

## Effect of long-term ozone fumigation on growth development and yield of spring wheat in open-top field chamber

Wang Xunling<sup>1</sup>, Huang Yunzhu<sup>1</sup> and Wang Jing<sup>1</sup>

(Received March 22, 1989)

**Abstract**— The experimental plants were grown in open-top chamber and exposed to 0.26 ppm of ozone for six hrs. per day from seedling stage till ripening. The results showed that the height of plants, rates of earing, flowering, grain forming, ripening and the weight/1000 kernels all declined in fumigated plants in comparison with the controls. The yield lost 76.7%. The actual actions of ozone were that it caused foliar injury and chlorophyll destruction accelerating leaf senescence, reduction of assimilation products. O<sub>3</sub> was unfavorable injurious to transport and accumulation of substances to the grains after flowering.

**Keywords:** ozone; growth-development; spring wheat.

Ozone (O<sub>3</sub>) is the most major air pollutant in Europe and America. For example, the crop yield loss caused by air pollutants in America was a value about 10-20 hundred million dollars per year, of which 90% was attributed to O<sub>3</sub> (Heck, 1982; Kress, 1985). Several reports about impact of O<sub>3</sub> on wheat were published (Kress, 1985; Wang, 1983). In recent years, researchers have carried out the studies on the effects of O<sub>3</sub> on cereals with great interest. They attempted to give a correct assessment of yield losses caused by air pollution and to expound the correlation between pollutant doses and crop yield. A National Crop Loss Assessment Network (NCLAN), funded by National Environmental Protection Agency, was established in U. S.. The experiments in fumigation of O<sub>3</sub> in open-top field chamber have been undertaken in north-east, south-west, south-east and central region of America since 1980. The long-term effects of lower concentration of O<sub>3</sub> on wheat growth have been noticed, and some valuable results were obtained (Heck, 1984a; 1984b).

In 1974, we discovered for the first time the photochemical smog pollution in the urban districts and suburbs of Lanzhou City by means of biological monitoring (Chen, 1978). Later,

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<sup>1</sup>Biology Department, Lanzhou University, China.

this discovery was confirmed by monitoring instruments (Gansu, 1980). In this work, the open-top field chambers were used to investigate the effect of O<sub>3</sub> on spring wheat. We have gained some valuable data of wheat yield affected by O<sub>3</sub>.

We hope to provide the necessary reference for environmental and agricultural departments to assess air pollution and to formulate counter-measure of reducing yield loss.

## MATERIAL AND METHOD

Spring wheat (*Triticum aestivum* L.) cultivar "Jin 2148" was used. Grains were sown in common cultivated soil in March 24, 1986. The tillers were cut off at tillering stage and 50 plants with main stems were put in each open-top field chamber to be fumigated from May 10. The controls were treated with charcoal-filtered air. The cultivation management of plants was as if that in the field.

Ozone was generated through ionization of oxygen in the air by high electrical pressure. The fumigation took 6 hrs. per day (from 7 A. M. to 1 P. M.) until ripening. Accumulative fumigation lasted 342 hrs (54 days). The O<sub>3</sub> concentration was measured with GSH-201 model ozone analyzer and recorded automatically. The O<sub>3</sub> concentration was adjusted to 0.26 ppm. The growth and development of both fumigated plants and controls were daily observed during experiment. The data obtained were the average of several measurements. The processing of related data was made. Environmental factors, such as temperature and humidity in fumigated chamber, were also recorded. The chlorophyll contents in second or third leaves from the top leaves downwards were determined using colorimetric method at different development stages. The voltage decreasing at middle segments of leaves was also measured three times at every stage by FI-3B model electric resistant meter for plant tissue (Yu, 1979; Zhu, 1982).

## RESULTS

### *Effect of O<sub>3</sub> on growth and yield of wheat*

#### 1. Effect of O<sub>3</sub> on height of plants

At tillering stage, the influence of O<sub>3</sub> on height of plants was not evident, but apparent at flowering stage (Table 1).

Table 1 Effect of O<sub>3</sub> on plant height (cm) at various stages

Development stage	Tillering stage	Jointing stage	Earing stage	Blooming stage	Milk maturity stage	Dough maturity stage
Measuring date	Apr. 2- May 13	May 14 -23	May 24 -30	May 31- Jun. 7	Jun. 9-20	Jun. 21 - July 3
Control	12.6±2.86	36.0±8.11	41.2±9.13	46.3±0.67	46.4±10.67	46.4±0.67
Treatment	11.8±2.87	34.6±11.16	41.0±9.12	43.5±9.90	43.5±9.90	43.5±9.90
Difference of hight	0.8	1.4	0.2	2.8	2.8	2.8
P value	>0.25	<0.25	>0.25	<0.10	<0.10	<0.10

## 2. Damage and senescence of leaves caused by O<sub>3</sub>

Leaves of wheat were damaged by O<sub>3</sub> treatment. The results are shown in Table 2.

**Table 2** The damage effect of O<sub>3</sub> on wheat leaves

Developmental stage	Tillering stage		Jointing stage		Earing stage		Blooming stage	
	Injury rate	Injury area	Injury rate	Injury area	Injury rate	Injury area	Injury rate	Injury area
Controls	0	0	0	0	0	0	0	0
Treated plants, %	40	30	50	30	50	70	70	80

Ozone fumigation showed an obvious influence to the leaves at tillering stage. At the earing stage, the injurious effect of O<sub>3</sub> fumigation remarkably increased. At blooming stage, the injured area of leaves were up to 80%.

The injured region of the leaves was related to the leaf age. Generally, the injury often occurred on the apex and front part for the young leaves, however, on the middle of leaf for the older leaves. The leaves at the lower part of the plants were injured more severely than that at the upper part. The initial injury symptoms expressed small dense flecks on the leaf apex and marginal area later, small flecks stretched downward along parallel veins and formed stringy pigmented spots. As damage developed, such spots joined together, so that the bifacial necroses occurred. A chlorosis band appeared between damaged tissue and health tissue. When the leaf margin rolled, the chlorosis band gradually disappeared.

The leaf blades of wheat usually have a definite lifetime. The lifetime of early developed leaves is longer than that of the later developed. Total area of green leaves decreased after booting stage, since a part of leaves became yellowing (Xia, 1962). The speed of leaf yellowing of O<sub>3</sub> fumigated plants was markedly faster than that of controls. The yellowed leaves of fumigated plants reached to 85% at milk maturity stage, but only 20% on controls.

**Table 3** Comparison of the number of yellowing leaves on a plant

Developmental stage	Earing stage	Blooming stage		Milk maturity stage	
Measuring date	May 29	May 31	Jun. 5	Jun.11	Jun.20
Control plants	1	1	2	3	4
Treated plants	2	2	5	6	6

## 3. Effect of O<sub>3</sub> on rate of earing

The effect of O<sub>3</sub> on earing is shown in Table 4.

**Table 4** The comparison of earing rates

Measuring date	unit: %			
	May 25	May 31-Jun. 7	Jun.8-11	Jun. 13-20
Control plants	23.2	79.7	100	100
Treated plants	20.3	72.5	84.1	84.4
Difference	2.9	7.2	15.9	15.6

The earing stage was delayed in the fumigated plants. The earing rate was finally decreased by 15.6% comparing with controls.

#### 4. Effect of O<sub>3</sub> on rates of blooming and fruiting

As showed in Table 5, O<sub>3</sub> obviously affected the flower development, pollination and embryogenesis. There were marked differences in the rates of blooming, empty ears, fruiting and the rate of immature ears between treated and untreated plants.

**Table 5** Comparison of blooming and fruiting status between controls and treatments

	Amount of ears	Rate of blooming, %	Rate of empty ears, %	Rate of fruiting, %	Rate of immature ears, %
Control	100	97.1	5	95	3
Treatment	85	86.5	16.5	83.5	9.4
Differences	-15	-10.6	+11.5	-11.5	+6.4

#### 5. Effect of O<sub>3</sub> on grain yield of wheat

The main elements of yield were listed in Table 6.

**Table 6** Comparison of essential elements of yield

	Grains/per ear, average	Weight/1000 kernels, g	Total yield, g
Controls	29	30.4	59.7
Treatments	18	11.9	14.0
Rate of reduce, %	37.9	60.9	76.7

#### *Effect of O<sub>3</sub> on some physiological functions*

##### 1. Effect of O<sub>3</sub> on electrical resistance of leaf tissue

The changes of electrical resistance of leaf tissue were measured three times at regular intervals at every development stage in order to express the effect of O<sub>3</sub> on leaf tissue. The average values obtained were shown in Fig.1.

The results showed that the electrical resistances of leaf tissue varied at various development stages, and this change exhibited as a single peak curve. The peak was reached at earing stage. The tendency of curve change of the treated plants was basically similar to that of the control. The maximum difference between them appeared at seedling stage. The electrical resistance of

leaf tissue in controls corresponded to 1.8 times of the treated plants. The minimum difference was seen at tillering stage. The lowest value in control was as 1.2 times as that in the treated plants (Fig. 1).

## 2. Effects of $O_3$ on chlorophyll content

The content of chlorophyll *a* and *b* were determined at different growing stages. The results are shown in Table 7.

The content of chlorophyll started to fall from blooming stage and decreased to zero at milk maturity stage on treated plants. The contents of chlorophyll in controls and treatments were not apparently different at early stages. It suggested that chlorophyll was intensively destroyed by  $O_3$ .

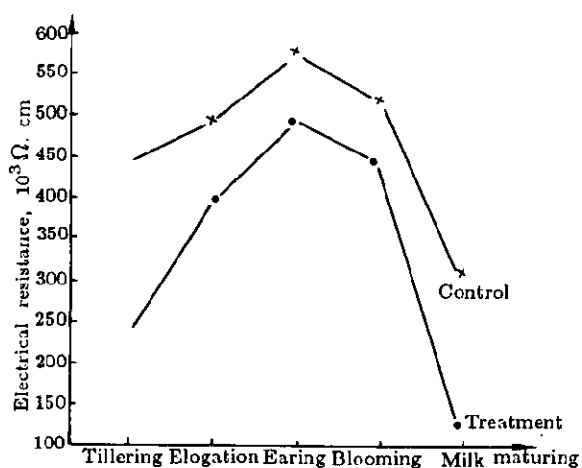


Fig. 1 Influence of  $O_3$  fumigation on electrical resistance of leaf tissue at various stages

Table 7 The contents of chlorophyll at various stages  
Unit: mg/g (fresh weight)

Developmental stage	Tillering stage			Jointing stage		
	<i>a</i>	<i>b</i>	<i>a/b</i>	<i>a</i>	<i>b</i>	<i>a/b</i>
Control	8.42	5.39	1.56	10.37	3.82	2.71
Treatment	7.97	5.13	1.56	8.36	3.17	2.63
Difference	-0.45	-0.26	-0.01	-1.99	-0.65	-0.08
Rate of duce	-5.34	-4.82		-19.19	-17.07	
<i>P</i> value	<0.05	<0.01		<0.05	<0.10	

(Table 7 continued)

Earing stage			Blooming stage			Milk maturity stage		
a	b	a/b	a	b	a/b	a	b	a/b
13.05	2.11	3.42	12.45	2.27	5.48	13.91	2.15	6.47
8.99	1.60	5.61	4.45	0.84	5.29	0	0	0
4.06	-0.51	2.19	-8.0	-1.43	-0.19	-13.91	-2.15	-6.47
-31.11	-24.7		-64.26	63.05		-100	-100	
<0.05	<0.01		<0.05	<0.10		<0.05	<0.10	

## DISCUSSION

Long-term fumigation with 0.26 ppm O<sub>3</sub> caused the yield loss by 76.7% in spring wheat cultivar "Jin 2148". It is obviously interesting to analyse the cause for this and to find the critical stage at which the yield loss took place. Hill and Little Field (Hill, 1969) suggested that the cause for yield loss induced by O<sub>3</sub> was that the assimilative area in leaves was reduced, hence the synthesis and accumulation of assimilate were correspondingly affected. Someone considered that O<sub>3</sub> might inhibit the development of vegetative and reproductive organs (Nagele, 1973). The others (Evans, 1973; Heath, 1975) thought that O<sub>3</sub> could induce changes of membrane permeability and affect the transport of water and nutritive substances. In this study, we have found that the cause for wheat yield loss caused by O<sub>3</sub> is comprehensive actions, and the harmful actions of O<sub>3</sub> is different at various development stages. The effect of O<sub>3</sub> at early stages of wheat reflected mainly on leaves, a little effect on stems. It is generally believed that the total leaf area of wheat reached to maximum at earing stage (Xia, 1962). In our work, 50% of leaves treated by O<sub>3</sub> was injured, and the injured area covered 70% of the total area of each leaf. Moreover, O<sub>3</sub> accelerated leaf senescence. For example, at earing stage, there was only 1 yellowed leaf on the control plants, but 2 on the fumigated plants. During blooming stage, there were 2 yellowed leaves on the controls, but 5 on fumigated plants. Even though, 20% of area on remained leaves of treated plants were green. Either in injured or yellowing leaves, the content of chlorophyll was reduced (Table 7). This inevitably affected the photosynthesis and then led to the reduction of photosynthetic products. The reduction of photosynthetic products before earing stage was unfavourable to the growth and development of vegetative organs, for example, lowering the plant height (Table 1). It also restarted the formation of flower organs, i.e. the rate of forming ears was reduced by 15% (Table 4). The decrease of photosynthetic products after blooming influenced the transportation of nutrients to grains. It was reported that 80–90% of photosynthetic products in wheat was accumulated at the late one-third period of life time (Xia, 1962). This point of view was also proved in our work. 60.9% of the weight of each 1000 kernels was lost due to O<sub>3</sub> effect.

Moreover, O<sub>3</sub> also markedly affected the formation of the reproductive organs and reproductive process in wheat. Manning *et al.* (Manning, 1976) observed that O<sub>3</sub> inhibited the elongation of pollen tubes in tomato, the rate of fructification dropped 37%. The yield declined

17%.

It was also noted that pollen tube growth were inhibited and the tip of tube was narrowed or died when the tobacco and petunia were affected by  $O_3$  (Nagele, 1973). We observed that the rate of pollen germination in some horticultural plants, such as apple "red delicious", fumigated with  $O_3$  was 72.19% lower than that in untreated plants. When plants were fumigated with  $O_3$ , the length of pollen tube was about 1/23 of controls (Wang, 1985). Since the rate of pollen germination decreased and the growth of pollen tubes was inhibited, the fertilization was certainly affected. The rate of empty ears in wheat in our experiment was 11.5% higher in  $O_3$  treated plants than in controls (Table 5). The amount of grains on each ear was reduced by 37.9% comparing with controls. Therefore, it should be noticed that the critical phase affecting wheat yield by  $O_3$  might be the duration from earing to filling stage.

$O_3$  impacted the growth and development of wheat through disturbing physiological activities to a great extent. It was reported that the primary site of  $O_3$  injury would be the cell membrane. Once the function of cell membrane was slightly changed, homeostasis was destroyed and eventually leading to metabolism disorder. Function change of cell membrane caused by  $O_3$  displayed increase of membrane permeability (Heath, 1975). Evans and Ting discovered that the influx of external water into leaf disks at the isotonic point was dramatically decreased by ozone fumigation on the plant (60%) and the efflux of internal water was increased about 20-30% (Evans, 1973). Transportations of water and nutritive substance as well as other metabolic activity were obviously impacted by  $O_3$ . The results obtained from this experiment showed that tissue electrical resistance of  $O_3$ -treated tissue was lowered just similar to that of  $SO_2$  (Yu, 1979). It is suggested that  $O_3$  increased permeability of plasma membrane at every stage and caused leakage of electrolytes. Loss of water from a plant cell and an efflux of ions and metabolites could deeply affect the normal function of the plant. The inevitable results were: leaves were injured, pollen germination was restrained and nutrient accumulation was decreased.

**Acknowledgements**—The author wish to thank Prof. Yu Shuwen for his advice and Prof. Zhang Chenlie for the useful discussion.

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