



## Adverse Effects of Elevated Ambient Ozone on Yield and Protein Loss of Three Thai Soybean Cultivars

**KANITA THANACHAROENCHANAPHAS**

*Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand*  
 Email: kanitat@nu.ac.th

**OROSE RUGCHATI**

*Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand*

Received 20 December 2009      Accepted 25 July 2010

**Abstract** Various studies in the past have shown that high ozone concentration significantly decreased yield and nutrition in soybean but there is little information in Thailand. Hence, an examination of the aforementioned problem is needed. The purpose of this study is to know the different adverse effects of elevated ambient ozone concentration on yield and protein content of Thai soybean. The research experiment was conducted during December 2007 to March 2008 at the Field Crops Research Center, Phitsanulok, Thailand. Thai native soybean 3 cultivars; Chiang Mai 60, Sorjor 5 and Srisumrong 1; were planted and covered with open top chamber (OTC) since seedling through maturing stage. The OTC with charcoal filtered and non-charcoal filtered were set to control the O<sub>3</sub> level at three different levels; at ambient level ( $32 \pm 11.1$  ppb), lower ( $12 \pm 10.1$  ppb) and higher than ambient level ( $62 \pm 10.8$  ppb). Results indicated that growth and yield loss at maturing stage obviously occurred in all 3 cultivars under high ambient O<sub>3</sub> concentration by statistical significance. However, we found the different reductions in number of total seed/plant of Chiang Mai60, Sorjor5 and Srisumrong 1, by 37, 28 and 33% respectively when they were exposed to 62 ppb O<sub>3</sub> compared to the lower ambient level. The significant reduction by 14% in plant height appeared in only one cultivar, Sorjor 5. The parallel result was shown in protein content; Sorjor5 cultivar showed the most sensitive to high O<sub>3</sub> concentration in protein content reduction by 2.3% but consistent effect was not found in Chiang Mai 60 and Srisumrong 1. The overall results in the study conclude that long-term high O<sub>3</sub> exposure caused different adverse effects in among 3 Thai soybean cultivars. Sorjor5 seems to be more an ozone-sensitivity cultivar than the other cultivars.

**Keywords** ozone, Thai soybean, open top chamber, yield, protein

### INTRODUCTION

Tropospheric ozone (O<sub>3</sub>) is one of the main pollutant gasses that cause air pollution. Source of O<sub>3</sub> in troposphere is from a complex, photochemically induced reaction between the hydrocarbons and nitrogen oxides released from motor vehicle exhaust (Manning and Feder, 1976). Many studies have shown that average concentrations of O<sub>3</sub> in many regions have increased, the changes may come about from climate changes and human-emitted precursors (Mohnen et al., 1993; Oltmans et al., 2006). It was anticipated that many factors will contribute to O<sub>3</sub> increase of 20-25% between 2015 and 2050 and 40-60% by 2100 (Mohnen et al., 1993; Singh et al., 2010).

In addition, O<sub>3</sub> which is a powerful oxidizing agent, is responsible for more damage to vegetation than any other air pollutant. Consequently, crop yield reduction caused by tropospheric O<sub>3</sub> is well known (Ostromsky et al., 2001; Singh et al., 2010). Exposure of plants and crops to O<sub>3</sub> usually results in reduction of yield and loss of physiological functions. It was estimated that O<sub>3</sub>, alone or in combination with other pollutants, accounts for approximately 90% of air pollution induced crop loss in U.S (David et al., 1994). Hence, among pollutants, tropospheric O<sub>3</sub> is one of the important species that has received considerable attention.

Soybean is one of the economic crops known for its sensitivity to O<sub>3</sub> (Miller et al., 1994). It has been estimated that soybean yields are suppressed by about 10% by 50 ppb O<sub>3</sub> concentration (seasonal mean 7h daily concentration) (Heagle, 1989). The ambient concentration of O<sub>3</sub> in central areas of Thailand is around 20-30 ppb and some supplemental factors can push this level to be 50-80 ppb (Pollution Control Department of Thailand, 2008), which is high enough to cause adverse effects on many components of the ecosystems including crop production in Thailand.

However, the possible adverse affects of O<sub>3</sub> elevation to agricultural crops especially soybean nutrition and production in Thailand is not well understood. Thus, in this study we carried out the experiment to evaluate and understand how elevated O<sub>3</sub> concentrations affect growth and yield of some Thai soybean cultivars.

## **METHODOLOGY**

### **Site and experimental design**

Thai soybean (*Glycine max* (L.) Merr.) 3 cultivars - Chiang Mai 60, Sorjor 5 (SJ5) and Srisumrong 1 - were planted in an open top chambers at the Phitsanulok Field Crops Research Center, Phitsanulok Thailand during December 2007 to March 2008.

Three replications of Randomized Complete Block Design (RCBD) were used in three treatments with different levels of O<sub>3</sub> in experiment. At vegetative growth stage - third node (V3) - all three cultivars were exposed to three different O<sub>3</sub> concentration levels for 7 hr exposure (9.00 am - 4 pm) in open top chambers until harvest.

### **Ozone exposure**

Exposure of three cultivars of soybean to different O<sub>3</sub> levels were carried out in a open top chamber. The open top chamber (3 m long, 3 m wide and 2 m high) was constructed from a transparent plastic. Ventilation fans were equipped on the front of the chamber to facilitate air circulation and to equilibrate the temperature difference between inside and outside of the chamber.

Three levels of O<sub>3</sub> concentrations - at ambient level, lower than ambient level and higher than ambient level - were set up. In the ambient chamber (non-charcoal filter; NCF treatment), the air was freely circulated in and out of the chamber without passing through any filter. In the chamber with O<sub>3</sub> lower than ambient level (CF treatment), the air was passed through charcoal filter.

O<sub>3</sub> was produced by O<sub>3</sub> generator (Belle Marketing Co.LTD, Thailand Model OZ-3020) via charcoal filter to control O<sub>3</sub> concentration at above ambient level in CF+O<sub>3</sub> treatment. O<sub>3</sub> concentrations in all chambers were measured by real-time ozone gas detector (BW technologies, Canada).

### **Yield and protein content determination**

Soybean plants and seeds were harvested from the experimental field at harvest stage (95 days). These three cultivars of soybean plant samples were determined of shoot length (height), number of total seed/plant. Protein content of soybean seed was analyzed based on analysis of protein and lipid content by AOAC (1995) method.

### **Statistical analysis**

The growth parameters, grain yield and nutrition value data were analyzed statistically with analysis of variance (ANOVA). Significant difference of parameters were reported at  $p < 0.05$  by DMRT.

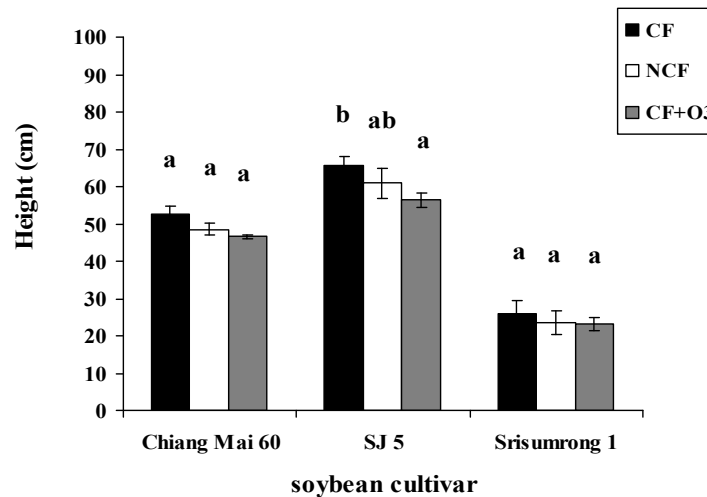
## RESULT AND DISCUSSION

### Ozone concentration

The O<sub>3</sub> monitoring data from 3 treatments in experiment are summarized in Fig 1. From the O<sub>3</sub> generator in the chambers, the detected O<sub>3</sub> concentrations for 7 hr exposure for each treatment were  $12 \pm 10.1$  ppb for CF,  $32 \pm 11.1$  ppb for NCF and  $62 \pm 10.8$  ppb for CF<sup>+O<sub>3</sub></sup>, respectively.

### Growth parameter

The results in growth parameter were visibly appearing in significant reduction by O<sub>3</sub> on height in Sorjor 5 (SJ5) at the maturity stage. However, their effects were not sufficient for significant reduction for Chiang Mai60 and Srisumrong 1. The most obvious negative result in height was found at high O<sub>3</sub> concentration in treatment CF<sup>+O<sub>3</sub></sup>; the percentage reduction was found to be approximately 14% when compared to CF treatment (Table 1). These results indicate that high O<sub>3</sub> level (above ambient level) induced height suppression and SJ5 could be considered more susceptible to O<sub>3</sub> than Chiang Mai60 and Srisumrong 1. The total changes in relative between enhanced O<sub>3</sub> concentration and height in 3 cultivars of Thai soybean are shown in Fig.1.



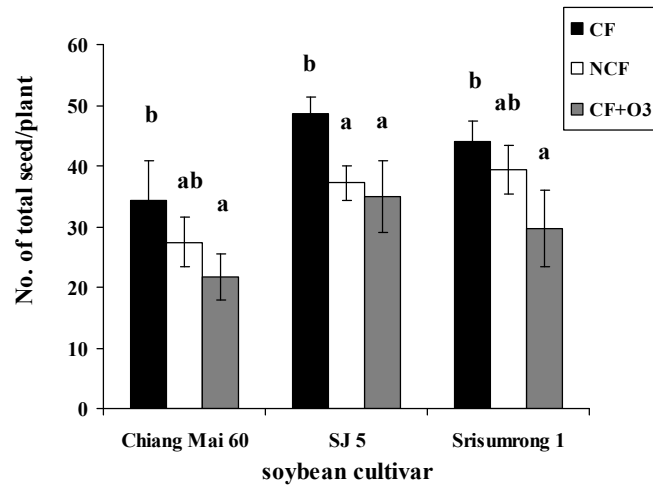
**Fig. 1 Effects of O<sub>3</sub> concentrations on height of soybean, 3 cultivars - Chiang Mai 60, Sorjor 5 and Srisumrong 1**

The different letters for each parameter indicate a significant difference at  $p \leq 0.05$ . Error bars above each histogram indicated standard deviations (S.D.) observed from samples of each treatment.

### Yield parameter

Grain yields were harvested at maturity stage. The parameters - number of total seed/plant - were determined to estimate grain quantity. The response in relative terms between O<sub>3</sub> concentrations (3 levels) and yield in 3 cultivars of Thai soybean are shown in Fig.2. The significant reductions in number of total seed/plant were found in all Chiang Mai60, SJ5 and Srisumrong 1 cultivars and showed the highest reduction in CF<sup>+O<sub>3</sub></sup>. When the significant reductions were considered, we found that the percentage reduction (compared between CF<sup>+O<sub>3</sub></sup> and CF) in Chiang Mai60, SJ5 and Srisumrong 1 were 36%, 28% and 32%, respectively (Table 1).

The results indicate the concomitant relationship between increasing O<sub>3</sub> concentration and decreasing in total seed /plant. In addition, we observed that Srisumrong 1 seems more susceptible to O<sub>3</sub> than other cultivars when considered in the highest reduction in grain yield parameter.

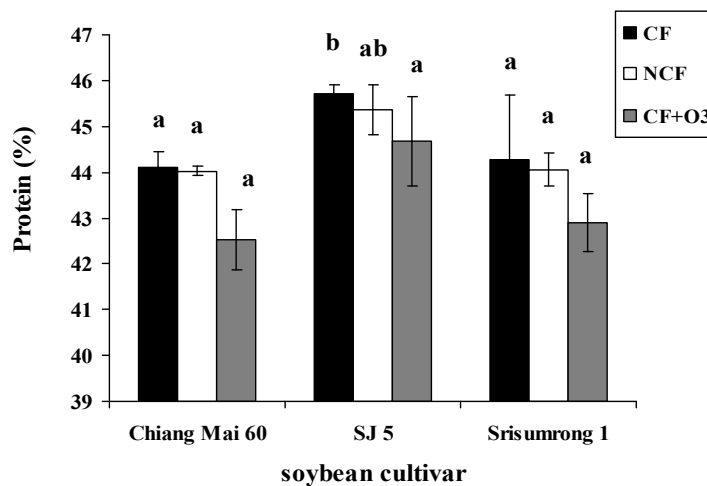


**Fig. 2 Effects of O<sub>3</sub> concentrations on yield of soybean, 3 cultivars - Chiang Mai 60, Sorjor 5 and Srisumrong 1**

The different letters for each parameter indicate a significant difference at  $p \leq 0.05$ . Error bars above each histogram indicated standard deviations (S.D.) observed from samples of each treatment.

### Protein content

Protein contents in this experiment were measured at maturing stage (Fig.3). The significant results appeared only in SJ5 cultivar when comparing CF<sup>+O3</sup> to CF. This significant result was not significant ( $p < 0.05$ , DMRT) when compared to the NCF (ambient level). Percentage of protein content in SJ5 seed indicated that sufficient O<sub>3</sub> concentration at about  $62 \pm 10.8$  ppb (above ambient level) could strongly decrease protein content in SJ5 cultivars. The significant reduction was found to be 2.3% (Table 1).



**Fig. 3 Effects of O<sub>3</sub> concentrations on protein content of soybean, 3 cultivars - Chiang Mai 60, Sorjor 5 and Srisumrong 1**

The different letters for each parameter indicate a significant difference at  $p \leq 0.05$ . Error bars above each histogram indicated standard deviations (S.D.) observed from samples of each treatment.

**Table 1 Percentage reduction of parameter in 3 cultivars when comparing CF<sup>+O<sub>3</sub></sup> with CF and NCF (in significant results)**

Parameter	Cultivar	Treatment			Percentage reduction
		CF	NCF	CF <sup>+O<sub>3</sub></sup>	
Height	Chiang Mai 60	52.5 ± 2.4 <sup>a</sup>	48.5 ± 2.5 <sup>a</sup>	46.5 ± 3.5 <sup>a</sup>	13.8%
	*SJ5	65.5 ± 1.6 <sup>b</sup>	60.9 ± 3.9 <sup>ab</sup>	56.4 ± 3.2 <sup>a</sup>	
	Srisumrong 1	25.8 ± 0.5 <sup>a</sup>	23.6 ± 1.9 <sup>a</sup>	23.1 ± 1.8 <sup>a</sup>	
Number of total seed /plant	*Chiang Mai 60	34.3 ± 6.6 <sup>b</sup>	27.5 ± 2.9 <sup>ab</sup>	21.7 ± 3.4 <sup>a</sup>	36.7%
	*SJ5	48.5 ± 4.0 <sup>b</sup>	37.2 ± 2.8 <sup>a</sup>	34.9 ± 3.9 <sup>a</sup>	28.0%
	*Srisumrong 1	44.0 ± 3.8 <sup>b</sup>	39.4 ± 5.9 <sup>ab</sup>	29.6 ± 6.3 <sup>a</sup>	32.8%
Protein content	Chiang Mai 60	44.1 ± 0.4 <sup>a</sup>	44.0 ± 0.2 <sup>a</sup>	42.5 ± 1.4 <sup>a</sup>	2.3%
	*SJ5	45.7 ± 0.1 <sup>b</sup>	45.4 ± 0.6 <sup>ab</sup>	44.7 ± 0.4 <sup>a</sup>	
	Srisumrong 1	44.3 ± 0.7 <sup>a</sup>	44.1 ± 0.9 <sup>a</sup>	42.9 ± 0.6 <sup>a</sup>	

The symbols (\*) indicate a significant result at  $p \leq 0.05$ .

The results obviously show that high O<sub>3</sub> concentration damaged the growth (in height), yield parameters and protein content of soybean examined in this study. However, the results were not as obvious as those observed in all cultivars of soybean. It is interesting that accumulative long-term O<sub>3</sub> exposure (to 62 ± 10.8 ppb) revealed the great significant reduction in whole 3 cultivars in number of total seed/plant. As regards the significant reduction results in all parameters, SJ5 showed the most sensitive cultivar to O<sub>3</sub> in this case.

Other researchers also observed similar results. For example, Miller et al. (1994) studied the effects of O<sub>3</sub> concentrations of 14-83 ppb (12 h daily) on soybean cultivars Coker 6955, Essex and S53-34. The results indicated that O<sub>3</sub> induced visible injury, suppressed net carbon exchange rate, water-use efficiency, accelerated reproductive development, and suppressed growth and yield. In terms of the effects of O<sub>3</sub> on yield loss, we found many consistent results with our investigation results. Studying in soybean at Pakistan Punjab, Wahid et al. (2001) found that O<sub>3</sub> caused significant losses of number of seed per pod and number of pod per plant. Kress and Miller (1983) found a greater reduction of the numbers of filled pods per plant and mean seed weight after O<sub>3</sub> exposure. Heagle et al. (1986) found the yield loss in soybean induced by O<sub>3</sub> in filled pod number per plant and mean seed size. In the field experiment by Kobayashi et al in Japan, they found that fraction of root and dry matter partitioning decreased with increasing O<sub>3</sub> level (Kobayashi et al, 1995). Ariyaphanphitak et al. (2005) describes that the grain growth of cereal is dependent on the production of carbohydrates and the translocation of assimilates from the source organs to the grains. The condition under O<sub>3</sub> exposure may induce the drastic decrease of sucrose and fructan content of the internodes, thus storage of assimilates in the culm could induce insufficient photosynthate supply to the grain and protein production. In addition, the various studies showed that O<sub>3</sub> via leaf then cellular constituents, nucleic acids, purine and pyrimidine derivatives, amino acid group can be oxidized by O<sub>3</sub> (Runeckles and Chevone, 1992). These may be the reason why grain filling and protein content in soybean seed in the research was greatly affected by O<sub>3</sub> exposure at high level. The investigation results show that protein loss due to O<sub>3</sub> exposure was found only in SJ5 cultivar, while the parallel results did not appear in Chiang Mai 60 and Srisumrong 1. Bell and Treshow (2002) reported that three amino acid residuals - tryptophan, cysteine, methionin - are particularly sensitive to O<sub>3</sub>. They reported that ozonolysis can open up the pyrrol ring of tryptophan and oxidize the sulphhydryl group (-SH) of cysteine and methionine to form disulphide bridge (-S-S-) or sulphoxides; alterations in these amino acids will lead to protein reduction in seed. Therefore, the O<sub>3</sub> tolerance of protein parameter in Chiang Mai 60 and Srisumrong 1 may result from an O<sub>3</sub> tolerance in some or all types of these amino acid residuals.

## CONCLUSION

The observation results in these experiments provide the evidences that elevated O<sub>3</sub> concentrations (above ambient level) induced suppression in yield production, growth and protein content in seed of soybean. Seed production/plant was the most susceptible parameter to O<sub>3</sub> among other parameters,

while SJ 5 was the most O<sub>3</sub> susceptible cultivar. The results also suggest that soybean planting under O<sub>3</sub> concentration at low levels, showed the best yield and protein production. Thus, the risk management by reducing O<sub>3</sub> emission, preventing further damages, and finding the proper countermeasures are needed and necessary.

## **ACKNOWLEDGEMENT**

We thank the Phitsanulok Field Crops Research Center for assisting in research field. We also thank Naresuan University for the research fund and use of laboratory facilities through this research.

## **REFERENCES**

- Ariyaphanphitak, W., Chidthaisong, A., Sarobol, E., Bashkin, V.N. and Towprayoon, S. (2005) Effects of elevated ozone concentrations on Thai jasmine rice cultivars, (*Oryza Sativa L.*). *Water. Air. Soil. Poll.*, 167, 179-200.
- Bell, J.N.B. and Treshow, M. (2002) *Air pollution and plant life*. John Wiley and Sons, Ltd, UK.
- David, T.T., David, M.O., Andrew, A.H. and Lee, E.H. (1994) Effects of ozone on crops. In J.M. David (ed.), *Tropospheric Ozone*, Lewis publisher, USA.
- Heagle, A.S. (1989) Ozone and crop yield. *Annu. Rev. Phytopathology*, 27, 397-423.
- Kobayashi, K., Okada, M. and Nouchi, I. (1995) Effects of ozone on dry matter partitioning and yield of Japanese cultivars of rice (*oryza sativa L.*). *Agric. Ecosystem. Environ*, 53, 109-122.
- Kress, L.W. and Miller, J.E. (1983) Impact of ozone on soybean yield. *J. Environ. Qual.*, 12, 276-281.
- Manning, W.J. and Feder, W.A. (1976) Effects of air pollutants on plants. In S.L. Allen (ed.), *Surface level ozone exposures and their effects on vegetation*, Lewis publishers, U.S., 93-156.
- Miller, J.E., Booker, F.L., Ficus, E.L., Heagle, A.S., Pursley, W.A., Vozzo, S.F. and Heck, W.W. (1994) Ultraviolet-B radiation and ozone effects on growth, yield, and photosynthesis of soybean. *J. Environ. Qual.*, 23, 83-91.
- Mohnen, V.A., Goldstein, W. and Wang, W.C. (1993) Tropospheric ozone and climate change. *Air. Waste*, 43, 1332-1344.
- Oltmans, S.J., Lefohn, A.S., Harris, J.M., Galbally, I., Scheel, H.E., Bodeker, G., Brunke, E., Claude, H., Tarasick, D., Johnson, B.J., Simmonds, P., Shadwick, D., Anlaf, K., Hayden, K., Schmidlin, F., Fujimoto, T. and Cuevas, H.E. (2006) Long term changes in tropospheric ozone. *Atmos. Environ.*, 40, 3156-3173.
- Ostromsky, T., Dimov, I., Tzavetanov, I. and Zlatev, Z. (2001) Estimation of the wheat losses caused by the tropospheric ozone in Bulgaria and Denmark. In L. Vulkov, J. Wasniewski and P. Yalamov (ed.), *Numerical Analysis and Its Applications*, Springer-Verlag, Berlin, Heidelberg, 636-643.
- Pollution Control Department (PCD) (2008) <http://www.pcd.go.th>.
- Runeckles, V.C. and Chevone, B.I. (1992) Crop responses to ozone, surface level ozone exposures and their effects on vegetation. In S.L. Allen (ed.), *Lewis publishers, USA*.
- Singh, E., Tiwari, S. and Agrawal, M. (2010) Variability in antioxidant and metabolite levels, growth and yield of two soybean varieties, An assessment of anticipated yield losses under projected elevation of ozone. *Agr. Ecosyst. Environ.*, 135, 168-177.
- Wahid, A., Milne, E., Shamsi, S.R.A., Ashmore, M.R. and Marshall, F.M. (2001) Effects of oxidants on soybean growth and yield in the Pakistan Punjab. *Environ. Poll.*, 113, 271-280.