.9 ppm, and 2.7 ppm, showed no significant differences in their variability. Group C covered both the control and 0.1 ppm $HgCl_2$. It can be concluded, therefore, that the critical level by which pollen abortiveness becomes significant is at 0.3 ppm and above. Below this concentration, pollen abortiveness is not much different from that of the control.

The absence of protoplasm in some aborted pollen grains of the experimented plants may be due to physiological damage (Micieta & Murin, 1996). It was also observed that aborted pollen grains have thicker exines. This occurrence was seen in *C. rutidosperma* (Fig. 1a) and *S. jamaice*nsis (Fig. 4). This observation was similar with the study of Rowley et al. (1997) wherein aborted pollen grains had thicker exines than the mature normal pollen grains. They concluded that the thicker wall represented early stages of development of the microspore, but its death prevented further development of the exine.

Bioindicators and bioremediators

From among the four species studied, *Stachytarpheta jamaicensis* can be considered as the best bioindicator of mercury pollution because its responses to the various treatments are clearly delineated in the Scheffe's test for variables. When determining only the presence of the pollutant, *Cleome rutidosperma* may be used initially. The two other species are highly variable in their responses to contamination. However, they can also be used only to determine the presence of contamination in the absence of *S. jamaicensis* and *C. rutidosperma*.

This study was also able to discover that the four species experimented have the ability to absorb mercury in their tissues. Thus, they may be considered as potential bioremediators of mercury pollution in contaminated sites.

CONCLUSION

Based on the results presented above, the accumulation of mercury in plants at higher concentration above the critical level of 0.3 ppm for plant tissues (Pfeiffer et al., 1988) resulted in pollen grain abortion. Pollen abortiveness has also been confirmed by the results of statistical analyses. The variability between treatments, as well as within the samples examined per laboratory treatment showed significant differences between the control and contaminated materials. Furthermore, pollen grain abortion was manifested by abnormalities in the structure of the pollen grains through staining deficiencies and reduction in sizes.

These results suggest that the experimented species can be used as bioindicators of mercury pollution. Likewise, the ability of the experimented plants to absorb mercury also imply that these species can be possible bioremediators of mercury-polluted areas.

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