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ANALYZING THE MONOCYCLIC PROCESS IN SHEATH BLIGHT OF RICE UNDER SEMI-CONTROLLED CONDITIONS

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ABSTRACT

Rice trap plants, used as probes, were exposed in quadrats that were inoculated with sheath blight (*Rhizoctonia solani* Kuhn) to study the spread of the disease. The effects of leaf wetness regime, leaf contact frequency and strength of inoculum source were quantified using this approach. Environmental variables were manipulated in the quadrats by covering them with plastic cages for different durations (leaf wetness), planting hills at different spacings (leaf contacts) and varying the amount and placement of inoculum in the canopy (source strength). Each experiment involved three successive batches of trap plants.

The infection efficiency increased with the accumulation of wet and dry daily cycles. Incidence and severity on sheaths increased with increased crop density. Increasing the amount of initial inoculum led to increase of disease incidence, leaf severity and number of infection points. Most disease variables were higher in treatments involving placement of initial inoculum at the leaf level compared to placement of the same amount of inoculum at the base of the plants. Disease spread declined with the successive batches of trap plants suggesting a decline in the number of infectious lesions over time.

INTRODUCTION

Sheath blight (ShB) is a fungal disease of rice caused by *Rhizoctonia solani* Kuhn (*Thanatephorous cucumeris*) (Frank) Donk. Under favorable conditions, the disease causes lesions on leaf sheaths, which coalesce, and spread to the upper leaf sheaths and on the leaf blades. In the last three decades, as modern, semidwarf nitrogen-responsive cultivars were introduced, the economic importance of sheath blight increased in many rice growing regions of the world (Teng, 1990). In both lowland and upland rice production areas, 25-50% yield loss could be incurred in rice as the disease develops on the flag leaf (Kannaiyan and Prasad, 1978). Roy (1979) found that 36% yield loss could be incurred at tillering stage and 11.73% at booting stage inoculation (Tsai, 1974). In the Philippines, the range of yield losses in farmers' fields was reported as negligible (0.4% – 23%) depending on the variety and the nitrogen input to the crop (Ou and Bandong, 1976)

Detailed experiments are necessary to study and quantify epidemiological mechanisms. Details of the different environmental factors associated with sheath blight (ShB) epidemiology can be derived from experiments under semi-controlled conditions where the host, the pathogen and some environmental factors can be manipulated. Factors possibly affecting ShB epidemics, such as leaf wetness, crop density and leaf contact frequency, and the strength on inoculum source, have been selected for screenhouse experiments. These factors have therefore been artificially manipulated (stimulus), in order to measure the disease response (Zadoks, 1972). Several biotic and abiotic factors can be manipulated in quadrats of rice hills that represent field plots. The disease response can be quantified by the use of trap plants that can probe the conduciveness of a given environment in a quadrat. Measurements of disease parameters can be derived from both the trap plants and the quadrat. Knowledge on the dynamics of sheath blight can help in developing a simulation model of ShB epidemiology that can be used to formulate methods for successful management of sheath blight.

Most epidemiological studies on sheath blight have been conducted in temperate countries. In Japan, detailed ecological studies on sheath blight have been conducted to develop a computerized forecasting system. Hashiba and Ijiri (1989) developed a computerized forecasting system of yield losses due to sheath blight (BLIGHTAS). However, this cannot be used under tropical conditions where effects of climate on sheath blight epidemiology might strongly differ from those of temperate regions. Some aspects of sheath blight epidemiology in the tropics have already been studied by many researchers. However, information on the effects of leaf wetness duration, the amount of initial inoculum and some cultural practices on sheath blight spread are still lacking.

The experiments were conducted from March to May 1992 in the screenhouse of the International Rice Research Institute, Los Baños, Laguna, Philippines. The objectives of the study were: to develop an epidemiological method for the study of monocyclic processes in sheath blight; to establish a functional relationship

between different leaf wetness durations and sheath blight spread; to establish a functional relationship between different crop density treatments and sheath blight spread; and to establish a functional relationship between different levels of initial inoculum and sheath blight spread.

MATERIALS AND METHODS

Experimental Environment, Land Preparation and Crop Establishment

Experiments (Table 1) were conducted in a screenhouse enclosed with wire mesh, with an average temperature of 31.7/28.5°C, relative humidity of 68.3/72%, and light intensity of 690/1050 watt/m^2, inside and outside of the screenhouse. The experimental area was plowed and harrowed 10 days before transplanting. Basal application of 80 kg/ha urea (45-0-0) was carried out during the final harrowing and levelling, a day before transplanting. A short-culmed rice cultivar, IR72, raised in a dapog seed bed, was used in all experiments. Plants were transplanted 10 days after sowing at 6 seedlings per hill, 20 x 20 cm spacing, except for spacing treatments in experiment 2 (Table 1). At maximum tillering stage, plants were topdressed with 40 kg/ha urea.

Inoculum Preparation and Inoculation

Sheath blight inoculum was prepared following Mew and Rosales (1986). Isolate AG-1-LR-1 of *Rhizoctonia solani* Kuhn was mass cultured in PDA plates for five days. Rice-grain-hull (RGH) was thoroughly mixed at 1:5 ratio. The mixture was soaked in water for two hours. Heat resistant bottles, measuring 8 x 20 cm, were filled with the RGH mixture to 80 percent, covered with aluminum foil and tied with rubber bands. The RGH-filled bottles were autoclaved at 100,000 Pascal at 121°C for two hours. A five-day old culture of *R. solani* in PDA plates was inoculated to RGH mixture at 1:4 (agar plate: RGH bottles) ratio after cooling. The inoculated mixture was used as source of ShB inoculum after 10 days of incubation at room temperature. Source hills were inoculated with ShB at maximum tillering stage, 40 days after transplanting. Except for experiment 3, stems and leaves were inoculated with 5 g of ShB inoculum following the insertion method of Yoshimura and Nishizawa (1954). The inoculum was placed directly at the base of each hill above the water line. In all experiments, leaves in each hill were held together by a rubber band tied at about 15 cm below the uppermost leaves and removed seven days after inoculation. To enhance infection, inoculated plants were sprayed with water three times a day and covered with plastic cages every night for seven consecutive days.

Quadrat and Trap Plant

An experimental unit was composed of a quadrat of 3 x 3 hills: 8 source hills and 1 trap plant. The latter was a disease-free hill that had been grown separately, and then transplanted at the center of the quadrat. The source hills were inoculated plants surrounding the trap plant. The quadrat was used to represent field plots where several biotic and abiotic factors could be manipulated. The trap plant was used to probe the conduciveness of a given environment, i.e., manipulated conditions prevailing in the quadrat, for disease spread. Any change on the trap plant with regard to ShB severity, number of infection points and incidence reflected the conduciveness of the environment to disease in the quadrat. Each quadrat was sprayed with water and covered with plastic cage for 12 hours, every night for three days except for leaf wetness treatments in experiment 1 (Table 1). After exposure, the trap plant was transferred into pots, sprayed with water and covered with plastic cage for another three days.

Treatments

Three experiments were conducted to test the effects of a series of environmental factors on the srpead of sheath blight using the trap plant and the quadrat. The treatments (Table 1) in all experiments were aimed at manipulating the quadrats prior to and during the exposure of the trap plants. Each treatment was represented by quadrats randomly distributed in replicates. Leaf wetness duration in experiment 1 was manipulated by covering the quadrats with plastic cages. In experiment 2, contact frequency between plant tissues (leaves and sheaths) was manipulated by varying the density of hills. In the first two experiments, 5 g of ShB inoculum per hill was used. The amount was either decreased or increased, and placed at different positions on source plants in experiment 3.

Experimental Design

All experiments were laid out in a randomized complete block design. Except for experiment 2, quadrats were separated by a row of border hills, while alleys (30 cm wide) were provided to separate replications. In experiment 2, the quadrats were assigned to 1 m x 1 m plots with hills planted at spacing similar to that of the assigned quadrat. Plots and replications were separated by 40-cm wide alleys. Three batches of trap plants were exposed successively into quadrats at three-day intervals.

Collection and Analysis of Data

Four variables (Table 2) for disease measurement were considered simultaneously because one variable may not sufficiently reflect the factors that contributed to the production of new lesions. A few infection points could for instance result in

a high severity. Infection efficiency was quantified as the ratio of the number of infection points on the trap plant to the number of infection points on the source hills. Infection points on the stems and on the leaves were considered to account for the spread of disease from the source hills to the trap plant by leaf-to-leaf and leaf-to-sheath contacts. Sheath blight severity, count of infection points and incidence on tillers (Table 2) were gathered from the source hills and from the trap plants. The source hills were assessed for ShB prior to exposure of the trap plants in the quadrats. One source hill was randomly chosen and five of its tillers were assessed for ShB. On reach of the trap plants, all the tillers and their leaves were assessed for ShB after the three-day incubation in pots. All variables used in ShB assessment (Table 3) were analyzed using a repeated-measures ANOVA (Madden, 1986). Arc-sine transformation was applied on data gathered from variables 1 and 4 while for variable 3, log transformation was used to normalize the distribution of values (Gomez and Gomez, 1984).

RESULTS

Experiment 1
Effect of Leaf Wetness on the Spread of Sheath Blight

The effects of leaf wetness regimes on sheath blight development on trap plants are presented in Table 4. Significant differences were found among the treatments for all variables, except for severity on stems (Ss). Disease parameters on trap plants were usually highest in treatment E (intermittent wet and dry 12/12 h periods) followed by treatment F (continuous wetness). Treatment E was found signficantly different from other treatments for all variables, except for incidence on tillers (Nit/Nt) and Ss.

Batches of trap plants were observed to strongly differ in sheath blight development as represented by significant ($P<0.01$) variance ratios for all variables. A strong decline among batches was observed for all variables, particularly infection efficiency (IE), which was highest in batch 1 (mean = 0.61), followed by batch 2 (mean = 0.09) and batch 3 (mean = 0.07) (Table 5).

The interaction of batches with treatments (A x B) was not significant for any variable except IE ($F = 3.44$, $P < 0.01$). The strong A x B interaction on IE indicates that treatments were ranked differently among batches (Table 5). Treatment D ranked second in batch 1, fourth in batch 2 and third in batch 3. Treatments E and B consistently ranked first and fifth, respectively, in all batches. Treatment C in batches 1 and 2 ranked third, while treatment F ranked second in batches 2 and 3.

Experiment 2
Effect of Crop Density on the Spread of Sheath Blight

There were no significant differences among crop density treatments with respect to SI, IPs + IPI and IE (Table 6). Nit/Nt, however, significiantly increased with increasing crop density. It was higher in treatment A (mean = 0.53, 15 x 15 cm spacing) than treatments C (mean = 0.17, 20 x 20 cm spacing) and D (mean = 0.15, 25 x 25 cm spacing). A similar effect was observed with respect to Ss, i.e., increase in sheath blight severity on the stem as crop density was increased.

A strong batch effect was observed on severity on leaves (SI), total number of infection points on stem and leaves (IPs + IPI) and IE as indicated by their variance ratios significant at $P < 0.01$. Values of some disease variables decreased among the batches of trap plants. This effect was not noted for Nit/Nt and Ss. No significant interaction of treatments with batches (A x B) was noted for any of the variables.

Experiment 3
Effect of the Strength of Inoculum Source on the Spread of Sheath Blight

Table 7 shows the effect of the strength of the inoculum source on sheath blight spread. Significant treatment effects were found on Nit/Nt ($F = 13.8$, $P < 0.01$), SI ($F = 19.5$, $P < 0.01$) and IPs + IPI ($F = 13.8$, $P < 0.01$). High amount of initial inoculum applied to the source hills accounts for high disease severity on trap plants as indicated by highest means attained for all variables on treatments F and G, where total amounts of initial inoculum were 10 g and 7.5 g per hill, respectively.

The position of inoculum on the leaves had a stronger effect on disease variables than positioning the same amount of inoculum on the stems. This is particularly shown in comparing means for SI and IPs + IPI between treatments D (SI = 0.24, IPs + IPI = 3.09) and E (SI = 0.48, IPs + IPI = 4.24).

There was a significant batch effect ($F = 4.76$, $P < 0.05$) on IPs + IPI. This suggests a decrease of infection points within the source hills of the quadrats with the successive batches of trap plants. The interaction of treatments with batches (A x B) was significant ($F = 1.94$, $P < 0.05$) on SI, i.e., ranking of treatments varied among batches. Treatment F had the highest mean in batches 1 (0.70) and 2 (0.71), but not in batch 3 where it ranked fourth (mean = 0.37). In treatment G, mean values in batches 1 (0.61) and 2 (0.51) ranked third but had the highest mean in batch 3 (0.46). Consistent ranking in all batches was observed for treatments C and H which ranked fifth and eighth, respectively. There was a decline of treatment mean values from the first to the third batch in most of the variables. This decline was strong in SI (Table 8) with mean values across treatments of 0.44 in batch 1, 0.41 in batch 2 and 0.35 in batch 3. Strong block effect was observed in SI ($F = 13.5$, $P < 0.01$) and IPs + IPI ($F = 5.92$, $P < 0.01$).

DISCUSSION

Screenhouse experiments were conducted to understand the dynamics of sheath blight. The effect of leaf wetness duration, crop density and strength of inoculum source on sheath blight spread was quantified using a probe (trap plant) exposed in a manipulated environment (quadrat).

Moisture on crop canopies has been the basis of attempts to develop several disease forecasting methods (Fry, 1982). Quantification of the influences of water-related variables, such as daily rainfall, rainfall intensity and duration, humidity and surface wetness, can help develop prediction rules for disease development. Among the water-related variables, leaf wetness duration often has a direct influence on pathogen activity. In some pathosystems, leaf wetness periods are often regarded as synonymous with infection periods and can account for a large amount of the variability in subsequent disease intensity (Royle and Butler, 1983).

The first experiment conducted, with strong differences between leaf wetness treatments, showed that leaf wetness regimes play an important role in ShB development and spread (Table 4). Comparison of treatment mean values indicated that ShB is particularly enhanced when subjected to intermittent wet and dry periods. Sheath blight severity and incidence were lower on trap plants subjected to continuous wetness (treatment F) than to intermittent wet and dry periods (treatment E). Increased leaf wetness period (treatments A to E) was associated with increased ShB intensity. Similarly, focus expansion of Rhizoctonia aerial blight of soybean is enhanced by prolonged free moisture (Yang et al., 1990). The mild effect of treatments on ShB severity on the stems may be due to the short exposure (3 days) of the trap plants in the quadrats.

The strong decline of ShB infection efficiency from batch 1 to batch 2 and batch 3 suggests that the number of infectious lesions on the source hills has declined during the exposure of the second and third batches of trap plants. This would mean that lesions in this experiment were infectious only for 3-10 days after inoculation. Zadoks and Schein (1979) called this phenomenon in the epidemic process as "removal", the transition from the infectious to the non-infectious state. Lesions that are no longer infectious were removed from the epidemic process.

The interaction between treatments and batches on infection efficiency suggests that treatment effects differed among batches of trap plants. Infection efficiency on trap plants subjected to continuous wetness (treatment F) ranked fourth in batch 1 and second in batches 2 and 3. Continuous wetness therefore appears to slow down the removal process, i.e., contributes in prolonging the infectious period of the inoculum.

Crop and canopy densities influence the microclimate within canopies and haveb een shown to increase ShB incidence and severity (Premalatha Dath, 1990). Results of the second experiment (Table 6) showed that incidence on tillers and severity on stems were significanlty affected by the spacing treatments. Incidence and severity were higher in the 15 x 15 cm (treatment A) and 15 x 20 cm (treatment

B) spacing treatments than in the 20 x 20 cm (C) and 25 x 25 cm (D) spacing treatments. In other words, disease intensity was higher with increased crop density. This result conforms with many reports (Roy, 1978; Srinivasan, 1980; Hori, 1982; Kannaiyan and Prasad, 1983; Ou, 1985). In this experiment, manipulating the plant spacing amounts to altering the contact frequency among healthy (trap plants) and diseased (source hills) plants in constant, favorable climatic conditions. There is high contact probability among the plants in dense planting so that disease is easily spread from plant to plant (Premalatha Dath, 1990).

The effect of crop density was mild on severity on the leaves, total number of infection points and infection efficiency. Although contact frequency was higher among the leaves than on the stems, temperature and relative humidity below the canopy were usually more favorable for ShB development (Ou, 1985). This could explain why the crop density variation had a significant effect on the stems (incidence and severity) and not on the leaves.

Strong differences were found among the batches of trap plants on severity on the leaves, total number of infection points and infection efficiency. For incidence and severity on the stems, batch effect was not significant. This could be attributed to the frequency of contact between the source hills and the trap plant where contact among the leaves was higher than contact between the stems.

The results of the third experiment (Table 7) indicate that the amount of inoculum in the source (strength) affects ShB spread. The effect of amount of initial inoculum was significant on incidence, severity on the leaves and on the total number of infection points. Except for infection efficiency, all variables had highest mean values on either treatments F or G which correspond to an inoculum amount of 10 g and 7.5 g per hill, respectively. All other treatments with low amount of initial inoculum had also low mean values in all variables. For severity on the leaves and the total number of infection points, significant differences were found when treatments F and G were compared to treatments A, D and H. This indicated that increasing the amount of initial inoculum from 2.5 to 7.5 and 10 g induced ShB severity, particularly the incidence on tillers, severity on leaves and the number of infection points. A similar result expressed by using area under the disease progress curve was obtained by Sharma (1989).

Positioning of inoculum on the leaves had a stronger effect on disease intensity than positioning the same amount of inoculum on the stems. The growth habit of transplanted rice (Vergara, 1979) allows contact between hills. During the third experiment, it was observed that leaf-to-leaf and leaf-to-sheath contacts markedly increased from early to maximum tillering stages. The frequency of leaf-to-leaf contacts was higher than the frequency of leaf-to-sheath contacts. This probably explains the higher ShB severity on trap plants exposed in quadrats that had been inoculated at the leaf level.

The interaction between batches and treatments with respect to severity on the leaves was indicative of changed treatment effects in each batch. For instance, treatment F (10 g of inoculum per hill) had the highest mean in batches 1 and 2, but

not in batch 3 where it ranked fourth (Table 8). Treatment G (7.5 g of inoculum per hill) had the third mean severity on leaves in batches 1 and 2, but had the highest mean in batch 3.

The quadrat and the trap plant were used to understand the effects of a series of environmental factors on the dynamics of ShB. This approach revealed that under semi-controlled conditions, intermittent leaf wetness, increased contact frequency, increased amount of initial inoculum and positioning of inoculum at the leaf level favor ShB spread. The methodology can be used to address a number of driving variables of the rice-Shb pathosystem.

Table 1. Screenhouse experiments with their corresponding treatments.

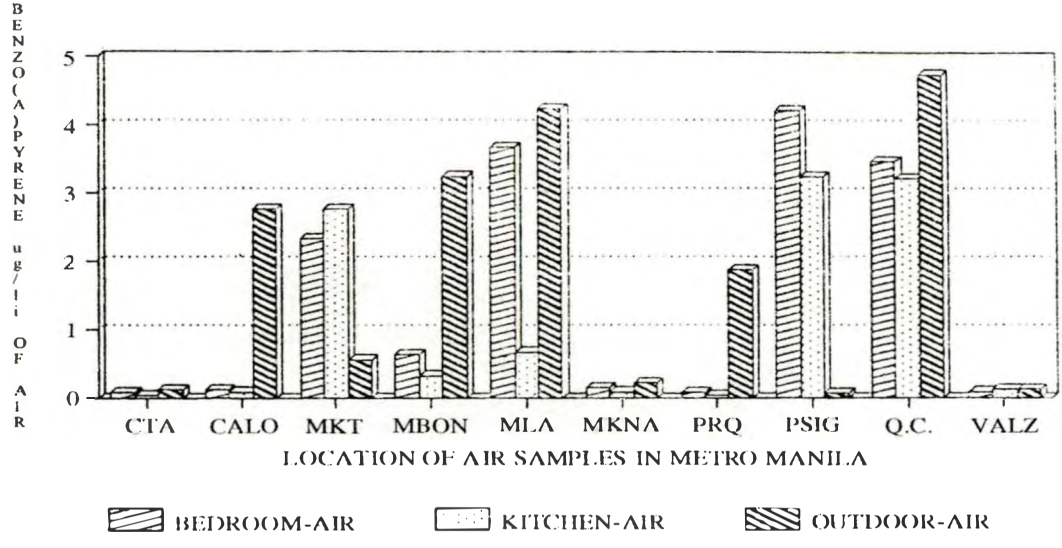
Experiment	*Treatment*
1 Leaf Wetness (5 reps)	A – non-inoculated quadrats; no water spray and no caging
	B – inoculated quadrats; no water spray and no caging
	C – inoculated quadrats; with water spray and 12-hour (1 night) caging
	D – inoculated quadrats; with water spray and 24-hour (2 nights) caging
	E– Inoculated quadrats; with water spray and 36-hour(3 nights) caging
	F – continuous wetness; treatment E plus water spray on day time, every hour for 3 days
2 Crop Density (4 reps.)	A – 15 cm x 15 cm spacing(49hills/m^2) B – 15cm x 20cm spacing(42hills/m^2) C – 20cm x 20cm spacing(35 hills/m^2) D – 25cm x 25cm spacing(25 hills/m^2)
3 Strength of inoculum source (5 reps.)	A – 2.5 g of inoculum on the stems
	B – 2.5 g of inoculum on the leaves
	C – 2.5 g of inoculum on the stems and on leaves (5 g/hill)
	D – 5 g of inoculum on the stems
	E – 5 g of inoculum on the leaves
	F – 5 g of inoculum on the stems and on the leaves (10 g/hill)
	G– 5 g of inoculum on the stems and 2.5 g on leaves
	H – no inoculum

Table 2. Operational definition of the variables used in ShB assessment

	Variable	*Definition*
1.	Incidence	The ratio of the total number of infected tillers over the total number of tillers per hill
2.	Infection point	A typical sheath blight lesion which may or may not expand and coalesce with other lesions
3	Infection efficiency	The ratio of the total number of infection points on the trap plants over the total number of infection points on the source hills
4.	Severity	The percent area covered by sheath blight lesions on the host tissues

Table 3. List of variables used for sheath blight assessment

Acronym	*Meaning*	*Unit*
Nit/Nt	Incidence on tillers	%
Ss	Severity on stem	%
Sl	Severity on leaves	%
IPs + IPI	Total infection points on stems and leaves	number
E	Infection efficiency	



Cta-Cainta; Calo-Caloocan; Mkt-Makati;
Mbon-Malabon; Mla; Q.C.; Prq-Parañaque;
Psig-Pasig; Valz-Valenzuela; Mkna-Marikina

Figure 3. Benzo(a)pyrene content of organic extracts of air particulates from indoor and outdoor air in Metro Manila

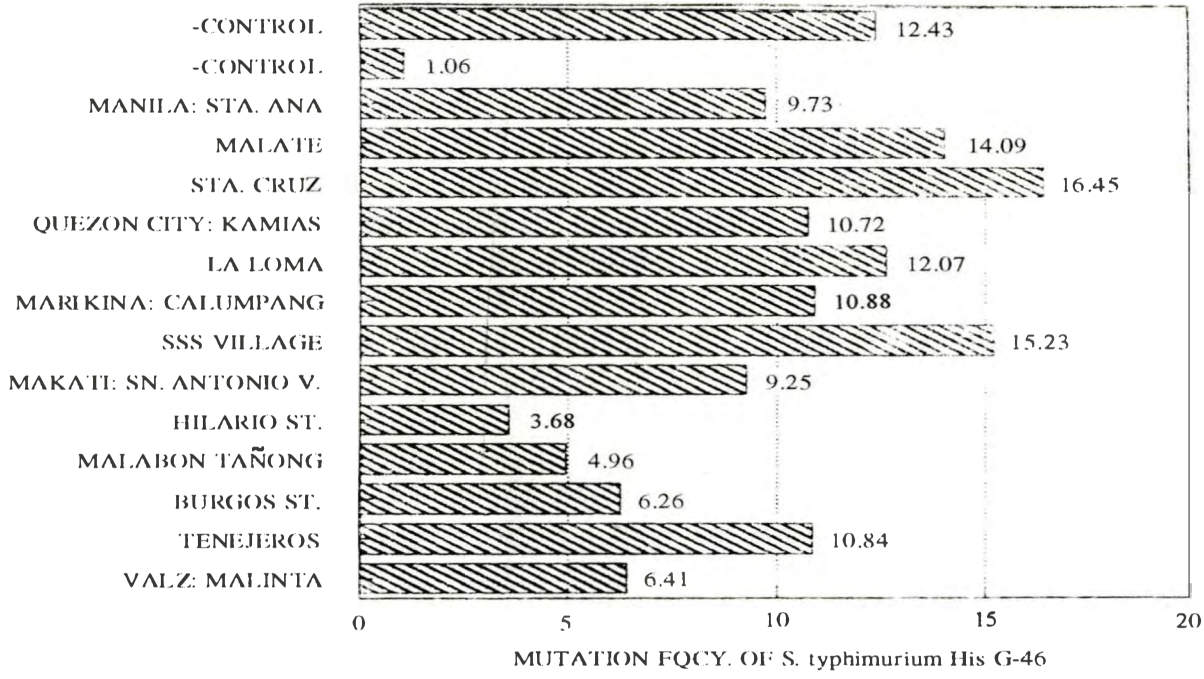
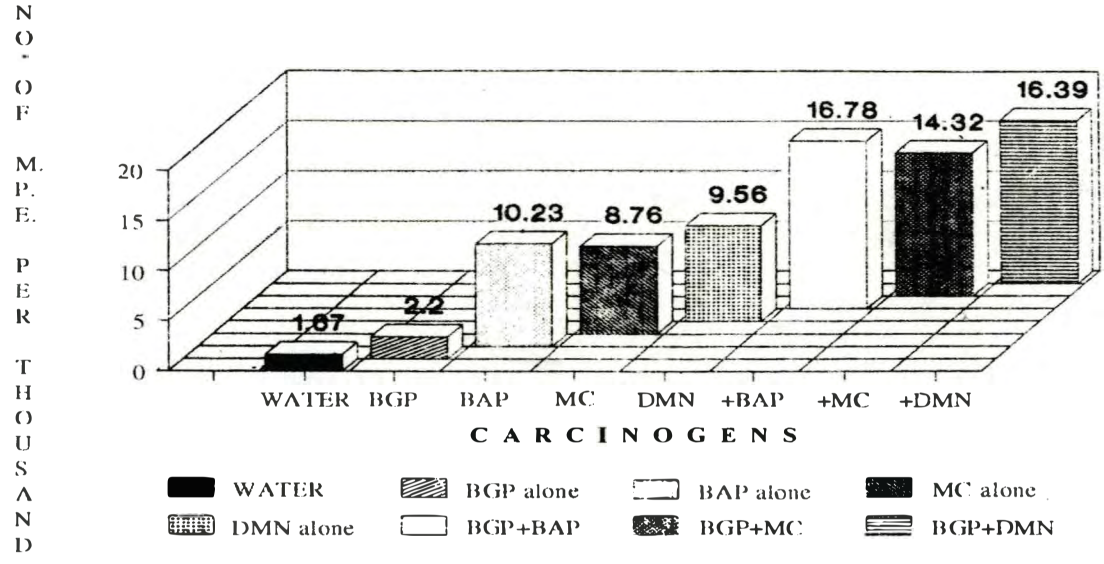
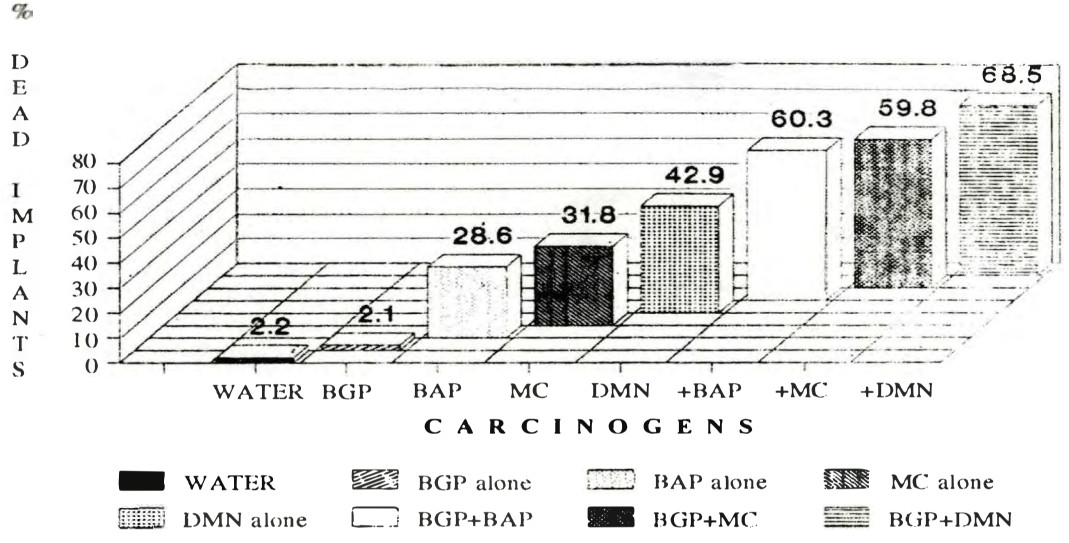


Figure 4. Mutagenicity after metabolic activiation of personal samples from student commuters to Diliman, Q.C.



BAP – Benzo(a)pyrene,
MC – Methylcholanthrene
DMN – Dimethylnitrosamine

Figure 5. Effects of benzo(ghi)perylene on the chromosome breaking effects of BAP, MC and DMN



BAP - Benzo(a)pyrene,
MC - Methylcholanthrene
DMN - Dimethylnitrosamine

Figure 6. Effects of Benzo(ghi)perylene on Germ Cell Genotoxicity of Benzo(a)pyrene, Methylcholanthrene and Dimehtylnitrosamine

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