VARIABLE TOLERANCE OF THE SILKWORM *BOMBYX MORI* TO ATMOSPHERIC FLUORIDE POLLUTION

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SUMMARY: The LC_{50} of fluoride in mulberry leaves to silkworms was investigated at the fourth instar stage for 50 varieties of silkworm *Bombyx mori* larvae from three localities in China. Large variations in fluoride tolerance among the silkworm varieties were found, which ranged from 19 to 693 mg F/kg of dried mulberry leaves for the LC_{50}. There was also considerable difference in the LC_{50} for the same variety of silkworm maintained in different localities, thereby indicating adaptation to atmospheric fluoride pollution. Significant differences in tolerance to fluoride were also found among commercial silkworm hybrids.

Keywords: Atmospheric fluoride; Fluoride pollution; Mulberry leaf fluoride LC_{50}; Silkworm *Bombyx mori* L.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L., is a very important economic insect that contributes substantially to the national economy of China. Because of its long history of domestication, some varieties have become extremely sensitive to environmental pollution, especially to atmospheric fluoride emitted from brick kilns and coal-burning power plants.[1-3] Moreover, its food source, the mulberry tree, *Morus*, is a high fluoride-accumulating plant.[4] As a result, serious loss of cocoon production often occurs in the main sericultural regions, even when atmospheric fluoride concentrations are lower than the 1980 human health standard in China.[3]

Much effort has been devoted to determining harmful effects of fluoride on silkworm growth and the mechanism of the action of fluoride in the larvae.[5-8] Considerable research has also dealt with the transfer of fluoride accumulated by insects near pollution sources.[9-13] Only a few investigations, however, have dealt with variations in tolerance to fluoride in different varieties of insects of the same species. The aim of this work was to investigate differences among varieties of silkworms in their response to fluoride pollution and to compare the fluoride tolerance of various silkworm hybrids used for commercial cocoon production.

MATERIALS AND METHODS

Silkworm varieties: Fifty silkworm varieties were collected from various sources and locations in China differing in climatic conditions and degree of atmospheric fluoride pollution. Special attention was given to two varieties, Hang 7 and Hang 8, which are maintained at different institutes, to test the response of their larvae to fluoride pollution.

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Leaf treatment and determination of F content: Freshly collected mulberry leaves were soaked in different concentrations of NaF solution for 10 min and then were spread to dry at 24–26ºC until no visible water drops remained on the leaf surfaces. Leaf samples were taken from each treatment before feeding the larvae, and the fluoride content in each sample was determined by the method of Wu[14] after it had been dried to constant weight at 80ºC.

Determination of the mulberry leaf half lethal fluoride concentration (LC_{50}) to silkworm larvae: Following the method developed by Tang and Wang,[15] a series of NaF solutions with concentrations of 0, 10, 20, 40, 80, 160, 320, 640, and 1280 mg F/L were prepared. Before the first feeding at the fourth instar stage,* 50 silkworm larvae were counted out for each rearing tray, with three replications of each treatment. After feeding the fluoride-treated leaves for 48 hr from the first feeding of the fourth instar, the larvae were fed with normal leaves.

Fluoride-tolerance among commercial silkworm hybrids: Seven hybrids that are popular for commercial silk production in China were tested for their cocoon yield when fed with mulberry leaves treated by NaF solutions at concentrations of 0, 40, and 80 mg F/kg from the first feeding of the third instar to the last feeding of the fourth instar. A total of 63 large trays (3 treatments × 7 varieties × 3 replications) were set up in a random arrangement with 400 larvae/tray. The actual F content in the dried leaves as determined by analysis showed the following levels: control (C) – 23 mg/kg; treatment I (TI) – 80 mg/kg; and treatment II (TII) – 128 mg/kg, respectively.

RESULTS

Variation of mulberry leaf fluoride LC_{50} among different varieties of silkworms: Results of mulberry leaf fluoride LC_{50} determinations for 50 silkworm varieties are summarized in the Figure, which shows there are large differences in fluoride tolerance. Most varieties fall into the LC_{50} categories between 50–99 and 100–199 mg F/kg of dried mulberry leaves, with similar numbers of varieties distributed among the other categories. The highest LC_{50} was 674 mg F/kg, and the lowest was 19 mg F/kg, representing a 35-fold difference in tolerance to fluoride among the tested varieties. Interestingly, when the data were compared and analyzed, no distinct relationship could be seen between fluoride tolerance and economic characteristics or adverse climate conditions.

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*Instar refers to successive molting stages of the larvae. Newly hatched larvae are in the first instar. The second instar begins after the first molting, the third instar after the second molting, and the fourth instar after the third molting. After about 7 or 8 days into the fifth instar the larvae are mature and begin to spin cocoons.

Response of silkworms to fluoride pollution: Two silkworm varieties, Hang 7 and Hang 8, obtained from three different locations with different levels of fluoride pollution, were tested for their mulberry leaf fluoride LC_{50}. These locations with their average fluoride content in the dried mulberry leaves are listed in Table 1. From the table it is evident that both varieties had substantial differences in their mulberry leaf fluoride LC_{50} when maintained in locations with different levels of fluoride pollution. Clearly, the LC_{50} of each variety was closely related to the fluoride concentration in the mulberry leaves on which the larvae fed, and this concentration in turn reflected the level of fluoride in the atmosphere in that region.

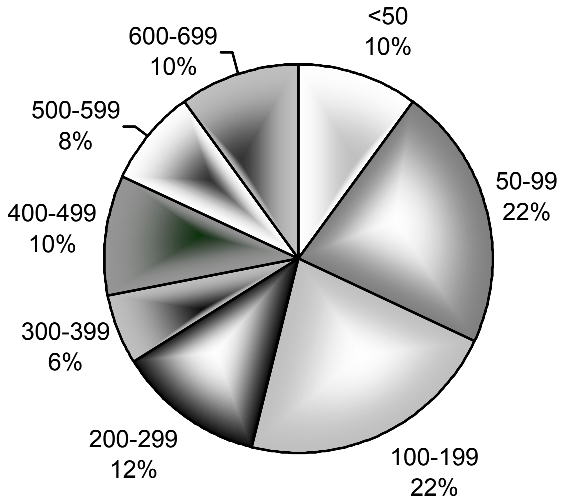

Figure. Mulberry leaf fluoride LC_{50} distribution of 50 different silkworm varieties (mg F/kg dried leaves).

Difference in fluoride tolerance among commercial hybrids: The effects of the three different fluoride test levels in mulberry leaves on larval viability and cocoon yield among seven different silkworm hybrids are presented in Table 2. The results indicated there was no appreciable difference in molting rate among the hybrids under the control conditions with 23 mg F/kg in dried leaves, the least being a molting rate of 99.72%. Large differences were found, however, under the TI conditions with 80 mg F/kg in the dried leaves. The seven hybrids then fell into two categories: the first four hybrids had molting rates higher than 97%, while the last three had rates lower than 60%. Furthermore, under the TII conditions with 128 mg F/kg in the dried leaves, all the hybrids had lower molting rates. Although the larval viability and cocoon yields of the first four hybrids under the TI conditions were all fairly close to those of the C groups, they were significantly lower for the last three hybrids. The first hybrid in Table 2, QiuFeng x BaiYu, proved to be the most fluoride tolerant of all the hybrids for larval viability and cocoon yields, even under TII conditions.

Table 1. Mulberry leaf LC_{50} F value of the same variety of silkworms from different locations

Location	Variety	LC_{50} F value (mg/kg dried mulberry leaf weight)	F content in mulberry leaves (mg/kg dried weight)	95% confidence
Parent Silkworm Egg Station	Hang 8	13.01	15	7.71–21.93
	Hang 7	21.00	15	20.10–31.09
National Sericulture Res. Institute	Hang 8	22.31	30	18.69–26.63
	Hang 7	29.91	30	25.37–35.27
Department of Sericulture & Apiculture	Hang 8	53.03	45–80	42.32–66.51
	Hang 7	73.92	45–80	64.94–84.15

Table 2. Effect of fluoride on molting, viability, and cocoon yield of seven silkworm hybrids

Variety name	Treatment	Molting rate (%)	Larval viability (%)	Cocoon Yield (g)
QiuFeng × BaiYu	C	100	100 ± 0.00	772 ± 3
	TI	99.92	99.84 ± 0.14	765 ± 2
	TII	97.24	97.07 ± 1.22*	727 ± 8*
Feng 1 × 54A	C	99.83	99.66 ± 0.16	749 ± 2
	TI	97.62	98.19 ± 0.76	741 ± 9
	TII	81.65	69.51 ± 7.33†	504 ± 35†
XinHang ×KeMing	C	99.92	99.74 ± 0.26	694 ± 5
	TI	98.44	97.02 ± 1.07	677 ± 7*
	TII	72.88	79.01 ± 4.83†	541 ± 23†
HuaFeng×XueShong	C	100	99.74 ± 0.25	778 ± 4
	TI	97.15	97.15 ± 1.89	759 ± 21
	TII	72.88	64.07 ± 1.44†	549 ± 62†
Chun.Lei× ZhengZhu	C	100	93.64 ± 2.35	728 ± 24
	TI	58.93	56.11 ± 1.24†	401 ± 4†
	TII	12.98	11.41 ± 2.86†	77 ± 4†
JingShong × HaoYue	C	99.92	93.32 ± 2.76	708 ± 35
	TI	35.62	33.61 ± 5.65†	211 ± 10†
	TII	1.33	1.33 ± 0.63†	17 ± 2†
ZheLei × ChuanXiao.	C	99.72	81.99 ± 2.32	603 ± 22
	TI	30.71	26.67 ± 6.51†	211 ± 15†
	TII	2.33	2.25 ± 0.43†	17 ± 1†

Data in the table are Mean ± standard deviation. *$P < 0.05$. †$P < 0.01$.

DISCUSSION

Earlier studies have shown that cocoon production or yield can be seriously reduced if the fluoride content of dried mulberry leaves exceeds 40 mg/kg.[1] One of the most direct negative effects of fluoride on silkworm viability is to decrease the molting rate. Other important indexes of silkworm sensitivity to fluoride are the viability of larval stages and cocoon mass or yield. The silkworm varieties chosen for the present study were geographically representative with different economic characteristics and fluoride sensitivity. Tolerance to fluoride was significantly different even among different varieties of the same species, possibly owing to differences in genetic constitution.

The significant difference in mulberry leaf fluoride LC_{50} of Hang 7 and Hang 8 maintained in different places with varied fluoride pollution could be caused mainly by a natural selection response to fluoride tolerance, *i.e.*, a biological adaptability to variations in environmental conditions.

Seven of the most popular silkworm hybrids tested for cocoon productivity under oral administration of low fluoride concentration showed that the four hybrids with higher tolerance to fluoride are all newly released to the field, whereas the three sensitive hybrids were all bred over 15 years ago. This result again suggests that increases in environmental pollution by fluoride can cause a natural selection to improve the fluoride tolerance of certain varieties of silkworms. Among the hybrids tested, QiuFeng × BaiYu proved to be the most fluoride tolerant and had the best performance in cocoon yield.

REFERENCES

1 Chen YY. Damage of fluoride to sericulture and its control. A review. Agricultural Information Research 1993;2:36-9.
2 Chen YY, Wu YC. Study on the effect of fluoride on the growth, development and economic parameters of silkworm *Bombyx mori* L. Sericologia 1994;34:627-35.
3 Tong ZL. An Approach to several aspects of silkworm toxicosis caused by fluoride pollution in the air in Hangzhou. Agro-Environment Protection 1988;7(5):46-8.
4 Chen YY, Lu SG. Fluoride accumulation and distribution in mulberry insects near fluoride pollution sources. Chin J Appl Ecol 2002;13:106-8.
5 Chen YY, Wu YC. Fluoride loading and kinetics in different tissues of larvae of fluorosis silkworm (*Bombyx mori* L.). Sericologia 1995;35:1-10.
6 Chen YY, Li JY. The formation and function of the specific spherocrystals of calcium salts in the midgut tissues of fluorosis silkworm larvae *Bombyx mori*. J Chin Electr Microsc Soc 1996;15:17-20.
7 Chen YY, Shao SH, Wu TJ, Wu YC. Effect of fluoride pollution on fecundity of *Bombyx mori*. Chin J Appl Ecol 1996;7:424-8.
8 Chen YY, Wu YC. Effects of Fluoride on Calcium, Copper and Iron Levels in *B. mori*. Sericologia 1998;38:69-77.

9 Buse A. Fluoride accumulation in invertebrates near an aluminum reduction plant in Wales. Environ Pollut 1986;41:199-217.
10 Davies FM. Accumulation of fluoride by *Xanthoria parietina* growing in the vicinity of the Bedfordshire bricks field. Environ Pollut A 1982;29:189-96.
11 Davies MT, Davison AW, Port GR. Fluoride loading of larvae of pine sawfly from a polluted site. J Appl Ecol 1992;29:63-9.
12 Czarnowski W, Wrzesniowsla K, Krechniak J. Fluoride in water, urine and egg shells as an indicator of environmental contamination. Fluoride 1994;17:141-4.
13 Walton KC. Fluoride in moles, shrews and earthworms near an aluminum reduction plant. Environ Pollut A. 1986;42:361-71.
14 Wu FZ. A rapid method to determine trace fluoride in mulberry leaves and silkworm. Bull of Sericulture 1982;4:30-2.
15 Tang ZH, Wang G. Pesticides Resistance of Agricultural Pests. Beijing: Agricultural publishers; 1982.

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