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GROUND-LEVEL OZONE: A THREAT TO RICE CROP IN MUDA IRRIGATION AREA OF PENINSULAR MALAYSIA

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Abstract: It has long been recognized that pollutant gases cause significant impacts on crops and forests in both developed and developing countries. Ground-level ozone (O_3) or better known as tropospheric ozone is recognised as the pollutant most likely to cause widespread crop damage. For this pollutant an AOT40 (accumulative O_3 concentration above a threshold of 40 ppb) value causing 5% yield loss for all agricultural crops has been established as 3000 ppb·h, which is applicable during daylight hours over a growing season (UN-ECE, 1996). In order to have a complete estimate of air pollution damage i.e., O_3 to paddy plantation area, a dose-response, or yield-loss function have to be developed. In this study, data was gathered from tests in open-top chambers (OTCs), whereby four OTCs were fabricated; two of which were exposed to ambient air pollution (NF) of which ozone is the major perpetrator whilst the remaining were provided with clean air i.e. charcoal filtered air treatment (F). The response of a popular local rice cultivar, MR-219 to current ambient air pollution of which O_3 is the overwhelming dominant pollutant was investigated for five successive seasons in Muda Irrigation Scheme Area (MADA); the largest rice growing area in Malaysia. The results of the study clearly indicate that at ozone concentrations even lower than the Malaysian air quality guidelines (60 ppb 8 hr mean) level, there exist a significant impact on the growth and yield of the popular rice cultivar MR-219. Even though weeds, diseases, and insect pests were absent, water and nutrients were in abundance, no adverse soil conditions, and that no extreme weather event such as typhoons occurs; the physiological, growth and development performances of rice plants exposed to ambient ozone were found to be significantly ($P< 0.05$) reduced by AOT40 compared to control rice plants in filtered chamber. This study discovered that the root was the most significantly affected component of MR-219 rice plant. Meanwhile, reproductive stage is the most vulnerable period of growth to ozone impact followed by grain filling and vegetative stages, respectively.

KEYWORDS: Air Pollution, Ground-level ozone, Open Top Chamber, Rice yield, MADA.

Introduction

Agricultural productivity is becoming more critical in Malaysia due to growing population along with land constraint. Domestic consumption of rice is projected to increase from 1.8 million tonnes in 1995 to about 2.3 million tonnes in 2010, as a result of population increase despite the projected decline in per capita of consumption of rice (Malaysian National Committee on Irrigation and Drainage [MANCID], 2002). Under the National Agricultural Policy, the local production of rice is expected to meet about 65 % of the domestic demand. Within Malaysia, the Muda Irrigation Area is of particular importance. It is a highly productive region in term of agriculture. It is also responsible for almost 52% of the nation's production of grains (EPU, MOA, 2003).

Air quality in Peninsular Malaysia has worsened since the last decades, due to rapid economic development of the region (Chameides *et al*., 1999a; Wang *et al*., 2001; Cheung and Wang, 2001; Wang *et al*., 2003; Streets and Waldhoff, 2000; Mauzerall *et al*., 2000; Bergin *et al*., 2001). Tropospheric ozone which is also known as ground-level ozone is a secondary air pollutant produced from photochemical reactions involving the oxidation of volatile organic compounds (VOC) and carbon monoxide (CO) in the presence of nitrogen oxides (NOx) (Baumgaertel *et al*., 1999). Enhanced concentrations of ozone are ubiquitous to most of the populated regions of the world, where the burning of bio fuels and/or fossil fuels used for cooking, heating, transportation and the generation of energy cause the emission of VOC, CO, and NOx and thus the photochemical generation of ozone. While the highest concentrations are typically found in urban centres, ozone pollution is a regional phenomenon that extends over thousands of square kilometres and thus can be of concern to agricultural areas. The ability of surface ozone to damage agricultural crops has been well documented by research projects conducted in the United States (US) and Europe in the 1980s and 1990s, e.g. the US National Crop Loss Assessment Network (NCLAN) (Heck *et al*., 1984) and European Crop Loss Assessment Network (EUCLAN) programs using Open Top Chamber (OTC). Field experiments have shown that many kinds of vegetation are affected upon exposure to elevated concentrations of surface ozone. In the case of agricultural crops, it has been found that the damage due to repeated exposures to elevated concentrations of ozone over a growing season tend to have a cumulative effect, ultimately leading to overall reductions in crop yields. The exposures are measured in terms of AOT40 (Accumulated exposure Over Threshold of 40 ppb). The threshold of 40 ppb has been accepted after several years of experimental research in open top chambers throughout Europe. As a result of these experiments, a critical level of 3000 ppb hours over a three month growing season applicable during daylight hours has been established for crops. This parameter was suggested to be applied to agricultural and economic assessments and subsequent modeling of benefits associated with reduced ozone exposure. The AOT40 parameter is commonly accepted now (Amann *et al*, 1999 and European Commission, 1999) whereby the value of 40 ppb is a practically determined threshold, below which the losses of crops due to ozone exposure could be neglected, and above which the losses are assumed to be linear with respect to the exposure. The choice of AOT40 is based on a large number of OTC experiments conducted in Europe and in the United States (Vulkov *et al*, 2000).

Materials and Methods

In this work, we use a local rice cultivar for plant growth and physiological data collection using open top chamber system for five consecutive rice growing seasons beginning from 2001 off-season (1/4/2001-31/7/2001) until 2003 main season (1/10/2003-31/1/2004) in Kampung Sungai Baru Tengah 'A' located at 6° 11.8' N, 100° 4.5' E, in Muda Irrigation Scheme Area (MADA), Kedah, north-west of Peninsular Malaysia. Rice seeds of local variety MR219 were used in this study because of its local agricultural importance, which accounts for more than 80% of rice production MADA area since 2000/2001 main season (MADA, 2002). In order to eliminate non-uniformity of soil being used in this experiment, 250 kg of clay-based soil collected from the study area rice field was thoroughly prepared in a large rectangular fibre container. Running tap water was added; then mixed well to ensure homogeneity of the aliquot. Approximately 3 kilograms of soil were then transferred into each plastic pot container. This study employed a standardised fertiliser regime, water level management and pesticide application, which was described in an official publication, entitled *"Senarai Semakan Tanaman Padi"* from MADA (2002). In this study, a one-way treatment structure in a completely randomised design (CRD) was employed as an experimental design. Twenty pots consisting of four plants per pot were planted inside each chamber with seven replicate assigned for each of the six treatments namely, charcoal filtered (F) air in OTCs as control and 5 non-filtered (NF) ambient air treatments in OTCs with different O_3 concentration.

Open Top chambers and Filters

Open top chambers (OTCs) ventilated with ambient (NF) or charcoal-filtered (F) air were employed as the experimental technique. Four OTCs were constructed from stainless steel pipes encased with transparent P.V.C plastic (Figure 1). Each was of octagonal shape, 2 m in height, 3.14 m^2 basal areas and a capacity of 6.2 m^3. The open-top end of the chamber is 0.74 m in diameter, and tilted slightly inwards in order to minimize the ingress of ambient air. Ambient air is passed through from a single air intake inlet for all the chambers to ensure that every chamber received the same air for the whole crop growth duration i.e., 120 days. The arrangement of blowers, filters box and how they were installed to the chambers is shown in Figure 1. Chambers number 1 and 4 were without filters whilst chambers number 2 and 3 were equipped with filters. These filters consisted of a cloth filter and a layer of activated carbon that was fabricated in box which connect the chamber to the blower units. Two types of filters were used, namely a charcoal filter and a dust filter, made of several layers of cotton cloth sandwiched between fine plastic mesh. In order to avoid the heating effect, blowers were placed inside a specially built hangar although part of the air inlet unit was exposed directly to the sun.

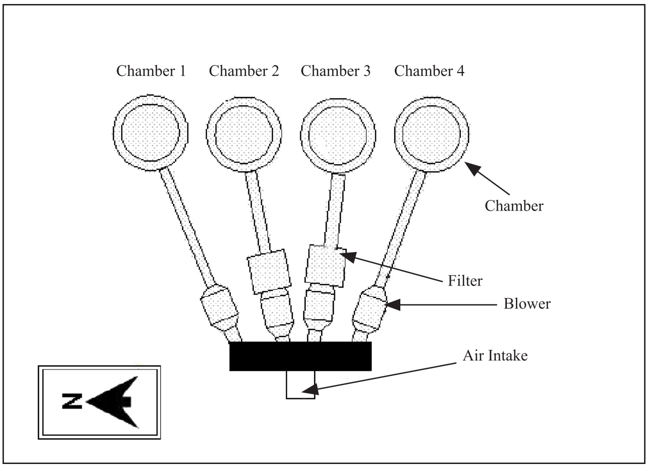

Figure 1: Plan view of chambers arrangements

Ambient Ozone Data Collection

Determination of hourly ozone concentration between Charcoal Filtered (F) and Non Filtered Chambers was performed using UV photometric O_3 analyser system (Dasibi 1008-PC, Dasibi, USA) which consist of internal ozone generating subsystem, internal manifold and capability for an ozonator-photometer feedback control system in which ozone generation may be controlled by the internal UV photometer subsystem. It is capable of monitoring low-level, ambient (0 – 1000 ppb) ozone concentrations. These data, which were in delimited ASCII file format, was transformed and compiled into a spreadsheet format and the sum of total ambient O_3 exposure for the whole growing period were calculated in Microsoft Excel spreadsheet using the following formula:

$$\text{AOT40 (ppmv-hour, ppmh)} = \sum_{i=1}^{n} (C_{O_3} - 40)_i \qquad \text{Equation 1}$$

For $C_{O_3} \geq$ 40ppb in the whole growing period i.e. 4 months
n = 1, 2, …………, 2880.

where C_{O_3} is the ozone concentration (in ppbv) based on hourly averages. For cross calibration purpose of the ambient ozone concentration, hourly ambient ozone data for the whole growing period were purchased from Alam Sekitar Malaysia Sdn Bhd (ASMA) whose station at Sekolah Agama Mergong, Alor Star is just only 500 meters away from the study area.

Result and Discussions

Influence of Open Top Chamber

Microclimatic properties of OTCs and ambient condition conducted in this study were photon flux density (PFD), relative humidity, temperature and wind speed. PFD inside the chambers (I_c) was suppressed by an average of 26% compared to ambient concentration (I_a) as depicted in Figure 2. It was shown that photosynthetic light saturation occurs at 800-to 1000 µmol·m^{-2}·s^{-1} for other Malaysian rice cultivar (cv. MR151) (Griffith, 1996). As the average hourly PFD of I_c plots (09:00 ~ 15:00) were approximately 800 ~ 1700 µmol·m^{-2}·s^{-1}, there was a strong possibility that the plants grown in OTCs received sufficient photosynthetically saturated level of PFD for optimum growth.

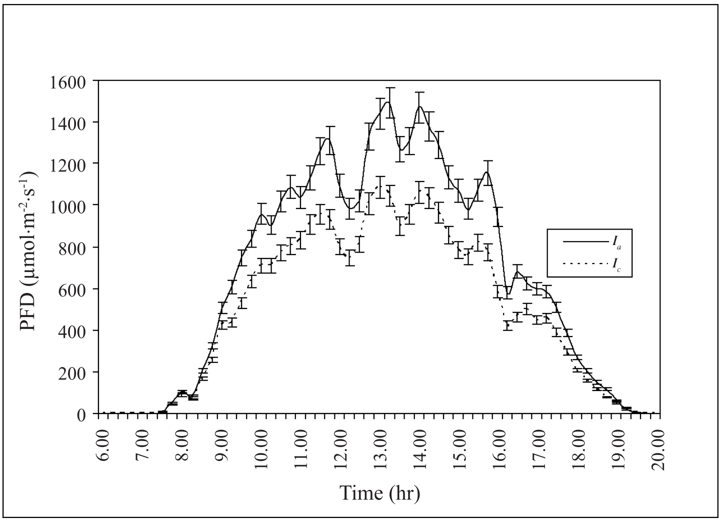

Figure 2: Diurnal mean pattern of I_a and I_c.

However, there is no statistically significant difference ($p < 0.05$) between ambient and OTC's for others parameter i.e. relative humidity (Figure 3), wind speed (Figure 4) and temperature (Figure 5), respectively. Similarly, there exist no statistically significant difference ($p < 0.05$) between the microclimates of the four chambers; confirming that all plants placed inside them experienced practically similar conditions throughout the experimental period.

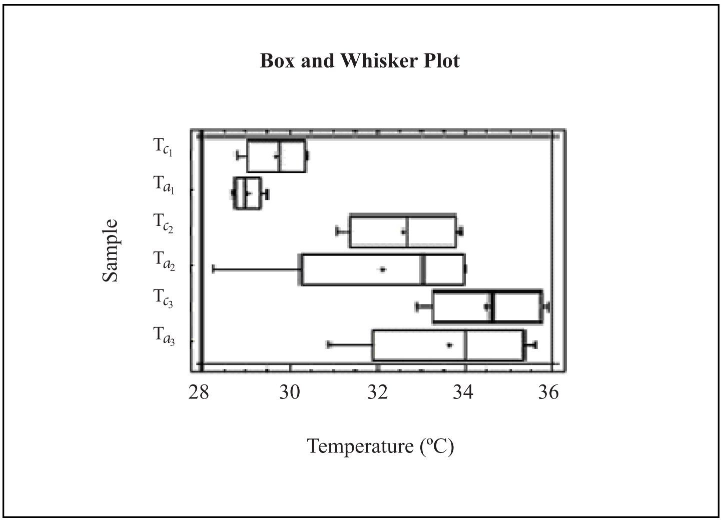

Figure 3: Comparison between ambient temperature (T_a) and chamber temperature (T_c) for 3 different days utilizing Box and Whisker plot

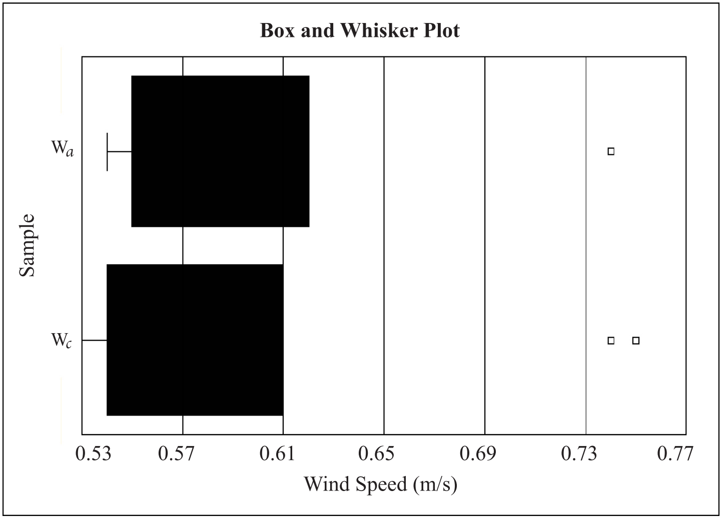

Figure 4: Comparisons of ambient wind speed (W_a) and chamber wind speed (W_c) utilizing Box and Whisker plot.

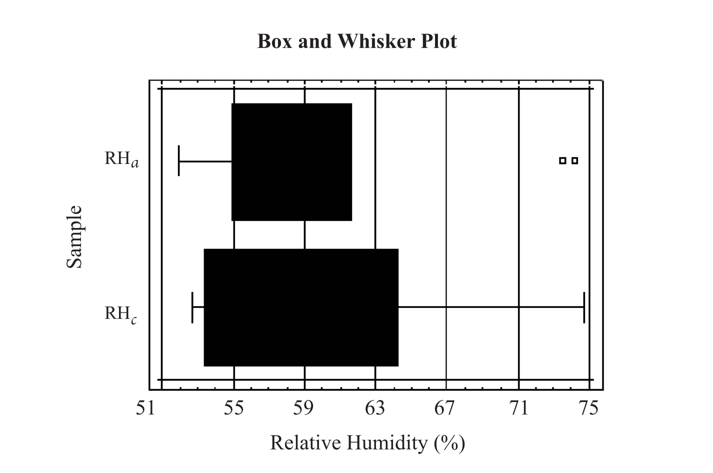

Figure 5: Comparisons of ambient relative humidity (RH$_a$) and chamber relative humidity (RH$_c$) utilizing Box and Whisker plot

Effect of AOT40 on Physiology

The productivity of a crop is significantly correlated with the amount of intercepted radiation received by the canopy. It has been shown that the maximum photosynthesis (A_{max}) of vegetation is proportional to the photosynthetic photon flux density (PFD) (Linder, 1984). Although daily CO_2 assimilation rate of a crop well supplied with water and nutrients at average daytime temperature i.e. 28ºC (as the plants in this study) is determined by light-use efficiency for the whole day duration; its physiological performances was found to be significantly ($P < 0.05$) reduced by ambient ozone above the threshold of 40 ppb h^{-1} (AOT40). The P_g saturation point for filtered MR-219 rice plant was around 700 µmol m^{-2} s^{-1} while P_g saturation point for non-filtered MR-219 rice plant (plant exposed to ambient ozone) was around 690 µmol m^{-2} s^{-1}, photon flux densities (PFD). The results of this study confirmed that maximum gross photosynthetic rate, P_{max} (µmol m^{-2} s^{-1}), light utilization efficiency (α), stomatal conductance (Figure 6) and transpiration rates (Figure 7) for filtered MR-219 rice plants (F) have higher value compared to that of plants exposed to non-filtered MR-219 rice plant (NF). For filtered MR-219 rice plant, P_{max} equals to 13.64; ε is 2.64 x 10^{-2} g CO_2 $J^{-1}m^2s^1$ and I_{net} equals 23168.05 $J^{-1}m^2s^1$. Meanwhile, maximum assimilation rate for non-filtered MR-219 rice plant, P_{max} equals to 13.30; ε is 2.62 x 10^{-2} g CO_2 $J^{-1}m^2s^1$ and I_{net} equals to 23168.05 $J^{-2}s^{-1}$. This is clearly shown in the amount of daily gross CO_2 (A_{gross}) assimilated between them; 471043.70 g/day for non-filtered and 482084.44 g/day for filtered MR-219 rice plant canopies, a difference of 11040.74 g/day. Daily assimilation rate (A_{gross}) was calculated from the instantaneous CO_2 assimilation rates at ambient radiation levels during the day and integrated over time; providing the CO_2 assimilation value over the entire day according to the relation expressed by Equation 2.

$$A_{gross} = P_{\max} * \exp^{\left(\frac{-I_{net} * \varepsilon}{P_{\max}}\right)} \qquad \text{Equation 2}$$

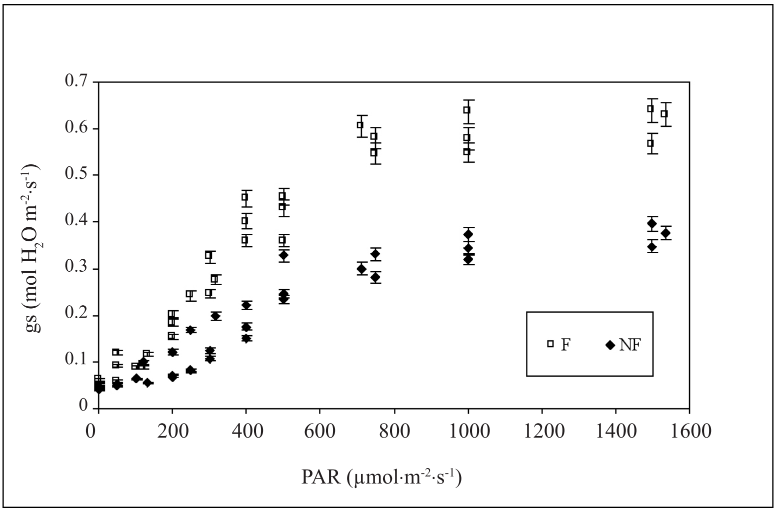

Figure 6: Stomatal conductance for Filtered (F) and Non-filtered (NF) MR-219 plant

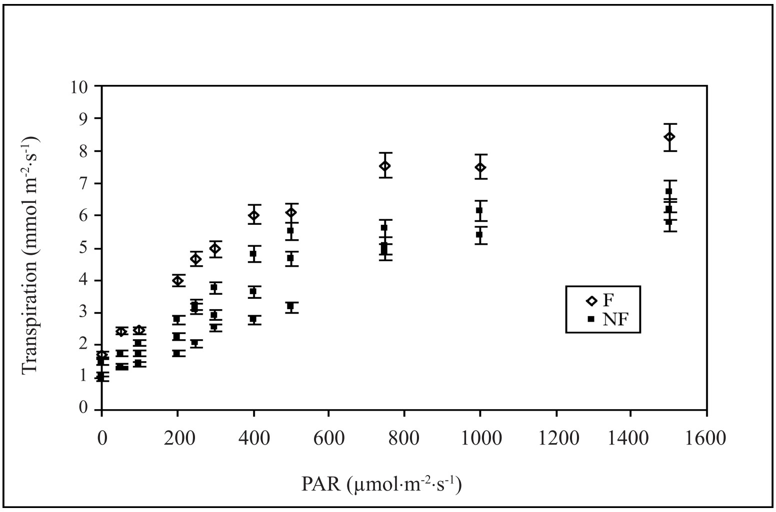

Figure 7: Plot of Transpiration Filtered (F) and Non-filtered (NF) MR-219 plant

Previous studies on exposure to O_3 treatment either in controlled environment or OTCs have also shown similar trend; whereby the earliest manifestations of ozone exposure on plants are reduced rates of photosynthesis and stomatal conductance (Hill and Littlefield, 1968; Reich *et al.* 1985; Olszyk and Tingey, 1986; Amundson *et al.*, 1987; Miller, 1987). However, the magnitude of this loss cannot be predicted from ozone exposure alone, as ozone must first gain access to the leaf and the effects will therefore be a function of stomatal conductance. The change of stomatal conductance may be regarded as a kind of resistance mechanism (Mansfield and Freer-Smith, 1984; Reiling and Davison, 1992).

Lack of visible injury might suggest that the Malaysian cultivar used in this study i.e. MR 219 was relatively tolerant to O_3 because a wide range of sensitivities in these parameters have been shown in some filtration studies in a controlled environment (Asakawa *et al.*, 1981a, b) and OTC

(Wahid *et al*., 1995). Nouchi *et al*. (1991) found that fumigation with O_3 concentrations below 50 ppb (8 hours mean) did not affect leaf number, although fumigation with 100 ppb showed some reduction. Percentage of more than 50% senescence leaves in the present study tended to be higher in the NF plots compared to F plot suggesting that plant maturity in the polluted environment could have been accelerated slightly, even without development of any foliar injury. Wahid *et al*. (1995) found a similar trend but with much greater extent in their OTC filtration study in Pakistan.

Effect of AOT40 on Plant Growth's Dry Weight (DW)

Logistic regression (logit) was utilized to model the growth of MR-219 rice crop which starts slowly, increases to a maximum rate and gradually slows approaching zero (Hunt, 1982; Clewer and Scarisbrick, 2001). Output of each logistic regression model describing the relationship between different ambient AOT40 exposure and an independent variable i.e. dry weight (DW) at days after planting can be generalized using the following equation:

$$y = \frac{\exp^{(a+bx)}}{\left(1 + \exp^{(a+bx)}\right)} \qquad \text{Equation 3}$$

Generalized growth model (DW) of MR219 rice cultivar subjected to different ambient AOT40 exposures was plotted in Figure 8. Unmistakably from the graph, it illustrated that the growth rate starts slowly; increases to a maximum rate and gradually slows approaching zero. As a result, three apparent stages can be identified i.e. Stage I from 0 to 65 days after planting (DAP), Stage II from 66 to 100 DAP and Stage III from 101 till 120 DAP and this augur well agronomically, whereby it is convenient to regard the life history of rice in terms of three growth phases: vegetative (Stage I), reproductive (Stage II), and ripening (Stage III), respectively. Graphs for each growth function exposed to different AOT40 concentrations from these three stages were then subjected to simple regression analysis to determine which stage is most susceptible to ozone.

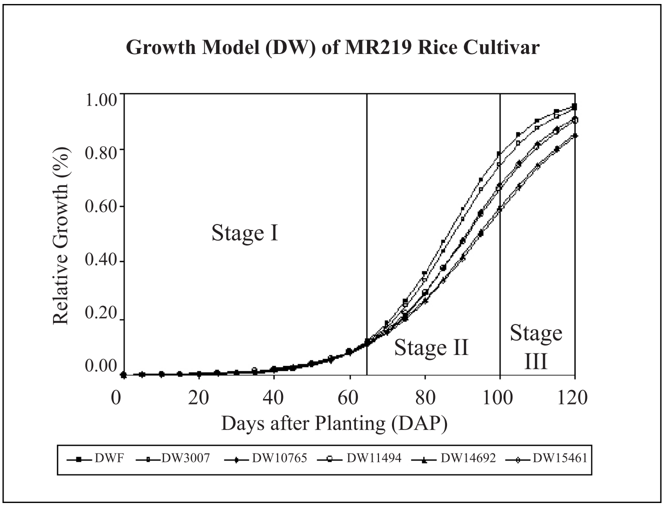

Figure 8: Growth function of MR219 rice exposed to different AOT40 concentrations

Results of statistical analyses performed on the three distinct stages of MR-219 rice plant indicated that ozone did not have any impact on its growth performance during vegetative stage. However, ozone effect on growth performance is clearly seen during reproductive and grain filling stages. Out of these two stages, the effect is more acute during the reproductive stage as shown by the gradient slope (*dy/dx*) of the linear regression for each stage fitted model. The correlation gradient for reproductive stage is -1.33×10^{-3} whilst the value is -8.61×10^{-6} for grain filling stage. Hence, the impact of AOT40 is 150 times (150) more severe during the reproductive stage as compared to the grain filling stage. In conclusion, severity order (from bad to worse) of ozone impact on growth performance of MR-219 rice plant is in the following stage order: vegetative (DW_v) < grain filling (DW_{gf}) < reproductive (DW_r).

Effect of Ambient O_3 Exposure on Growth Rate of MR-219 plant

The normalised logistic curve of equation 4 for each AOT40 exposure integrate all the growth data that was gathered and can be differentiated with respect to t (time) i.e. DAP, giving the second differentials as in Equation 4. The second differential with respect to t (time) is the sum of three terms below, and if *t* is being replaced by *–a/b*, the whole expression reduces to zero (i.e. this means it is a point of inflexion).

$$\frac{2b^2\exp(3a+3bt)}{(\exp[a+bt]+1)^3}-\frac{3b^2\exp(2a+2bt)}{(\exp[a+bt]+1)^2}+\frac{b^2\exp(2a+bt)}{(\exp[a+bt]+1)} \qquad \text{Equation 4}$$

The logistic curve have been normalised (applying the mean and standard deviations of the standardised scores in the normal distribution equation), so the slopes of the growth curves represent the relative growth rate. When these differentials are zero (2nd differential) they respectively define the point of inflexion to the curve, i.e. the maximum relative growth rate. These mathematical exercises enable all the growth data is utilized and express as a relative growth rate for each of the logistic curves. For logistic curve (0 -1), its mathematical property indicated that the point of inflexion occurs half way, i.e. at 0.5. The non-linear equations were solved using the DOS program MERCURY to give the values of maximum growth rate (2nd Differential) for each ozone exposures (Table 1).

Table 1: The values of maximum growth rate at the point of inflexion

AOT40 (ppb h^{-1})	**a**	**b**	**Point of inflexion (t50)**	**Maximum growth rate (2nd Differential)**
0	-7.96	0.092	86.52	0.023
3007	-7.781	0.089	87.43	0.02225
10765	-7.365	0.081	90.92	0.02025
11494	-7.064	0.078	90.56	0.0195
14692	-6.573	0.0695	94.58	0.01738
15461	-6.5728	0.069	95.26	0.01725

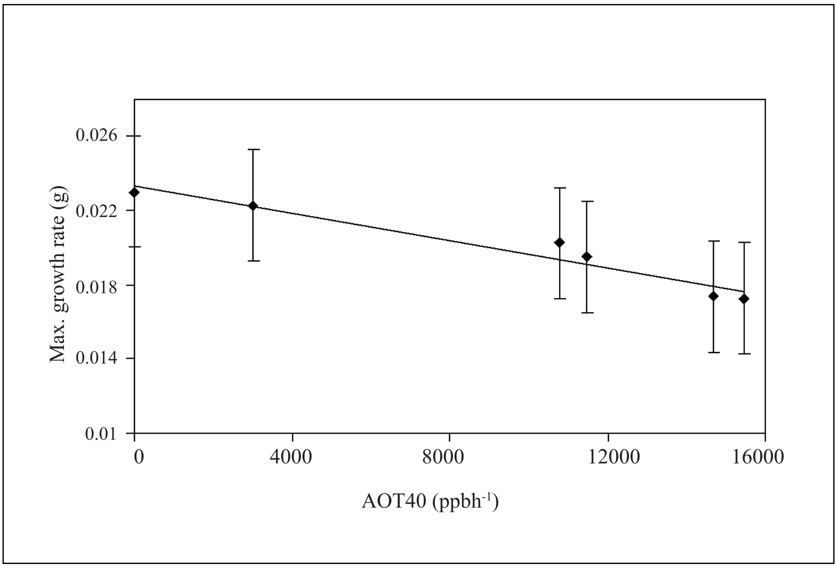

Figure 9: Relative maximum growth rate versus AOT40

The maximum relative growth rates seem to show a good relation to ozone dose (AOT40), whereby the higher the ozone amount the lower the slopes is, as illustrated in Figure 9. The result of fitting a linear model ($p<0.05$) for growth rate of MR219 rice plant subjected to different AOT40 produced the following equation with a correlation coefficient equals -0.97 (Equation 5).

$y = 2.335\text{x}10^{\Sigma 2} - \Sigma 3.693\text{x}10^{\Sigma 7} * x$ Equation 5

where y is maximum growth rate and x is AOT40.

Effect of AOT40 on Dry Weight at Final Harvesting (DWF)

At final harvesting i.e. 120 days after planting, the dry weight of total biomass and plant components dry weight's of interest, namely root, leaf, tillers and grain exposed to different concentration of AOT40 revealed the following findings. There exits statistically significant ($p<0.05$) weight reduction on total biomass, roots and grains of MR-219 rice plant exposed to different concentrations of ozone (AOT 40), but although there exist a reduction trend in leaf and tillers weight, the effect is insignificant. The graph of weight reduction on total biomass, roots and grains of MR-219 rice plant exposed to different concentrations of ozone (AOT 40) are shown in Figure 10, 11 and 12, respectively. Linear equation was the best-fitted regression model for total biomass and roots weight function to ozone (AOT40). The generalized model for total biomass (DWF_T) is = (DWF_T) 0.997 - $\Sigma 5.66 \text{ x } 10^{\Sigma 6}$ AOT40. Meanwhile, the root's weight (DWF_R) function to ozone (AOT40) is: (DWF_R) = 0.983 – (1.044 x 10^{-5} * AOT40. For grain weight, a square root-Y equation is the best-fitted model for describing its relationship with ozone (AOT40): (DWF_G) = 0.983 – (1.044 x 10^{-5} * AOT40)^2.

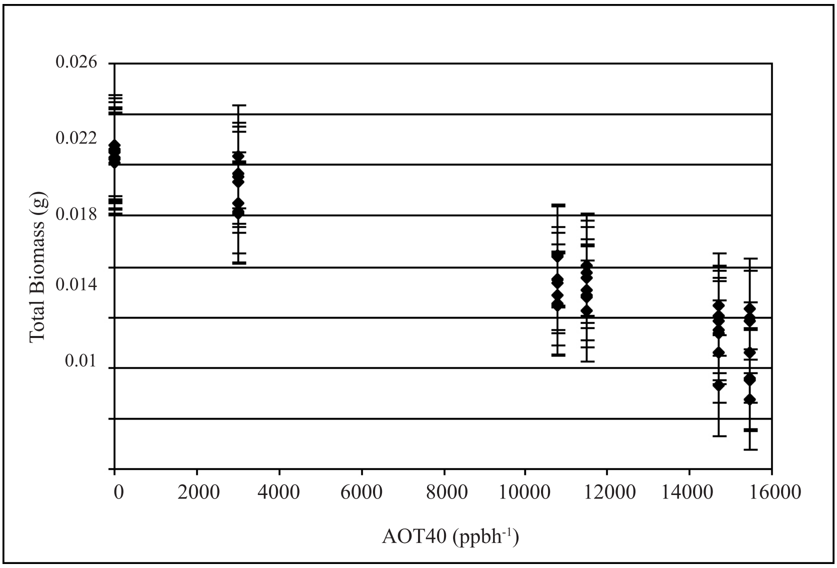

Figure 10: Total Biomass of MR-219 function to AOT40

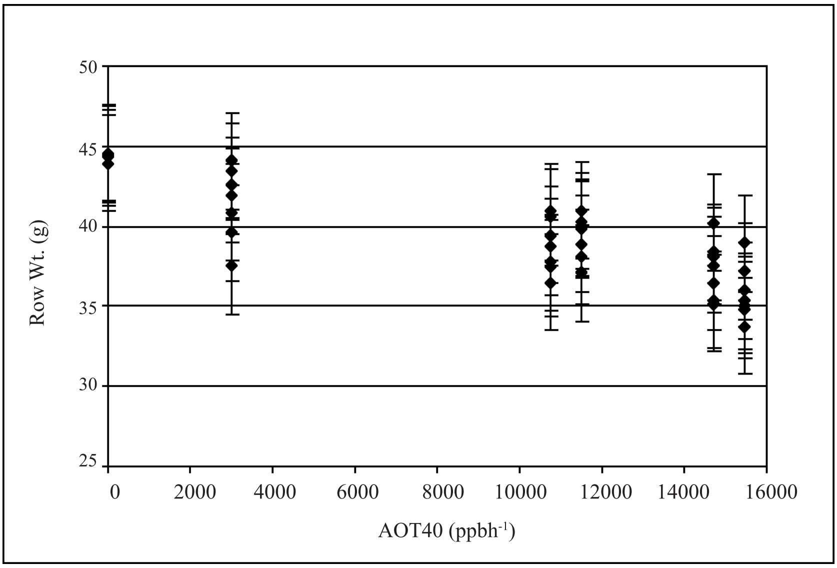

Figure 11: Root weight of MR-219 function to AOT40

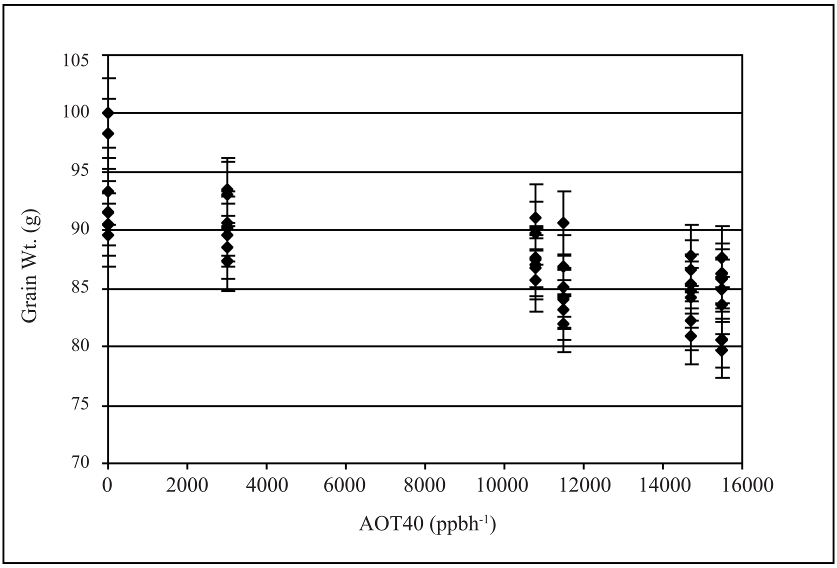

Figure 12: MR-219 grain weight function to AOT40

Previous studies on exposure to O_3 treatment either in controlled environment or in OTCs have also shown similar trend; that is reduced total biomass accumulation in rice (Kats *et al*., 1985; Kobayashi *et al*., 1995) and soybean (Kress and Miller, 1983). In addition, the detrimental effects of ambient ozone on crops, even at relatively low concentrations, are well established (Heck and Brandt, 1977; Heagle *et al*., 1979; Heck *et al*., 1982; Mulchi *et al*., 1986; Amundson *et al*., 1987; California Air Resources Board, 1987; Olszyk *et al*., 1988; Fuhrer *et al*., 1989, 1992; Ashmore, 1991; Pleijel *et al*., 1991; Wahid *et al*., 1995; Mulholland *et al*., 1998; Ojanpera *et al*., 1998).

Conclusion

Even though weeds, diseases, and insect pests were absent; water and nutrients were in abundance, no adverse soil conditions, and no extreme weather occurred; the physiological, growth and development performances of rice plants exposed to ambient ozone were found to be significantly ($P< 0.05$) reduced by AOT40 compared to control rice plants in filtered chamber.

In conclusion, the results of the study clearly indicate that at ozone concentrations even lower than the Malaysian air quality guidelines (60 ppb 8 hr mean) level, there exist a significant impact on the growth and yield of the popular rice cultivar MR219. This scenario can be seen clearly whereby the results reported in this study indicated that the current levels of AOT40 is on the rise and are causing substantial losses on rice yield in the study area especially during the off-season (October till January). Therefore, the algorithms established in this study will be very useful for determining the total loss of yield due to ozone in other rice growing regions in Malaysia such as Barat Laut in Selangor, and KEMUBU area in Kelantan. Finally, ozone yield loss function established in this study can be utilized to predict the total impact of air pollution on national rice production.

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