INFLUENCE OF SIMULATED ACID RAIN ON POPULATION DYNAMICS OF CARMINE SPIDER MITE, *TETRANYCHUS CINNABARINUS* (BOISDUVAL) (ACARI: TETRANYCHIDAE) AND ITS HOST PLANT

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ABSTRACT -The effect of simulated acid rain on the population dynamics of *Tetranychus cinnabarinus* (Boisduval) and the physiology of its host (eggplant) was measured in a series of laboratory experiments. The number of *T. cinnabarinu*s on plants treated with pH 4.0 and 5.6 acid rain was higher than and the population declined later than the control. The number of mites on plants treated with pH 3.0 acid rain was less than the control, but the population declined later. However, the number of mites on plants treated with pH 2.5 acid rain was less than and the population declined earlier than the control. The pH value, water content, soluble sugar, reduced sugar, phosphorus (P) and soluble protein content of the leaves of host plants varied according to treatment with acid rain of different pH values and different treatment times. The pH values of eggplant leaves decreased with decreasing pH of acid rain. As the acidity increased, the P content and soluble protein increased significantly at the beginning of treatment with acid rain (15 days after first treatment) and reached the highest level in pH 4.0 or 3.0, respectively, then declined with lower pH of acid rain. Soluble sugars followed an opposite trend to P and soluble protein. Reduced sugar content increased linearly with the decline in pH of acid rain. Water content of eggplant leaves did not show any significant changes among different treatments. With the increase of treatment time, the water content, soluble sugar, reduced sugar, and P content also varied significantly. These results suggest that treatment of plants with acid rain (pH > 4.0) increased the levels of nutritious substances (reduced sugar, P, and soluble protein). These changes may favor growth and development of eggplant and *T. cinnabarinu*s. However, strong acid rain (pH < 3.0) inhibited both plant and mite growth.
Key words - Acidic rain, *Tetranychus cinnabarinus*, nutriment, population dynamics, China.

INTRODUCTION

The threat of acid rain to the global biosphere has become an environmental problem of worldwide importance. Increasing acidity of many lakes and soils, alteration of the bio-communities equilibrium, and forest decline have been shown to be related to acid rain (Shaefer, 1992). Acid deposition is also capable of influencing host-plant susceptibility and suitability to insect herbivores (Bedford, 1987; Riemer and Whittaker, 1989; Heliövaara and Väisänen, 1993). Plant tissue concentrations of water, nitrogen, soluble proteins, amino acids, and defensive compounds have been shown to be affected by acid deposition (Trumble and Hare, 1989; Dercks *et al*., 1990; Paine *et al*., 1993). Stinner *et al*. (1988) reported that after corn plants were treated with a simulated acid rain (pH 2.8 and 4.2), food utilization by black cutworm larva [*Agrotis ipsilon* (Hufnagel)] was increased and the developmental time was shortened.

Chongqing Municipality, China is seriously polluted by acid rain. Acid rain in Chongqing has caused soil acidification, forest decline, and crop yield reduction and intensified insect pest infestation (Zhao *et al*., 1999). Based on our investigations, the infestation of the carmine spider mite [*Tetranychus cinnabarinus* (Boisduval)], a serious mite pest of vegetable and fiber crops, is positively correlated with the frequency of acid precipitation (Zhang *et al*., 2004; Wang *et al*., 2004, 2006). These studies showed that population development and reproduction of the mite were restrained by direct application of acid rain (pH 4.0-3.0) on mites. However, population development and reproduction of the mite was enhanced by the host plant-mediated impact of simulated acid rain (pH 5.6-3.0). Wang and Zhao (2002) found that the number of mites and the degree of injuries on host plants were significantly increased compared to the control after exposure of both mites and host plants to acid rain (pH 4.0). This study is a continuation of our previous work and seeks to determine the effects of acid rain on the quality of eggplant foliage and population dynamics of *T. cinnabarinus*.

MATERIALS AND METHODS

Mites - The stock culture of carmine spider mite [*Tetranychus cinnabarinus* (Boisduval)] originated from cowpeas (*Vigna sinensis* Endlicher) in Chongqing, China. The culture was maintained on potted kidney bean (*Phaseolus vulgaris* L.) (30-40 cm tall) in a walk-in insect rearing room at 28 ^{o}C, 75-80 % RH, and a photoperiod of 14:10 (L:D) h. This colony was maintained for more than two years before use. Voucher specimens (Lot No. 02056) were deposited at the insect collection of the Southwest University, Chongqing, P. R. China.

Simulated acid rain treatment - An initial solution was created by adding mean ambient concentration of 5.35mg/l $(NH_4)_2SO_4$, 0.36mg/l Na_2SO_4, 0.97mg/l $MgSO_4$, 6.80mg/l $CaSO_4$, 1.52mg/l KNO_3, 0.51mg/l $NaNO_3$, 0.77mg/l $MgCl_2$, and 0.29mg/l NaF as determined from data of Tongyuan Monitoring Station, Chongqing, to deionized water (Wang and Qin, 1989). Specified amount of H_2SO_4 was added to bring the initial solution to pH 5.6, 4.0, 3.0, and 2.5. Deionized water of pH 6.8 was used as a control. The pH values of all solutions were checked with a pHS-4C meter (Chengdu instrument Inc., Chengdu, China).

Host plant - Eggplant (*Solanum melongena* L.) seedlings grown to about 10 cm tall in pots (7.5 cm height, 4.5 cm diameter) were used as mite hosts. The experiment was conducted in the insect rearing room at 25 ^{o}C, 80 % RH, and photoperiod of 14:10 (L:D) h. The pots with one seedling each were arranged randomly in the insect rearing room for acid rain treatment. The plants were fertilized with a controlled nutritional solution (Hoagland nutritional solution) and watered with deionized water as required. Before spraying with acid rain, plants with similar growth conditions were selected for the experiment. In all experiments, simulated acid rain of different pH values (6.8, 5.6, 4.0, 3.0, and 2.5) was applied to 20 potted eggplants for each treatment from a portable overhead sprinkler system at a height of 75 cm. The plants were sprayed every two days with 150 ml acid rain per plant each time. The host plant leaf tissues were randomly selected from the eggplant seedlings for each acid rain treatment, and the biochemical assay was conducted at 15, 30, and 45 days after commencement of acid rain applications.

Population dynamics of mite under acid rain impact - Prior to placement on experimental plants, the test mites were treated using 150 ml of acid rain per plant every other day for one year. The reason for this was to investigate the long-term effects of acid rain on population dynamics of the mite. For each treatment, about 150 young adults (females and males) of the same age from colonies treated using acid rain were transferred to each eggplant at 30-40 cm tall for egg production. The mites were held on the plants at 25 ^{o}C for a 24 h oviposition period, and then the adults were removed. Six eggs were left on a leaf of each eggplant, and were confined with the cotton strips at the beginning. Plants with eggs were placed in an insect rearing room randomly at 25 ^{o}C, 70-80 % RH, and a photoperiod of 14:10 (L:D) h. Eggs and eggplant were treated using acid rain the same way as eggplants that were used for biochemical assay. The number of mobile mites was counted using a stereomicroscope. Observations were made every three days until the leaves fell. The number of mites on ten eggplants was counted for each treatment.

Biochemical assay of host plant - On 15, 30, and 45 days after first application of acid rain, the third leaf (from the top of stem) of each eggplant was collected for biochemical assay. The number of mites on the leaf samples was counted. The fresh weight of each foliage sample was determined immediately then the leaf was stored at -80 ^{o}C until assayed. The pH values of leaves were determined based on the method described by Zhang (1992). The contents of water, soluble sugar, and reduced sugar of leaves were measured using methods of Li (2000). Bradford reagent (Bradford, 1976) was used to assay the soluble protein content with bovine serum albumin as the standard. Triplicate readings were taken on each extraction using UV-VIS8500 spectrophotometer (Shanghai, China). Each treatment had three temporal replications.

Data analysis - The contents of nutritious substances of eggplant leaves were subjected to a two-way ANOVA (pH value and treatment period, GLM procedures, SPSS), and regression analysis was also used to investigate the relationship between nutrients and pH values of acid rain.

RESULTS

Effects of simulated acid rain on population dynamics of mites - On eggplant, the numbers of *T. cinnabarinus* increased slowly with the same trend under the acid rain treatment during first 20 days after eggs were laid on eggplant leaves. After 20 days, the numbers of mites increased rapidly and numbers of mites on leaves treated with pH 4.0 and pH 5.6 acid rain were greater than those on other acid rain treated plants. Growth velocities of mite populations among all treatments were the fastest

Table 1. Effects of simulated acid rain acidity and treatment period on pH value of eggplant leaves (M ± SE) [Means within the same column followed by same letters are not significantly different ($P > 0.05$)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	6.00 ± 0.05a	5.84 ± 0.02c	5.67 ± 0.01c
pH 5.6	5.99 ± 0.01a	5.68 ± 0.01b	5.61 ± 0.00b
pH 4.0	5.94 ± 0.03a	5.57 ± 0.01a	5.62 ± 0.01b
pH 3.0	5.94 ± 0.03a	5.55 ± 0.01a	5.60 ± 0.01b
pH 2.5	5.96 ± 0.01a	5.56 ± 0.01a	5.54 ± 0.01a

after 28 days and peaked about 35 days. Generally, the numbers of mites on leaves treated with pH 4.0 and 5.6 acid rain were higher and declined later than control. The numbers of mites on leaves treated with pH 3.0 acid rain was less than control, but declined later than control. However, the numbers of mites on leaves treated with pH 2.5 acid rain was less and declined earlier than that of control (Fig. 1).

Effects of simulated acid rain on foliage quality of eggplant - The effect of different acidities of simulated acid rain and treatment periods on pH value of eggplant leaves were different (Table 1). After 15 days treatment,

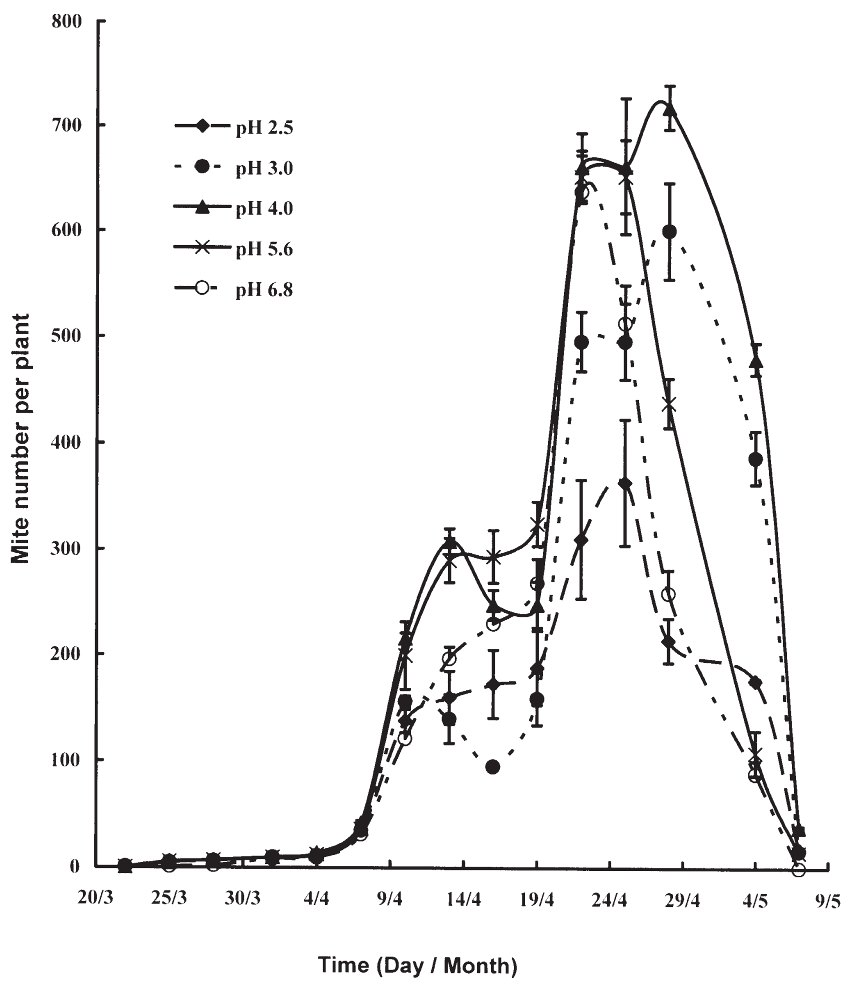

Fig. 1. Effects of acid rain impact on the population dynamics of *Tetranychus cinnabarinus*.

Table 2. Effects of simulated acid rain acidity and treatment period on H_2O content of eggplant leaves (M ± SE, %) [Means within the same column followed by same letters are not significantly different ($P > 0.05$)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	0.94 ± 0.01a	0.87 ± 0.02a	0.84 ± 0.03a
pH 5.6	0.94 ± 0.03a	0.87 ± 0.01a	0.83 ± 0.01a
pH 4.0	0.94 ± 0.02a	0.88 ± 0.01a	0.84 ± 0.01a
pH 3.0	0.91 ± 0.01a	0.87 ± 0.02a	0.84 ± 0.01a
pH 2.5	0.87 ± 0.01a	0.85 ± 0.01a	0.84 ± 0.01a

pH values of eggplant leaves among different treatments were similar ($P > 0.05$), but after 30 days and 45 days , pH values of eggplant leaves among different treatments were significantly lower than the control ($P < 0.05$). The pH values of eggplant leaves declined significantly with increasing treatment periods. The interaction between acidities and treatment period was also significant ($F = 4.203$; $df = 8, 30$; $P = 0.002$) (Table 1). Regression analysis showed that the pH value of the leaves was linearly decreased with the increased acidity of acid rain ($Y = 5.59 + 0.03X$, $r = 0.965$, $P < 0.01$).

The water content of eggplant leaves declined with increased treatment period ($F = 33.571$; $df = 2, 30$; $P < 0.001$), but the water content did not vary between different acid rain treatments ($F = 1.928$; $df = 4, 30$; $P = 0.133$), and the interaction between acidities and treatment period was also not significant ($F = 0.971$; $df = 8, 30$; $P = 0.478$) (Table 2). Regression analysis also did not show a significant relationship between water content of eggplant leaves and pH value of acid rains ($r = 0.681$, ns).

Acidities and treatment periods of acid rain also had a significant effect on soluble sugar content of host plant leaves (acidities: $F = 6.231$, $df = 4, 30$, $P < 0.001$; treatment period: $F = 34.794$, $df = 2, 30$, $P < 0.001$). The interaction between acidities and treatment period was also significant ($F = 2.713$; $df = 8, 30$; $P = 0.020$). Among the acid rain treatments, the soluble sugar content of eggplant leaves was the lowest under the pH 4.0 acid rain with 30 days treatment (77.6% of control, Table 3). No linear relationship was found between the soluble sugar content of host plant leaves and pH value of acid rain ($r = 0.528$, ns).

There was no significant difference in reduced sugar content of acid rain treatments and control after 45 days. The interaction between acidities and treatment period was significant ($F = 13.457$; $df = 8, 30$; $P < 0.001$). Among the treatments, the reduced sugar content of eggplant leaves was the highest under the pH 2.5 acid rain with 30 d treatment (Table 4). The reduced sugar content of eggplant leaves was linearly increased with the increasing of the acidity of acid rain ($Y = 1.24 - 0.03X$, $r = 0.896$, $P < 0.05$).

The P content of eggplant leaves was significantly different between acid rain treatments, and between the treatment periods (acidities: $F = 16.949$, $df = 4, 30$, $P < 0.001$; treatment period: $F = 92.147$, $df = 2, 30$, $P < 0.001$). The interaction between acidities and treatment period was also significant ($F = 8.978$, $df = 8, 30$, $P < 0.001$). The highest P content of eggplant leaves was recorded for the leaves treated with pH 4.0 acid rain (Table 5). No linear relationship was found between the phos-

Table 3. Effects of simulated acid rain acidity and treatment period on soluble sugar content of eggplant leaves (M ± SE, g/g FW) [Means within the same column followed by same letters are not significantly different ($P > 0.05$)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	0.07 ± 0.01b	0.06 ± 0.00c	0.08 ± 0.00ab
pH 5.6	0.05 ± 0.00a	0.05 ± 0.00ab	0.08 ± 0.00b
pH 4.0	0.05 ± 0.01a	0.05 ± 0.00a	0.07 ± 0.00a
pH 3.0	0.06 ± 0.00ab	0.05 ± 0.00abc	0.07 ± 0.00a
pH 2.5	0.06 ± 0.00ab	0.06 ± 0.00bc	0.07 ± 0.00a

Table 4. Effects of simulated acid rain acidity and treatment period on reduced sugar content of eggplant leaves (M ± SE, mg/g FW) [Means within the same column followed by same letters are not significantly different (*P* > 0.05)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	0.96 ± 0.00a	1.12 ± 0.02a	1.01 ± 0.05a
H 5.6	1.04 ± 0.01b	1.14 ± 0.01a	1.02 ± 0.01a
pH 4.0	1.05 ± 0.01b	1.13 ± 0.00a	1.09 ± 0.04a
pH 3.0	1.05 ± 0.01b	1.28 ± 0.01b	1.00 ± 0.02a
pH 2.5	1.23 ± 0.02c	1.35 ± 0.00c	1.01 ± 0.00a

phorus content of host plant leaves and pH value of acid rains (r = 0.099, ns).

The soluble protein content of eggplant leaves varied significantly between different acid rain treatments and different treatment periods (acidities: F = 11.720, df = 4, 30, P < 0.001; treatment period: F = 284.092, df = 2, 30, P < 0.001). The interaction between acidities and treatment period was also significant (F = 2.593; df = 8, 30, P = 0.028). Soluble protein content of eggplant leaves treated with pH 4.0 acid rain increased, but that of pH 2.5 decreased compared with the control (Table 6). No linear relationship was found between the soluble protein content of eggplant leaves and pH value of acid rains (r = 0.091, ns).

DISCUSSION

Previous research showed that population development and reproduction of *T. cinnabarinus* was restrained by direct application of acid rain of pH 5.6~4.0 (Zhao *et al*., 1999). Mite behavior, development and reproduction were affected by the exposure of host plants to simulated acid rain of pH 5.6~3.0 (Wang *et al*., 2004). After acid rain (pH 4.0) acting both on mite and host-plant, the number of mites on the host-plant and the degree of injury of plants significantly increased compared with the control. The present study showed that acid rain of pH 5.6~4.0 promoted population of *T. cinnabarinus* while pH 2.5 inhibited development. The difference might be in the present study the mites before transferred to host plant were treated for almost 30 generations by acid rain and the mites studied in our earlier study (Wang and Zhao, 2002) were sensitive to acid rain. Exposure of plants to acid deposition caused increase in the foliar concentration of soluble protein and nitrogen (Paine et al., 1995). Nitrogen is a particularly key nutritious component of food quality for phytophagous insects (Mattson, 1980). Further study revealed that the effects of acid deposition on plant insect system are plant-mediated (Redak *et al*., 1995). Our previous study found that the adults of carmine spider mite prefer to aggregate on eggplant leaves treated with pH 4.0 acid rain. In addition, mites feeding on kidney bean leaves treated with pH 4.0 acid rain had faster developmental rate, higher survival rate, and greater fecundity, which in turn led to a higher intrinsic rate of population increase compared with other treatments (Wang *et al*., 2004). All of these results suggest that population increases of mite under acid rain treatment was mainly due to changing host plant quality. We also found that the activities of peroxidase, superoxide dismutase, catalase, and acid phosphatase of the mite increased significantly with the increase in the acidity of the acid rain, reaching the high-

Table 5. Effects of simulated acid rain acidity and treatment period on phosphorus content of eggplant leaves (M ± SE, µg/g FW) [Means within the same column followed by same letters are not significantly different (*P* > 0.05)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	441.87 ± 4.37b	475.86 ± 3.32a	535.43 ± 24.02a
pH 5.6	554.89 ± 3.83d	647.68 ± 22.49c	683.91 ± 7.82b
pH 4.0	462.98 ± 7.09c	655.41 ± 26.80c	705.75 ± 63.78b
pH 3.0	464.39 ± 6.98c	534.17 ± 11.05b	634.70 ± 47.35ab
pH 2.5	278.60 ± 2.17a	650.08 ± 2.30c	664.57 ± 26.85b

Table 6. Effects of simulated acid rain acidity and treatment period on soluble protein content of eggplant leaves (M ± SE, mg/g FW) [Means within the same column followed by same letters are not significantly different ($P > 0.05$)].

Treatment	Treatment period		
	15 d	30 d	45 d
pH 6.8	42.87 ± 1.35ab	64.78 ± 3.89a	46.02 ± 1.99b
pH 5.6	45.53 ± 2.78ab	68.68 ± 1.36a	47.80 ± 0.67b
pH 4.0	48.52 ± 2.77b	77.10 ± 0.40b	47.65 ± 1.00b
pH 3.0	47.19 ± 1.09ab	79.48 ± 2.21b	45.44 ± 1.64b
pH 2.5	40.74 ± 1.66a	65.90 ± 2.14a	39.05 ± 2.02a

est levels at pH 4.0 or 3.0, and then declined (Zhang *et al.*, 2004). The changes in activity of these enzymes might be relevant to population changes of the mite. We noticed that leaves treated with low pH acid rain were smaller and had some damage symptoms. Furthermore, the changes of the secondary plant defenses and leaf surfaces, such as hardness or toughness, in response to induction by acid rain, might be also related to population dynamics of the mite (Zhao *et al*., 1999).

Several other studies have also demonstrated a plant-mediated effect of air pollution upon insect herbivores (Kainulainer *et al*., 1993; Hughes, 1988; Heliövaara and Väisänen, 1993). Number of *Cinara* spp (Homoptera: aphididae) had a positive response to air pollution, especially SO_2, but high levels of air pollution may cause rapid collapse of *Schizolachnus pineti* L. (Homoptera: Lachnidae) populations due to a nutritious value of the host plant (Holopainen *et al*., 1993). *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) fed on different host plant leaves disks treated with acid rain and ozone had different food assimilation, weight gain and different relative growth rate due to changes in leaves quality in response to simulated acid rain or ozone (William and Cannon, 1993). Paine *et al*. (1993) found that application of acid fog altered the California coastal sage scrub, *Encelia farinose* (Compositae: Asteraceae), foliage quality (increased concentrations of nitrogen and soluble protein) so that the food consumption of *Trirhabda geminata* Horn (Coleoptera: Chrysomelidae) larvae and adults increased. The evidence from these studies on the effects of air pollutants on insect-plant relationship suggest that the effects of pollutants are mostly mediated via the plant. It is well known that many biochemical and physiological changes are induced in plants under acid rain (Paoletti *et al*., 1989; Valentini *et al*., 1989; Koricheva *et al*., 1997; Shumejko *et al*., 1996; Norby *et al*., 1986; Mersie and Foy, 1986; Guttenberger *et al*., 1998; Scalet *et al*., 1995). Our study clearly demonstrated that pH value, water content, soluble sugar, reduced sugar, and P varied according to the treatment of acid rain of different pH values and different treatment periods.

Intracellular pH changes as a consequence of acidic precipitation were shown by Wellburn and Wolfenden (1986) who found barely significant decreases in vacuolar pH by 0.3 units after a spray of pH 3.0. Meanwhile, the pH of a cell homogenate and the buffer capacity of beech seeding were significantly lowered after a treatment of acid mist. The pH changes of plant can alter solubility and ingredient of nutrition substance that insects and mites need. Maybe this is an important factor that acid rain affect feeding and development of insect via plant. Our studies showed that acid rain cannot alter pH value in a short time (15 days), but can reduce leaf pH value if the leaf is treated over a longer period (more than 30 days). Soluble sugar content of eggplant decreased under low acidic rain (pH > 4.0) and increased under higher acidic rain (pH < 3.0). Zhang (1992) reported that injury degree of plant by spider mites was negatively related to soluble sugar, so lower soluble sugar of eggplant may provoke mite development under low acidic rain (pH 4.0). Suski *et al*. (1975) found that P content of bean was significant relative to survivorship, sex ratio and r_m of spider mite. Higher P content of eggplant with acid rain of pH 4.0 and 5.6 treatment promotes the development of mite. Low acidity could promote synthesis of soluble protein and higher acidity inhibits synthesis, which is consistent with an earlier study (Heliövaara and Väisänen, 1993). Dohmen (1988) stated that higher acidity would produce larger effects. However, if acidity becomes high enough to damage severely or even kill the host plant, it will be unfavorable to the herbivore.

In general, the present study has provided some basic information on the effects of acid rain on mite growth and host plant quality. This will contribute to a more complete understanding of the interaction between mites and host plants. As acid rain affect significant areas of China, including areas of agricultural and natural biotic importance, further studies investigating both the direct and plant-mediated impact of acid rain upon plant-mite interactions are warranted.

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