Journal of Environmental Sciences Vol.17, No.6, pp.1034—1037, 2005

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2005)06-1034-04 **CLC number**: X517 **Document code**: A

Effects of simulated acid rain on fertility of litchi

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Abstract: The regulatory role of calcium in fertility of pollen and pistil under simulated acid rain was investigated. The germination percentage of pollen treated with acid rain of pH 4.5 was 9.42% lower than that of control, and that of pH 3.5, pH 2.5 and pH 1.5 were 22.47%, 45.49% and 71.62%, respectively. Simultaneously, the injury character of pollen was obviously observed when flowers were treated with acid rain of pH 3.5. The difference in fruit setting rate between the female flower treated with acid rain of pH 4.0 and the control was significant at $p < 0.05$. $Ca(NO_3)_2$ of 0.2—0.4 mmol/L could promote pollen germination under the stress of acid rain. The beneficial function was reduced when calcium concentration surpassed 0.8 mmol/L. Spraying 2 mmol/L $Ca(NO_3)_2$ reduced the injury of acid rain to pistil and increased fruit-setting rate significantly. The physiological importance of calcium during pollen germination and pistil development was also discussed.

Keywords: simulated acid rain; litchi(*Litchi chinensis* Sonn.); pollen; pistil; fruit-setting; ultrastructure

Introduction

In recent years, acid rain has become a serious problem in agriculture of China. The frequency of acid rain has kept increasing. The pH value of rain in some areas has been lower than 4.0(Chen, 1997). For example, acid rain with pH 3.55 was detected in Zhangzhou City, Fujian Province, China(Xiao, 1991). The average pH of rains in Guangdong Province in 1997 was 3.81 (Qi, 1995). The crop areas influenced by acid precipitation in south-east of China have reached 9.91 million hm^2(Zhang, 1997).

Acid rain induces changes in the cellular biochemistry and physiology of the whole plant. Biological effects of acid precipitation on plants are numerous and complex, and include visible symptoms of injury (chlorosis and/or necrosis), and invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance, variation of several enzyme activities, changes of pollen physiology and ultrastructure (Qiu, 2001a; 2002a; 2002b; Velikova, 2000; Bellani, 1997). Fruit trees have received little attention despite their economic importance, and such a findings as there are lack consistency: injury to the trees without reduction in production was reported by Practor (Practor, 1983) and Forsline *et al*. (Forsline, 1983b); reduction in production and impaired fruit marketability due to alternations of the peel(Rinallo, 1996; 1992; Forsline, 1983a). Litchi, mainly grown in south China, is a famous and delicious fruit in subtropical areas. It is of a remarkable economic significance in the areas of south China. However, few studies have been done on the harmful effects of acid rain on litchi production(Qiu, 2001b). Due to the need for further evidence regarding atmospheric pollution in the form of acid rain and its interaction with and influence upon the reproductive processes of litchi, the study was undertook concentrating on one stage of reproduction. It is known that pollen germination is significantly regulated by the transport Ca^{2+}, K^+ and other inorganic ions across the plasma membranes of pollen. Moreover, it is known that pollen germination is significantly promoted by calcium (Feijó, 1995; Taylor, 1997; Fan, 2001). Thus, the aim of this study was to determine what extent pollen germination and pistil may be affected by exposure to simulated acid rain and by calcium regulation. Such data will be useful in assessing the response of acid rain to litchi production.

1 Materials and methods

1.1 Material

Six mature bearing litchi trees(*Litchi chinensis* Sonn.) of uniform height, vigor and cluster amount were used in this study. They are the main cultivar in Fujian Province. The trees had been grafted and cultivated for 8 years in the campus of Fujian Agricultural University.

1.2 Preparation of simulated acid rain

In the southern part of Fujian, sulfuric acid and nitric acid contribute to the acidity of precipitation. In general, the mole ratio of sulfuric acid to nitric acid is about 5 to 1(Tang, 1996). Based on this ratio, sulfuric acid and nitric acid were selected for the preparation of artificial acid rain. Taken into account the dissolution of carbon dioxide in water to form carbonic acid, the pH value of the solution is 5.6, and it was regarded as one control. A series of solutions of simulated rains with pH 4.5, 4.0, 3.5, 3.0, 2.5 and 1.5 were prepared. Dilution of reagent grade sulfuric acid and nitric acid were done with distilled water and determined by a pHSJ-4 acidity analyzer(Shanghai, China). A sprayer was used to apply the acid solution to clusters.

1.3 Experimental design

1.3.1 Pollen

Flower clusters of uniform size were thoroughly wetted by spraying the acid solution. Sprays were repeated 3 times at 10:00 am, 1:00 pm and 4:00 pm. Symptom was observed every day after the sprays. Anthers were collected 1 d after treatments, and split open at 25℃ and were taken as samples for examining pollen germination percentage with scanning electron microscopy.

1.3.2 Pistil

Seven clusters of uniform size and cluster number in each tree which located on middle of out part of crown were selected for seven different pH spraying, About 50—60 female flowers were counted for each treatment. At each

Foundation item: The National Natural Science Foundation of China(No. 30400061) and the Natural Science Foundation of Educational Department of Fujian Province(No. K20029); * Corresponding author application, clusters were thoroughly wetted after the male flower had been excised. Spray of flower-clusters for examination of fruit-setting rate was repeated six times during 3 d and done at 10:00 am and 4:00 pm every day. Each treatment was repeated 6 times. Fruit setting percentage were investigated 4 times at 7 d intervals. Fruit setting rate was defined as the number of young fruits as a percentage of the number of female flowers on each clusters.

1.3.3 Examination of pollen germination percentage

Changes in the concentrations of $Ca(NO_3)_2$ in the medium as well as medium pH are indicated in the text. The medium was prepared with distilled water and heated to 100℃ for 2 min. Following pollen application, the dishes were immediately transferred to a chamber at 25℃. The total and germinated pollen grains were counted under a microscope after incubation for 8 h. All experiments were repeated three times and each treatment in one experiment included three agar plates (replicates). For each agar plate 300 pollen grains were counted for calculation of germination percentage.

1.4 Calcium treatment

The solution of $Ca(NO_3)_2$ with various concentration of 0, 2.0, 4.0, 8.0 mmol/L were prepared. Pistils sprayed with $Ca(NO_3)_2$ after having been treated with the acid rain of pH 2.5 for 30 min, and the solution without $Ca(NO_3)_2$ was taken as control.

1.5 Scanning electron microscopy

Anthers were ground after they were soaked in acetic acid glacial for 24 h. After filtration, sediments was incubated for 1 h in 5 ml acetic acid anhydride, and then centrifuged. Sediment was soaked in absolute ethanol and centrifuged. This process was repeated for three times. The sediment was stored in absolute ethanol. samples were coated in JFC-1200 sputter and examined and photographed with a JSM-5310LV.

1.6 Statistical analysis

The data were analyzed by factor analysis, and means were compared with Duncan's multiple range test (DMRT) calculated with $p = 0.05$.

2 Results

2.1 Injury of acid rain to flower organ

No injury symptom in calyx appeared in flowers treated with the acid rain of pH 4.5, pH 3.5, pH 3.0 while small browning speckles could be observed in flowers with treatment of pH 2.5 and they became more seriously with the increment of dosage. Browning speckles in calyx could be observed at 6 h after the first spray of flowers with acid rain of pH 1.5 while the browning speckles in pH 2.5 was observed at 2 d after sprays. Speckles in sepals became larger with the delay of time and finally flowers dropped.

The structure of pollen in treatment of pH 4.5 observed in low amplification was almost the same as that of pH 5.6 (Fig.1A), while in high amplification the indistinctly black spots could be observed (Fig. 1E). The corrosive-like structure in wall of pollen treated with acid rain of pH 3.5 was ambiguously observed (Fig. 1B) while that of pH 2.5 could be distinctly observed in low amplification. The line decoration of pollen wall was corroded and dissolved in pH 3.5 (Fig.1F).

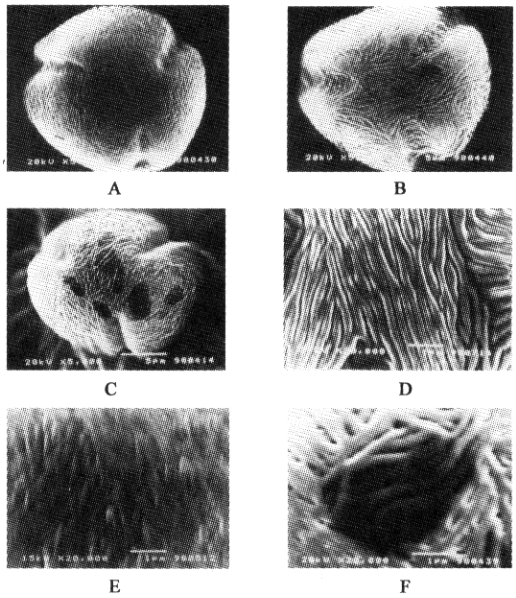

Fig.1 Effects of simulated acid rain on the pollen ultrastructure of litchi
Pollen ultrastructure of litchi treated with solution of A: pH 5.6(×5000); B: pH 3.5(×5000); C: pH 2.5(×5000); D: pH 5.6(×20000); E: pH 4.5 (×20000); F: pH 3.5(×20000)

2.2 Effect of acid rain on pollen germination percentage

The germination percentage of pollen dropped with the decrease of pH value of acid rain. Germination percentage of pollen reduced by 9.42% in the treatment of pH 4.5, 22.77% in pH 3.5 and 45.49% in pH 2.5 as compared with pH 5.6. Pollen treated with pH 1.5 hardly has the ability of germination (Fig. 2). The germination percentage was positively correlated with the pH value of acid rain $Y = 55.774\ln X - 12.173$, $R^2 = 0.9965$.

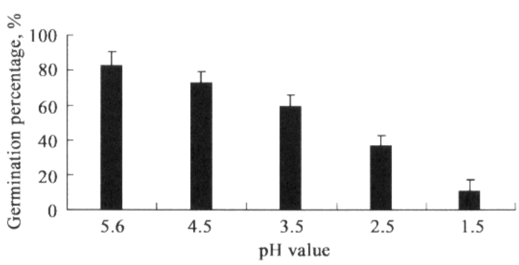

Fig.2 Effects of acid rain stress on pollen germination of litchi

With the aim to verify the effect of acid rain on pollen fertility, pollen were cultured in the acid medium which were regulated by citric acid buffer of pH 5.6, pH 4.5 and pH 3.5. Fig. 3 clearly showed that external pH dramatically influenced pollen germination. The results showed that the medium with pH 5.6 was the most suitable for pollen germination, germination percentage of pollen dropped with the decrease of the pH value of medium. Pollen germination was significantly inhibited at or below 4.5, The germination percentage of pollen in the medium of pH 4.5 was 73.81% of pH 5.6, and that of pH 3.5 was 47.23%.

2.3 Effect of acid rain on rate of fruit-setting

The fruit-setting rate at pH 4.0 was significantly lower than that of control (Fig. 4). Fruit-setting rate (y) was positively correlated with the pH value of acid rain (X). $Y_{13/4}$

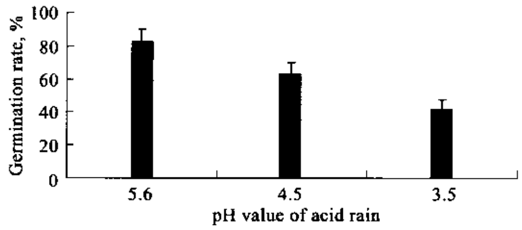

Fig.3 Effects of pH value of medium on pollen germination of litchi

$= 21.491\ln X + 33.846$, $R^2 = 0.9317$; $Y_{20/4} = 17.926\ln X + 1.5588$, $R^2 = 0.9649$; $Y_{27/4} = 13.591\ln X - 2.6689$, $R^2 = 0.9801$; $Y_{3/5} = 10.487\ln X - 3.5285$, $R^2 = 0.9858$.

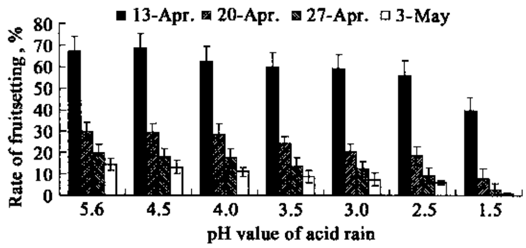

Fig.4 Effects of acid rain on fruit-setting rate of litchi

2.4 Effects of Ca^{2+} on fertility of pollen and ovary of litchi under acid rain stress

Pollen were cultured in the mediums of pH 3.5, pH 4.5 with various concentration of $Ca(NO_3)_2$. The Ca^{2+} concentration suitable for the pollen germination in the medium was determined based on the results of repeated experiments. As shown in Fig.5, 0.4 mmol/L Ca^{2+} resulted in the highest pollen germination percentage after the treatment of pH 3.5, pH 4.5, either higher or lower content of Ca^{2+} reduced the beneficial function.

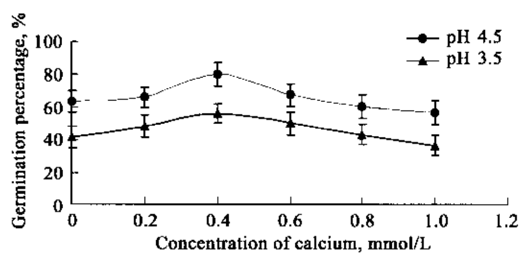

Fig.5 Effects of calcium on pollen germination of litchi

Calcium can also increase the rate of fruit-setting. Calcium with concentration of 2 mmol/L increased significantly the fruit-setting rate (Table 1). The beneficial function reduced when the concentration was 4 mmol/L. The fruitlets dropped dramatically when the concentration was above 8 mmol/L.

Table 1 Effects of calcium on the pistil fertility of litchi under acid rain stress

Treatments	Fruit-setting rate			
pH + $Ca(NO_3)_2$, mmol/L	Apr.13	Apr.20	Apr.27	May 3
2.5 + 0	56.23b	18.54b	9.19 b	6.08c
2.5 + 2.0	62.29a	23.66a	15.86a	11.31a
2.5 + 4.0	54.11b	19.18b	10.07b	8.26b
2.5 + 8.0	50.67c	17.25b	8.26 c	6.21c

3 Discussion

3.1 Injury of acid rain to fertility of litchi

As for the most fruit trees, formal pollination is essential factor to develop fruits. The yield and quality of fruits were influenced by bad pollination which should be avoided in fruits production. Good fertility of pollen is one factor of the formal pollination. Pollen fertility not only depends on its quality, but also is related to temperature, mineral nutrient and different plant growth regulators etc. in germination environment(Lu, 1995). Our results showed that the acidity in germination environment is one of the important factors which is closely related to pollen germination of litchi. Germination percentage dropped with the decrease of pH value of acid rain. The germination percentage of pollen in the treatment of pH 4.5 was 9.42% lower than that of control. Injury symptom of pollen could be observed in the treatment of pH 3.5 (Fig. 1 B, F), and the germination percentage of pollen was 22.47% lower than that of control (Fig. 2). These deduced that pollen is injured and its germination is influenced. This result is consistent with the report in apple pollen(Munzuroglu, 2003).

The development of pistil was influenced by acid rain, and physiological fruit-drop was promoted which resulted in the decrease of fruit-setting. The lower pH of the acid rain is, the worse the pistil and fruit-setting rate are influenced. The data showed that there was significant difference in fruit-setting rate between the treatment of pH 4.0 and the control. The fruit-setting rate of pH 2.5 was only 42.43% of the control(Fig.4). These might be the result of acid rain stress which caused the injury of floral organ, especially the stigma, stylus and ovary, and resulted in bad pollination and ovary development.

3.2 Possible physiological roles of calcium in fertility of pollen and pistil under acid rain stress

Proton can contribute to both the loosening and strengthening of the cell wall. It was observed in the our study that pH 5.6 did enhance pollen germination. The reason may be that pH 5.6 spray may create one proton condition which may not only reduce Ca^{2+}-pectase cross-bridges and stimulated pectin gel degradation thus weakening the apical cell wall, but also enhance the activity of acidic isoforms of PME, which together with pectin hydrolases can cause the random degradation of pectin gels and weaken the cell wall. As a result, it promotes pollen germination. Also, the hyperpolarization of the plasma membrane is associated with entracellular acidification that is included by H^+-ATPase activity that pumps H^+ out of the cell. However, low pH such as pH 4.5, pH 3.5 inhibited pollen germination may contribute to that low pH enhances the leaking of calcium which is essential to pollen germination. Our results that $Ca(NO_3)_2$ of 0.2—0.4 mmol/L could promote pollen germination under the stress of acid rain support the opinion (Fig.5). In this point, application of calcium is useful for lessening the injury of acid rain to pollen germination(Qiu, 2002a). But an excessive increase of external Ca^{2+} concentration inhibited the inward K^+ which is required for both pollen growth and tube growth(Feijò, 1995).Therefore, It was found that the beneficial function reduced when concentration of $Ca(NO_3)_2$ surpassed 0.8 mmol/L.

4 Conclusions

The litchi pollen is more sensitive to acid rain than the fruit. The acid rain with pH value below 4.5 might have contributed to the reduce of pollen germination. The development of pistil was influenced by acid rain with pH value below 4.0, which resulted in the significant increase of fruit dropping. $Ca(NO_3)_2$ of 0.2—0.4 mmol/L could promote pollen germination under the stress of acid rain. The beneficial function reduced when calcium concentration surpassed 0.8 mmol/L. Spraying 2 mmol/L $Ca(NO_3)_2$ reduced the injury of acid rain to pistil and increased fruit-setting rate significantly.

Acknowledgment: The author thanks Ms. Huang B H and Mr. Chen L. for technical support.

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(Received for review March 31, 2005. Accepted July 13, 2005)