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# Secondary effect of $SO_2$ pollution: enhanced growth of the army worm, *Mythimna separata* (Walker)

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**Abstract**—Effects of $SO_2$ pollution on growth of the army worm, *Mythimna separata,* were investigated by rearing the larvae for 12 days on the wheat plants being exposed to $SO_2$ levels ranged from 50 to 200 ppb in the field open-top fumigation devices. Larval period shortened while their mean relative growth rate (MRGR) increased as $SO_2$ dose elevated. Similar experiment with the insect on the artificial diet excluded the possibility that $SO_2$ pollution directly stimulated growth of the army worm.

The patterns of change in amino acid hydrolysates in the host foliage suggested that contents of methionine, and perhaps, arginine in food material exposed to unpolluted air were at such levels that they could only support sub-optimum growth of the insect, and $SO_2$ pollution increased concentrations of these limiting amino acids, resulting in better performance of the larvae.

**Keywords:** environmental pollution; $SO_2$; wheat plant; amino acids; army worm (*Mythimna separata).*

Air pollution has been making a great contribution to climatic change. A warming global climate due to greenhouse effect can serve as a typical example. Sulfur dioxide has been recognized as an important air pollutant with adverse effects on the health of humans, other animals and plants (Petters, 1982). Its impacts on plants are particularly striking and have been documented (Mandl, 1975; Linzon, 1978; Cao 1985). Recently, the secondary effect of the pollutant on plants has been discovered. This is the effect which is not caused directly by it, but $SO_2$ represents a causal predisposing factor. One such effect is improvement in growth and fecundity of a few species of insects on their host plants in $SO_2$-polluted environments.

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There are many surveys describing larger populations of insect herbivores and greater damage to their hosts in areas adjacent to $SO_2$ emitting source and some suggestions to explain the relationship between them (Stark, 1968; Port, 1980; Alstad, 1982; Yu, 1988). However, experimental evidence was absent for these hypotheses until 1980s. Hughes *et al.* in 1982 presented the first example in which faster growth and greater egg production of the Mexican bean beetle, *Epilachna varivestis,* were testified on soybean plant fumigated with $SO_2$ (Hughes, 1982). Since then, similar results have been reported for several species of aphids (Dohmen, 1984; 1985; Warrington, 1987a; 1987b).

Such secondary effect of $SO_2$ pollution on plants may be considered as a general but individual response of insects to the stressed hosts. To prove it, a set of laboratory experiments were carried out with the army worm, a species of Lepidopteral insect, on the wheat foliage which had been exposure to lower doses of $SO_2$ and their better performance was detected (Wu, 1990). The present paper deals with growth of the army worm on the wheat plants in the environments simulating $SO_2$ pollution, and a possible mechanism responsible for increased growth of the larvae is suggested on basis of change in amino acid pattern in the host plant.

## MATERIALS AND METHODS

*Food plant and insect*

Pot-sown winter wheat (*Triticum aestivum* cv. Chang Feng First) was used for food plant. After turn-green stage the plants were exposed to different doses of $SO_2$ in a series of top-open fumigation devices. The chamber received charcoal-filtered air served as the control. The colony of the army worm, *Mythimna separata* (Walker) has been raised for several generations in the room before tested. For the details of plant fumigation and management and insect rearing, to see the recent paper (Wu, 1990).

*Army worm treatment*

Two sets of experiments were designed to investigate direct and indirect effects of $SO_2$ on the insect, respectively.

1. Growth of the larvae on wheat plants. The neonate larvae in groups were raised separately with the wheat foliage which had been exposed to different levels of $SO_2$ for 1 week at 25°C, natural photoperiod in the laboratory. Then, identical developmental stage of the worm, i.e., newly-moulted 3rd-instar larvae were selected from each group and transferred, respectively, to the wheat plants which were fumigating with corresponding doses of $SO_2$. After freely feeding for 12 days they were searched out and reared on the artificial diet in the laboratory until pupated. During growth period of the larvae on the host daily temperature averaged 22.3°C with a range from 16.0°C to 27.9°C.

2. Direct impact of $SO_2$ pollution on the insect. The larvae after hatching were reared on the artificial diet for 1 week at the same condition as above in the room. The worm entered the

3rd instar on the 8th day were chosen and put on a piece of the diet in 25 ml beakers, individual per container. The beakers covered with gauze were divided into groups at random and placed at the center of fumigation chambers. Fumigation was run daylight. Every afternoon when the machines were turned off the beakers were removed into the room and the diets were replaced. 12 days later, the larvae were reared again with the diet in the laboratory until the pupae formed. Daytime temperature during 12 days varied between 14.1°C and 35.4°C with a mean of 28.2°C.

Mean relative growth rate (MRGR) of the larvae was measured according to Van Emden (Van, 1969).

$$MRGR = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Growth time in day}}$$

*Measurements of sulfur and amino acid content*

At the beginning of raising the larvae, the wheat foliage were sampled and their sulfur contents were examined with the combustion method. When feeding experiment came to end the foliage were taken, dried and ground. After hydrolyzed with 6 mol/L HCl concentrations of amino acids were determined by the high pressure liquid chromatography (HPLC) with reversed phase column at the standard condition.

## RESULTS AND ANALYSES

*Performance of the larvae on the plants*

Certain differences in developmental progresses of the larvae appeared after 12 day's treatment. The higher the $SO_2$ dose, the greater the percentage of the 6th instar larvae out of the total found, and those searched out from the plants received 200 ppb of $SO_2$ were all at the last instar. During this period MRGR of the larvae on the host exposed to $SO_2$ levels above 100 ppb were also higher than that of the control (Table 1).

The whole duration of the larvae shortened with elevating $SO_2$ dose and a significant linear correlation existed between them (Fig. 1).

During fumigating period MRGR of the larvae on the plants exposed to 50 ppb of $SO_2$ did not differ from that of the control, but this treatment had a hysteresis effect, resulted in higher MRGR for the whole period of larvae in comparison to that of the control. Thus, MRGR during larval period was linearly related to $SO_2$ level with which the plants and the larvae were fumigated for 12 days (Fig. 2).

**Table 1** Growth of army worm on wheat plants exposed to different concentrations of $SO_2^*$

| $SO_2$ level, ppb | Control | 50 | 100 | 200 |
|---|---|---|---|---|
| No. of larvae tested | 32 | 32 | 32 | 32 |
| No. of larvae found after 12 days | 12 | 12 | 25 | 21 |
| Distribution of larval instars, % | | | | |
| 4th instar | 8.3 | 8.3 | 0 | 0 |
| 5th instar | 16.7 | 8.3 | 8.0 | 0 |
| 6th instar | 75.0 | 83.3 | 92.0 | 100.0 |
| MRGR during 12 days | 0.3018 (0.0111)[a] | 0.3014 (0.0172)[a] | 0.3298 (0.0082)[ab] | 0.3477 (0.0060)[b] |
| Pupal weight, mg | 302.2 (14.7) | 293.1 (10.4) | 291.2 (7.2) | 286.8 (9.2) |
| Pupal duration, Days | 10.91 (0.09) | 11.0 | 10.88 (0.13) | 10.83 (0.09) |

* Values in parentheses indicate standard errors ($SE$). Means followed by different letters differ significantly at $P<0.05$.

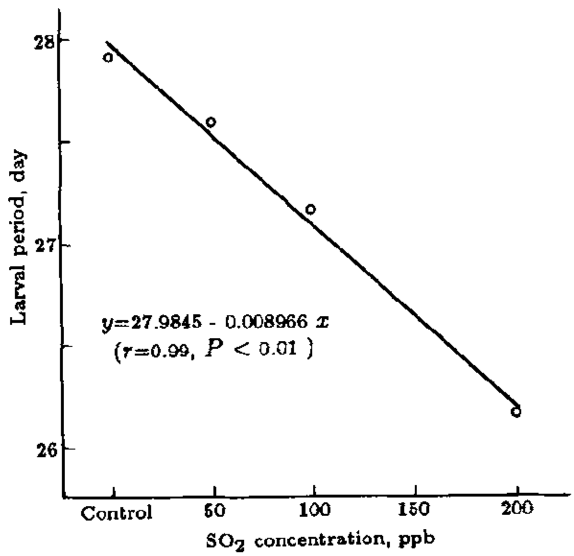


**Fig. 1** Developmental period of larvae on wheat plant in relation to ambient $SO_2$ level

*Direct impact of $SO_2$ pollution on the larvae*

The similar experiment with the animals on the artificial diet showed no any favorable

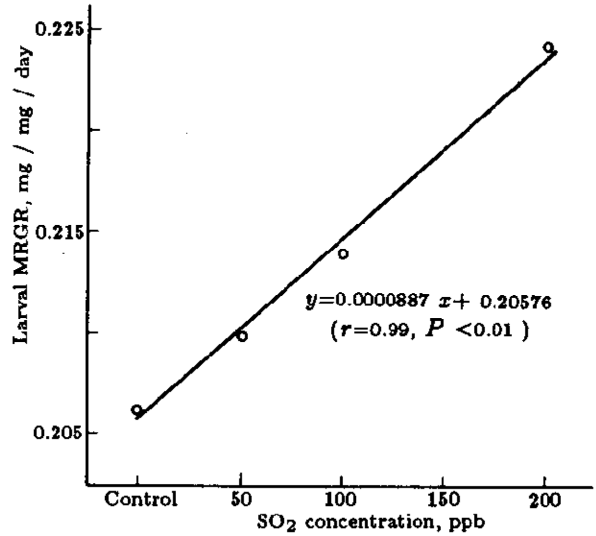


**Fig. 2** Relationship between MRGR during larval period and $SO_2$ level in ambient air

effect of the pollutant on growth of the army worm (Table 2). In contrast, their growth was significantly retarded when $SO_2$ concentration was at 200 ppb. Within the range of $SO_2$ doses tested either pupal size or their duration did not differ significantly among the treatments.

**Table 2** Direct effect of $SO_2$ fumigation on growth of army worm

| $SO_2$ level, ppb | Control | 50 | 100 | 200 |
|---|---|---|---|---|
| No. of larvae tested | 40 | 40 | 40 | 40 |
| No. of survivor after treatment | 15 | 19 | 23 | 21 |
| MRGR during 12 days | 0.2966 | 0.2839 | 0.2910 | 0.2750 |
| | (0.0032)[a] | (0.0053)[ab] | (0.0038)[a] | (0.0036)[b] |
| Larval duration, day | 30.20 | 30.89 | 29.43 | 30.95 |
| | (0.70) | (0.62) | (0.44) | (0.57) |
| Pupal weight, mg | 318.2 | 320.8 | 339.4 | 326.9 |
| | (12.4) | (11.4) | (6.4) | (10.5) |
| Pupal duration, day | 11.31 | 11.41 | 11.36 | 11.32 |
| | (0.13) | (0.12) | (0.10) | (0.13) |

A combination of the data from these two sets of tests indicates that improved growth of the larvae resulted from $SO_2$ pollution was mediated via their host plant and some changes in components and/or their concentrations of the food plant exposed to the pollutant should occur in favor of the insect.

*Change in foliar sulfur content*

Sulfur content in the foliage was greatly influenced by $SO_2$ dose. With the dose-tested range foliar sulfur content increased as $SO_2$ concentration went up. There existed a linear relationship between them (Fig. 3). Sulfur content in those fumigated with 200 ppb of the pollutant was 2.3 times that of the control.

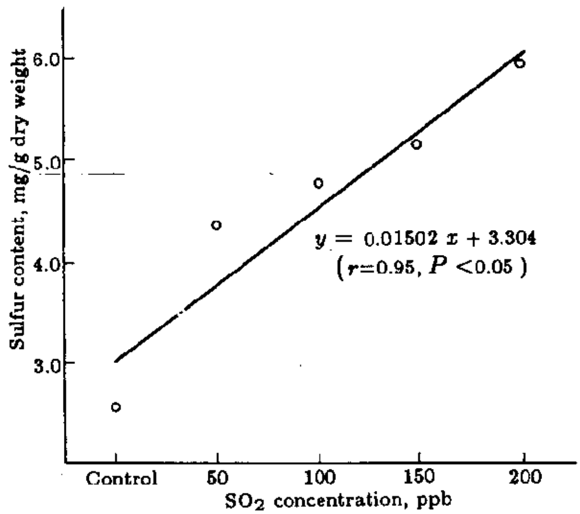


**Fig. 3** Relationship between sulfur content in wheat foliage and $SO_2$ level in air

*Change in amino acid contents of the foliage*

Total amount of amino acid hydrolysate as well as quantities of individual ones varied in the food materials treated with different levels of $SO_2$. Sum of them gradually declined with increasing $SO_2$ dose above 50 ppb and it reduced by 40% at the highest concentration of the pollutant compared to that of the control (Fig. 4). A majority of individual amino acids in the foliage exposed to 50 ppb of $SO_2$ increased more or less. Of 9 detected amino acids essential to insect increased contents occurred in 6 ones among them arginine doubled, and methionine, lysine and valine increased by more than 35% relative to that of the control (Fig. 5). Although total amino acids in the foliage fumigated with 200 ppb of $SO_2$ sharply dropped and most individual ones reduced to different extent, methionine and arginine were still much greater than that of the control.

Among 15 amino acids determined change in methionine, a S-containing amino acid, is most impressive. Either its absolute content or relative concentration in terms of the percentage accounting for the total amino acids increased with elevating $SO_2$ level (Fig. 5), which Seemly resulted from $SO_2$ participating in plant metabolism.

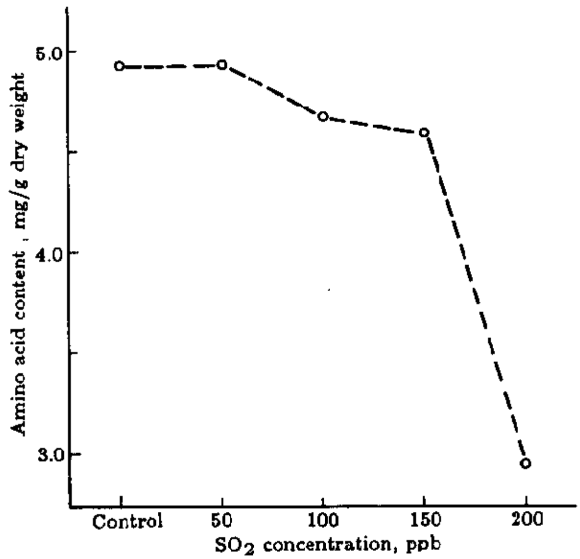


**Fig. 4** Change in total quantity of amino acids in the foliage exposed to different doses of $SO_2$

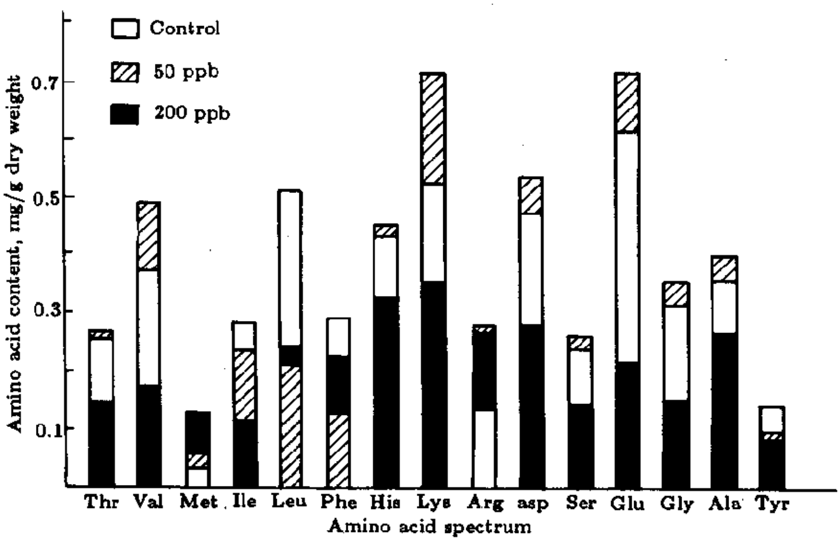


**Fig. 5** Amino acid contents in the wheat foliage exposed to different doses of $SO_2$ (The first 9 ones are essential to insect growth)

## DISCUSSION

Damage of $SO_2$ pollution to plants has been well known for long time whereas its secondary effect was recognized only in the past decade (Hughes, 1982; Duhmen, 1984; Dohmen, 1985; Warrington, 1987; Wu, 1989). A laboratory examination showed that growth and reproduction of the army worm were enhanced when the larvae were reared on the wheat foliage exposed to lower levels of $SO_2$ (Wu, 1990), which is further confirmed in the present field experiment.

Improved performance of phytophagous insect by $SO_2$ pollution can be considered as its direct effect or indirect consequence mediated via their host plants, or both of them (Alstad, 1982; Dohmen, 1984; Wu, 1988). In general, $SO_2$ treatment reduced growth and fecundity of most insect species (Alstad, 1982). In some cases no obvious influence was detected (Petters, 1982). Of the insect species examined only exception was the large milkweed bug. *Oncopeltus fasciatus,* which was difficultly explained even by the authors (Fair, 1983). The results presented here also deny the possibility of the pollutant directly stimulating growth of the army worm and indicate importance of its indirect effect.

The precise mechanism with which insects performed better in air-polluted environments is yet to be elucidated. It is commonly assumed that food plants submitted to pollution would undergo certain change in their composition, especially protein and /or amino acids, which would benefit the animals (Dehmen, 1984; Braun, 1985). But there is little evidence derived from experiments directly relating insect performance with the changes in plant components induced by the pollutants. The recent paper by Bolsinger *et al.* (Bolsinger, 1989) dealing with the relationship between motorway air pollution and infestation of the aphids, *aphis fabae,* has made some contribution to the issue. They found an increase in almost all of the detected amino acids in the host plant due to the pollutants. By translating amino acid pattern analyzed into artificial diet they demonstrated better performance of the aphid on the simulated diet. Our analyses revealed that $SO_2$ fumigation altered amino acid pattern in the wheat foliage with a substantial effect on methionine and arginine. Of all the detected amino acids in the foliage exposed to charcoal-filtered air methionine was the smallest, only accounting for 0.7% of the total quantity of amino acids, and arginine, the second smallest, 2.7%, which was about half the other amino acids essential to insects. Methionine and arginine in the foliage treated with 50 ppb of $SO_2$ increased by 60% and doubled, respectively. With the higher doses of the pollutant sum of the foliar amino acids declined in order and many individual ones decreased to varied extent, but methionine continuously increased, and other necessary amino acids kept at properly high levels (Fig. 6).

These measurements indicate that improved growth of the army worm on the wheat plants grew in $SO_2$-polluted environment could be attributed to increase in individual amino acids, especially methionine and arginine, rather than all ones induced by the pollutant. It is rea-

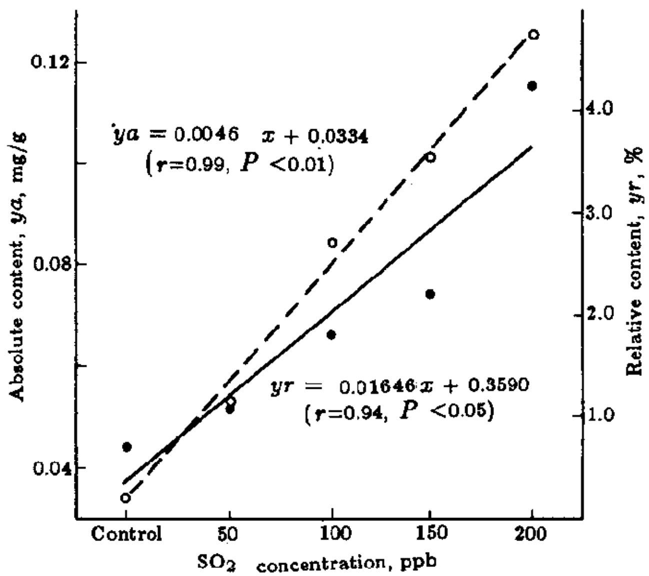


**Fig. 6** Methionine content in wheat foliage in relation to ambient $SO_2$ level

sonably suggested that very low levels of methionine and perhaps, arginine in the host plant grew in unpolluted environment could only support sub-optimum growth of the army worm, and increase in these limiting amino acids caused by $SO_2$ pollution improved host nutritional value in terms of amino acids-balanced food for the insect, resulted in better performance of the army worm.

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## REFERENCES

Alstad, D.N., Edmunds, G. F. Jr. & L. H. Weinstein, Ann. Rev. Entomol., 1982, 27:369

Braun, S. & W. Fluckiger, Environ. Pollut., 1985, 39:183

Bolsinger, M. & W. Fluckiger, Environ. Pollut., 1989, 56:209

Cao Hongfa, Liu Houtian, Shu Jianmin & Gao Yingxin, J. Environ. Sci. China, 1985, 6:59

Dohmen, G. P., McNeill, S. & J. N. B. Bell, Nature, 1984, 307(5946):52

Dohmen. G. P., Environ. Pollut., 1985, 39:227

Fair, D. & R. Hale, Int., J. Environ. Studies, 1983, 20:269

Hughes, P. R., Potter. J. E. & L.H.Weinstein, Environ. Entomol., 1982, 11:173

Hughes, P. R., Dickie, A. I. & M. A. Penton, J. Environ. Qual., 1983, 12:565

Linzon, S. N. In: Sulfur in Environment, Part II. Ecological impacts (Ed. by Nriagu, J. O.), New York: John Wiley, 1978, 109

Mandl, R. H., Weinstein, L. H. & M. Keveny, Environ. Pollut., 1975, 9:133

Petters, R. M. & R. V. Mettus, Environ. Pollut., 1982, 27:155

Port, G. R. & J. R. Thompson, J. Appl. Ecol., 1980, 17:649

Stark, R. W., Miller, P. R., Cobb, F. W. Jr., Wood, D. L. & J. R. Parmeter Jr., Hilgardia, 1968, 39: 121

Van Emden, H. F., Entomol. Exp. Appl., 1969, 12:125

Warrington, S., Environ. Pollut., 1987, 43:155

Warrington, S., Mansfield, T. A. & J. B. Whittaker, Environ. Pollut., 1987, 48 285

Wu Kunjun, Gong Peiyu, Li Xiuzhen, Shu Jianmin & Cao Hongfa, Acta Sci. Circum. Sinica, 1990, 10:78

Wu Ya, Edward, H. L. & M. B. Edward, Acta Ecol. Sinica, 1989, 9:336

Wu Kunjun, J. Entomol. Knowl. Sinica, 1988, 25:122

Yu Shuwen, Yu Ziwen, Ma Guangjing *et al.*, J. Environ. Sci. China, 1988, (3): 77