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Effects of fluoride on growth and reproduction of the army worm, *Mythimna separata* (Walker)

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Abstract— This paper deals with effects of airborne fluorine on the army worm, *Mythimna separata* (Walker), by rearing the larvae on the wheat foliage exposed to HF or fumigating the larvae on the artificial diet with the pollutant. Larval relative growth rate (GR) and index of population trend of the insect reduced by 5% and 11%, respectively, when the larvae were reared on the foliage taken from the wheat plants exposed to 0.87 $g \cdot dm^{-2} \cdot day^{-1}$ of fluorine compared with those of the control. An extra instar appeared in a majority of the larvae treated. Survival rate and GR of the larvae on the wheat plant being exposure to the same concentration of fluorine in field open-top fumigation device were 40% and 15% lower than that of the control, respectively. Similar experiment with the insect on the artificial diet also showed that direct impact of the pollutant on the army worm was greater than its indirect effect via their host plant.

Keywords: air pollution; fluoride; wheat plant; army worm (*Mythimna separata).*

In recent years, rural industries have developed rapidly in China and have resulted in air pollution and affected agriculture, horticulture and forestry seriously. Among important air pollutants, the hydrogen fluoride (HF) is emitted from brick kilns, phosphate fertilizer plants, coal and other industrial activities. Impacts of fluoride on insects have been reviewed by Alstad and so on (Alstad, 1982), and more recently by Wu (Wu, 1988). This problem is vital to silkworm industry in China and Japan where damage from ingestion of F-polluted mulberry leaves has been reported frequently (Fujii, 1972; Imai, 1974; Wang, 1980; Qian, 1984; Bian, 1985). The present work aims to investigate impacts of airborne fluorine on the army worm, *Mythimna separata,* and its direct and indirect effects were compared.

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MATERIALS AND METHODS

Food plant

Winter wheat (*Triticum aestivum* cv. Chang Feng First) for food plant was sown in pots. After turn-green stage they were exposed to different doses of HF in a series of field open-top fumigation devices (Shu, 1986). The control one received charcoal-filtered air. Fumigation was run in the daytime, 7 hrs per day. Three weeks later, the foliage were cut to raise the larvae of the army worm. Atmospheric fluorine concentration in the chambers was determined with NaOH treated paper (Jocobson, 1977).

Insect

A colony of the army worm, *Mythimna separata* (Walker) was established from the moths collected at Beijing Suburb and had been raised on the corn leaf-wheat germ artificial diet for several generations before the test. Three sets of experiments were arranged.

1. Larvae were reared on HF-exposed wheat foliage

Newly-born larvae were raised at 24±1°C in the room with the foliage exposed to HF. Their performances were monitored. Larval relative growth rate (GR), and approximate digestibility (AD), gross and net conversion efficiencies of the foliage by the larvae (ECI and ECD, respectively) were measured according to Waldbauer (Waldbauer, 1968). Couples of adult moths were provided with 10% honey solution and their fecundities were examined.

2. Larvae were reared on wheat plants being fumigated with HF. The larvae after hatching were raised with the artificial diet for the first two instars, then 1-day-old larvae of the 3rd instar were selected and transferred to the wheat plant being fumigated with the pollutant. 12 days later, the larvae were looked for and their GR were determined.

3. Larvae were reared on the artificial diet in F-polluted air

After the neonate larvae being reared on the artificial diet at 24°C for 8 days the newly-exuviated 3rd instar larvae were picked out and placed on the diet in the desiccators where HF-containing air was driven into and went out. 12 days later, larval GR were measured.

RESULTS AND ANALYSES

Performance of larvae on the treated foliage

Table 1 shows that survival rates of the insect during their immature stage in all treatments were more than 90% while larval period significantly prolonged with increasing fluorine dose, resulting in less index of develop-survival of the larvae on the foliage exposed to higher fluorine level. F-polluted foliage also reduced larval GR. Furthermore, an additional instar appeared in a majority of the larvae on the food material treated by the highest fluorine concentration.

Table 1 Effects of HF-fumigated wheat foliage on growth and development of army worm*

F does, $\mu g \cdot dm^{-2} \cdot day^{-1}$		0.05	0.43	0.61	0.81	0.87
Larvae tested		120	80	120	80	120
Larvae period, days		17.11^a ±0.15	17.31^b ±0.10	17.42^c ±0.12	17.78^d ±0.08	17.92^e ±0.12
% 7th instar larvae		0	8.5	8.5	22.2	70.4
GR, $mg.mg^{-1} \cdot day^{-1}$		0.117^a ±0.001	0.116^b ±0.001	0.115^c ±0.002	0.113^d ±0.002	0.112^d ±0.002
Pupal weight, mg	Female	335.3^a ±8.8	305.6^b ±6.0	302.5^b ±4.7	315.6^c ±6.0	317.5^c ±6.7
	Male	328.7^a ± 5.5	290.5^b ±6.2	296.7^c ±3.0	304.9^d ±5.2	329.0^e ±5.7
Pupal period days	Female	10.63^a ±0.16	10.21^b ±0.11	9.41^c ±0.28	9.58^d ±0.39	9.61^e ±0.17
	Male	11.09^a ±0.18	10.63^b ±0.15	9.77^c ±0.09	10.21^d ±0.10	9.94^e ±0.06
% adult eclosion		92.2	91.4	93.6	94.5	92.3
Index of develop-survival		5.79	5.56	5.45	5.40	5.33

* Values in the table indicate means with standard errors ($M \pm SE$). Values followed by different letters differ significantly at $P < 0.05$. The same footnotes are applied in following tables.

These data indicate that growth of the army worm was retarded due to ingestion of the foliage fumigated with the pollutant.

Digestion and utilization of the foliage by the larvae

Nutritional parameters of F-exposed foliage for the larvae during the first two days of the 6th instar are presented in Table 2. Generally, food ingestion and fecal excretion rates by the larvae tended to decline as increase in ambient fluorine, also demonstrating an adverse effect of the pollutant on their feeding. Similar trend was observed for larval AD in spite of greater ECI and ECD for the worms on the foliage fumigated with fluorine doses greater than 0.8 $\mu g \cdot dm^{-2} \cdot day^{-1}$.

Table 2 Digestion and utilization of F-fumigated wheat foliage by the larvae (n=14)

F dose, $\mu g \cdot dm^{-2} \cdot day^{-1}$	0.05	0.43	0.61	0.81	0.87
Ingestion, $mg. mg^{-1} day^{-1}$	3.08^a ±0.190	3.10^a ±0.114	2.94^a ±0.184	2.68^b ±0.109	2.64^b ±0.124
Egestion, $mg. mg^{-1}. day^{-1}$	1.82^a ±0.050	1.84^a ±0.084	1.70^b ±0.044	1.71^b ±0.060	1.66^b ±0.084
AD, %	40.25^a ±1.26	40.64^a ±1.48	42.08^b ±0.77	36.19^c ±1.31	37.12^c ±0.79
ECI, %	46.31^a ± 2.73	48.90^b ±2.62	44.96^a ±1.70	64.39^c ±2.20	66.74^d ±2.15
ECD, %	18.36^a ±0.85	19.12^b ±0.87	18.58^a ±0.50	24.49^c ±0.70	21.97^d ±0.35

Performance of the adult moths

Three groups of adult moths were chosen for determination of their fecundities and longevities (Table 3). Significant poorer egg production and viability were recorded for the females from the larvae on the polluted foliage and their life spans shortened in comparison to the control group. A comparison among indices of population trend which integrate effects of environmental factors on animal survival rate and fecundity indicates that the population size of the insect on the foliage exposed to over 0.6 $\mu g \cdot dm^{-2} \cdot day^{-1}$ of pollutant after one generation would be 12% less than that on unpolluted food material.

Table 3 Performance of the adults from the larvae on F-fumigated wheat foliage

F dose, $\mu g.dm^{-2}.day^{-1}$		0.05	0.61	0.87
Fecundity	Eggs laid	1938.6 ± 162.8^a	1789.4 ± 124.7^b	1801.9 ± 99.2^b
	Eggs remained	21.7 ± 10.4^a	17.4 ± 12.1^a	17.3 ± 11.2^a
	Total	1960.3 ± 157.3^a	1806.8 ± 125.3^b	1819.2 ± 99.9^b
	% Hatch	99.8 ± 0.33^a	98.68 ± 0.95^b	97.00 ± 2.67^b
Life spens, days	Female	15.67 ± 1.04^a	14.61 ± 1.18^b	12.14 ± 1.24^c
	Male	20.98 ± 1.50^a	19.50 ± 1.40^b	15.78 ± 1.91^c
Index of population trend		957.4	860.8	851.6

Joint effect of atmospheric fluorine and F-fumigated plant on the larvae

The larvae on the wheat plant in fumigation devices were affected by both HF in air and fluoride in the plant. Table 4 shows retardation of larval development caused by fluorine pollution. During 12 days 3 quarters of the larvae had entered the 6th instar on the control plant while number of the same instar larvae accounted for less than 50% of the total in the treated groups with a few of them only passing one instar. Comparing the data from Table 1 and 4, it is apparent that growth and survivalship of the larvae were affected much severer on the plant being fumigated than on the foliage cut from the plant exposed to the same concentration of fluorine.

Table 4 Effects of airborne fluorine and F-fumigated plant on growth of the larvae

F dose, $\mu g.dm^{-2}.\ day^{-1}$		0.05	0.61	0.87
GR, $mg.mg^{-1}.\ day^{-1}$		0.135	0.119	0.117
	6th	78.79	48.57	48.00
Distribution of	5th	21.21	48.57	48.00
larval instars, %	4th	0	2.86	4.00
Survival rate, %		80.00	76.00	56.00

Direct impact of atmospheric fluorine on the larvae

Performances of the insect feeding on the artificial diet in HF-containing air again indicated a serious impact of the pollutant on their growth (Table 5). The animals submitted to direct exposure of HF grew a little and all were at the 4th instar. During the same period most larvae in charcoal filtered air were at 5th instar, and a few, 6th one. Larval GR and survivalship were greatly affected by HF in air, too.

Table 5 Effects of airborne fluorine on growth of the larvae

F dose, $\mu g.dm^{-2}.\ day^{-1}$		0.05	0.61	0.87
GR, $mg.mg^{-1}.\ day^{-1}$		0.0921	0.0756	0.0744
	6th	5.88	0	0
Distribution of	5th	94.12	0	0
larval instars, %	4th	0	100	100
Survival rate, %		75.00	62.00	55.00

DISCUSSION

Airborne fluorides have been recognized for long time as a pollutant affecting health of man and animal as well as affecting plants and ecosystems as a whole. Its effects on insects varied from promoting growth to entirely killing them, depending on insect species tested and its dose used (Alstad, 1982; Hughes, 1985). Influence of airborne fluorine on mulberry-silkworm ecosystem have been extensively studied. It is established that the toxicity of F-containing mulberry leaves to silkworm could be attributed less to the total quantity of fluorine ingested than to the kind or form of the fluorine compounds provided. Ingestion of fluorine salts depressed feeding rate of the larvae, caused softening of the cuticle, reduction in growth rate, and increased mortality (Fujii, 1972; Qian, 1984; Bian, 1985; Hughes, 1985; Kuribayashi, 1972; Wang, 1980; Wang, 1988). There was a close correlation between survival rate of the silkworm and the distance from F-emitting source (Wang, 1988). The results presented here show heavy impact of atmospheric fluorine on the army worm with most symptoms similar to that of silkworm. A comparison among the data from 3 sets of experiments revealed that direct damage of HF in air on the insect was greater than its indirect consequence through their host plant. Based on their study on the cabbage looper *Trichoplusia ni*, Hughes and so on (Hughes, 1985) suggested that fluorine absorbed by plant reacted with components of its tissues to form insoluble salts or complexes. These bound fluorides were mostly or entirely excreted, therefore, not accumulated by insects and less toxic to them. This hypothesis seems an explanation reasonable for the present case, but the real story is not clear until fluorine accumulation by the army worm larvae and the oral toxicity of fluorine salts in artificial diet are tested.

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