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# Effect of acid solutions on plants studied by the optical beam deflection method

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**Abstract**

The optical beam deflection method was applied to study the effects of acid solution on both a terrestial and aquatic plants *Egeria* and *Cerastium*, which are common aquatic plant and terrestial weed respectively. A probe beam from a He-Ne laser was passed through a vicinity of a leaf of the plants, which were put in culture dishes filled with acid solutions. Deflection signals of the probe beam were monitored and compared for acid solutions with different pH values. The results of *Egria* showed that the deflection signals changed dramatically when pH values of acid solutions were 2.0 and 3.0, while little at pH of 4.0 and 5.0. For *Cerastium* when pH were below 3.0, deflection signals changed greatly with time at the begining. After a certain period of time, deflection signals changed little with time. When pH value was above 4.0, deflection signals of *Cerastium* were still changing with time even after 20 hours. The results suggested that the damage threshold of pH was between 3.0 and 4.0 for both the land and aquatic plants.

**Key words**: acid rain; plant; optical beam deflection; *Egeria*; *Cerastium*

## Introduction

Acid rain can cause many changes in both biochemistry and physiology on plants including the changes of morphology and anatomy, respiration and photosynthetic rate, and concentrations of sugar and starch (Ferenbaugh, 1976; Evans et al., 1981; Velikov et al., 2000). So far, most studies of acid rain effect on plants are based on visual observations of symptoms of injury (Walker and Mclaughlin, 1993). Many analytical methods have been used for studies of acid rain effect on plants. For example, atomic absorption spectroscopy was used for determination of minerals in the plant treated with acid rain (Hindawi et al., 1980). Fluorescence method was used in acid rain effect on plants by monitoring fluorescence of chlorophyll *a* (Qiu et al., 2002).

Recently we applied the optical beam deflection (OBD) method for detecting materials movements at different locations of a plant (Wu et al., 2009). The OBD method used a He-Ne laser probe beam passing through a vicinity of aplant. If the plant was alive, a change of the probe beam deflection would be induced by changes of refractive index. The changes of refractive index were generated by concentration and/or temperature changes in the materials' movement across the plant surface. If the plant was not alive, no or little change of the probe beam deflection would be induced, because no or little active materials movements across the plant surface occurred. The experimental results showed that the OBD method could be used for noninvasive sensing and monitoring of the mass transfer process occurred on the plant surface. It did not give any stimulation on the plant during the measurement.

As illustrated in **Fig. 1**, when a plant is put in an acid rain or acid solution, $H^+$ ions move into the surface of the plant, which induces movements of other chemical components and chemical/biochemical reactions involved in the plant physiology. These materials movements and reactions generate changes of concentration and temperature gradients in the vicinity of the plant surface, which in turn generate changes of the probe beam deflection (Wu et al., 2009). Therefore, theoretically, effects of acid rains on the plant physiology including materials movements across the plant surface can be explored by monitoring and analyzing the deflection signals. In this article, we apply the OBD method to study effects of acid solutions on plants.

## 1 Experimental

The OBD experimental setup is similar to that reported by Wu et al. (2009). As illustrated in **Fig. 2**, a He-Ne

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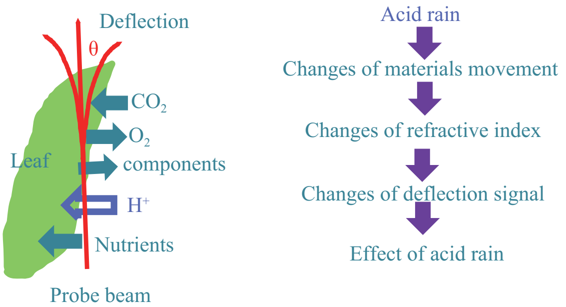


**Fig. 1** Illustration of the principles of the method.

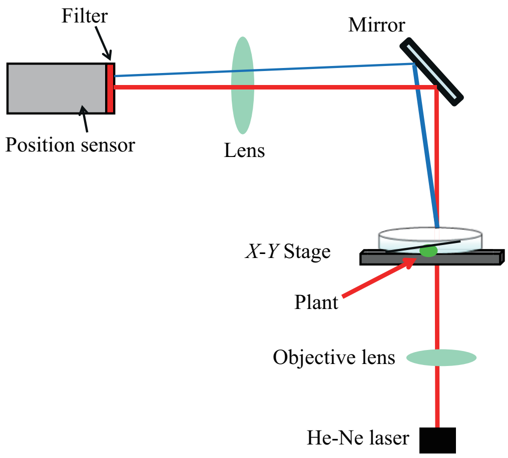


**Fig. 2** Experimental set-up for the OBD method.

laser (output power: 3 mW; wavelength: 633 nm) was used as a light source of the probe beam. The probe beam was focused to a vicinity of a plant in a culture dish by an objective lens. The culture dish was mounted on an *X-Y* microstage, which was used to adjust the distance between the the probe beam and the plant surface or edge. After the deflected probe beam was reflected by a mirror, it was focused to either a position sensor (in the experiments of aquatic plant) or a bi-cell photodiode (in the experiments of land plant) by a 5 cm-focal length lens. A filter, which allowed light longer than 640 nm passed through, was placed infront of the position sensor or the bi-cell photodiode.

*Egeria*, a common aquatic plant, was used as a model aquatic plant. A short piece of the plant was put in a 10 cm-culture dish, which was filled with 30 mL HCl solution with a certain pH. A slide glass was placed on the plant for preventing its possible movements during the experiments. Then, the distance between the probe beam and a leaf edge of the plant was adjusted to about 1 μm by the *X-Y* microstage (**Fig. 3**). Value of pH of the HCl solution in the dish was 2.0, 3.0, 4.0, or 5.0. The blank experiment was also tested under the same conditions without the plant.

*Cerastium*, a common terrestial weed, was used as a model terrestial weed. The plant was put in a 5 cm-culture dish, filling with 4.5 mL distilled water, and covered with a slide glass. After 1 hr, 0.5 mL HCl solution of 1, 0.1, 0.01, or 0.001 mol/L was added in the culture dish. The final

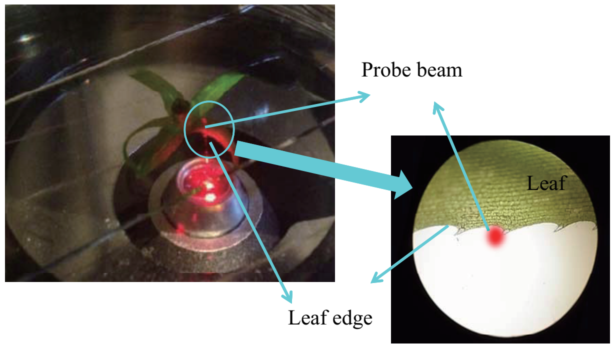


**Fig. 3** Photoes of the leaf/probe beam in the experimental set-up (left) and microscope (right).

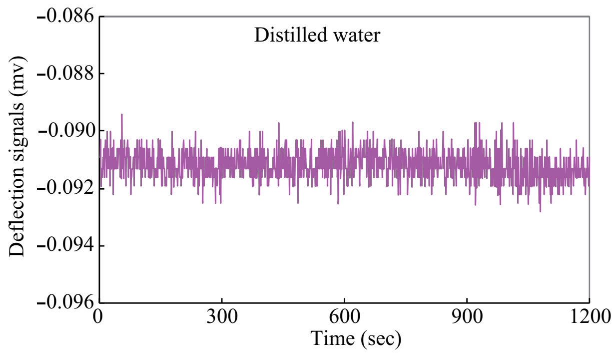


**Fig. 4** Deflection signals obtained in distilled water without the plant.

HCl concentration was 0.1, 0.01, 0.001, or 0.0001 mol/L, corresponding to a pH of 1.0, 2.0, 3.0, or 4.0. Then, the probe beam was adjusted to a vicinity of a leaf edge of the plant, as illustrated in **Fig. 3**, and the deflection signals were monitored.

## 2 Results and discussion

**Figure 4** shows a typical deflection signal for a blank experiment without a plant. The deflection signals changed from –0.092 to –0.090 mV, i.e., the difference between the maximum and minimum the deflection signal was 0.002 mV during a monitoring time of 1200 sec. This change was the noise level of the experimental set-up, mainly caused by the pointing noise of the laser beam, andmechanic and/or electronic instability of the experiment set-up.

**Figure 5** shows the deflection signals of *Egeria* in acid solutions at different pH levels and photos of *Egeria* before and after the experiments. In **Fig. 5a**, deflection signals of pH = 2.0 and 3.0 changed significantly, while those of pH = 4.0 and 5.0 changed little with time. Deflection signals changed sooner after being put in the acid solution of pH 2.0. After about 2500 sec, the deflection signals were out of range, which was because the probe beam had been completely deflected to the plant side. In other words, the probe beam was blocked by the plant and no probe beam was detected by the position sensor. On the other hand, deflection signal of pH = 3.0 changed little until about 1500 sec, then, it began changing significantly and finally

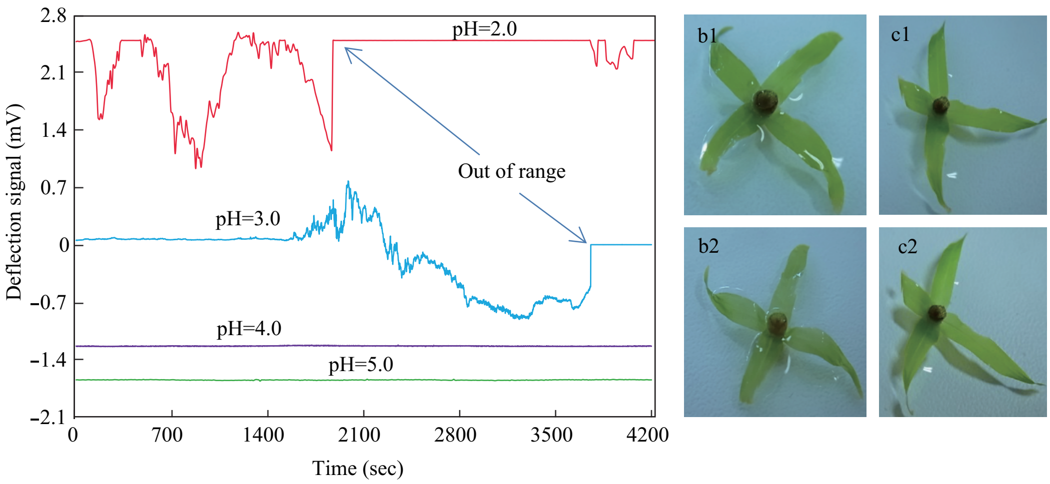


**Fig. 5** Deflection signals of *Egeria* obtained at HCl solutions with different pH (a), and photos of *Egria* taken before and after the experiment at pH 3.0 (b1, b2) and 4.0 (c1, c2).

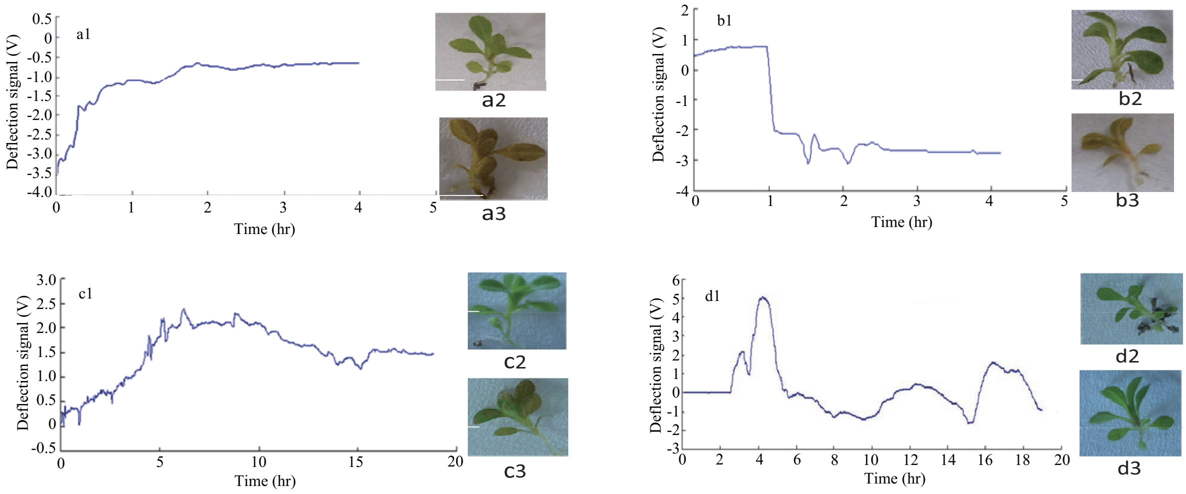


**Fig. 6** Deflection signals of *Cerastium* obtained at pH 1.0 (a1) and 2.0 (b1), 3.0 (c1) and 4.0 (d1), and photos of *Cerastium* taken before and after the experiment at pH 1.0 (a2, a3), 2.0 (b2, b3), 3.0 (c2, c3) and 4.0 (d2. d3).

was out of range after about 4000 sec. Large changes in the deflection signal with time meant large change of the concentration gradients in the vicinity of the plant surface, which was due to either a large amount of $H^+$ ions absorbed by the leaf, or a large amount of components leached from the leaf and dissolved into the acid solution. When pH value of the acid solutions was 4.0 and 5.0, little change in deflection signals was observed, in comparison with those at pH 2.0 and 3.0. This indicated that much fewer of $H^+$ ions were absorbed by the leaf, and also much fewer of other components leached out at pH 4.0 and 5.0.

It is well known that there is a pH threshold for damaging plants by acid rain (Haines et al., 1980). **Figure 5a** suggests that the damage pH threshold of acid solution for *Egeria* was between 3.0 and 4.0. As far as we knew, this was the first report on the damage pH threshold for *Egeria.*

**Figure 5b1** and **b2** was photos before and after the experiments at pH 3.0, **Fig. c1** and **c2** was those at pH 4.0, respectively. At pH 3.0, **Fig. 5b1** and **b2** showed that the green color faded a little after the experiments in comparison with that before the experiment. On the other hand, photos of *Egeria* before and after the experiments seemed to be the same at pH 4.0.

**Figure 6a1** and **b1** is the deflection signals from the *Cerastium* obtained at pH 1.0 and 2.0. They showed that the deflection signals changed greatly at first 2 or 3 hr. After 3 hr, the deflection signals changed little. This meant that at the first 2 or 3 hr, large concentrations changes occured in the vicinity of the leaf edge, i.e., large materials movements across the leaf surface existed. Small or little change in the deflection signals after 3 hr meant that the materials movements had almost stopped. This suggested that the plant was greatly damaged and even led to dead by the acid solutions of pH 1.0 or 2.0. **Figure 6a2–b3** were the photos of the plant before (**Fig. 6a2** and **b2**) and after (**Fig. 6a3** and **6b3**) the experiments. It was also obviously that the plant was geatly damaged or even killed by the acid solutions since the color of leaves had changed greatly.

**Figure 6c1** is deflection signals obtained in the acid solution of pH 3.0. The signals changed greatly until 16 hr, after that the signals had little change. This also suggested that great materials movements occurred at the first 16 hr, and then, little materials movements across the plant surface. **Figure 6c2**, **c3** shows that a few leaves changed their color from green to brown after the experiments, but the color change was not so remarkable as those shown in **Fig. 6a2–b3**. Therefore, damage of the acid solution of pH 3.0 on the plant was lighter than those of pH 1.0 and 2.0.

**Figure 6d1** was deflection signals obtained in the acid solution of pH 4.0. During the monitoring time of 20 hr, the signals kept changing with time. This meant that even after 20 hr, active materials movements still existed across the plant surface. This suggested that the pH 4.0 acid solution had not given the plant serious damage. **Figure 6d2, d3** showed little change in color of the plant. This also suggested that the acid solution of pH 4.0 had little damage on *Cerastium*.

Above results revealed that the HCl solutions of pH of 1.0, 2.0 and 3.0 had great damage on *Cerastium*, while the HCl solution of pH 4.0 had little. Therefore, the damage pH threshold of acid solution for *Cerastium* was between 3.0 and 4.0. Also, this was the first report on the damage pH threshold for *Cerastium*, as far as we knew.

The deflection signals changed little with time for the *Egeria* while greatly for the *Cerastium* when pH was larger than the damage pH threshold. This might be caused by the following reasons. Firstly, the terrestial plant *Cerastium* did not get used to the aqueous environment, thus there must have some materilas movements at the surface/interface of the plant/aqueous solution until it got used to the aqueous environment. Actually, when the terrestial plant was put into distilled water, deflection signals changed greatly with time even two days later. Secondly, the leaf of *Cerastium* was much thicker than that of *Egeria*. That meant that the probe beam light-path passing through the concentration gradients was longer in the vinicity of the former than the later. This generated the larger deflection signal. Thirdly, the sensitivity of the position sensor was lower than that of bi-cell photodiode.

## 3 Conclusion

Changes of the deflection signals from the plants in the acid solutions revealed the impact of acid solutions on the plants. When pH values of the acid solutions were 2.0 and 3.0, the deflection signals from *Egeria* changed dramatically while little when pH values were 4.0 and 5.0. On the other hand, although the deflection signals from *Cerastium* behaviored differently from that of *Egeria*, they changed greatly with time at the beginning, then became unchange after a certain period of time when pH of the acid solutions was 1.0, 2.0, and 3.0. When pH was above 4.0, the deflection signals from *Cerastium* kept changing even after 20 hr. For both *Cerastium* and *Egeria*, the pH threshold was suggested to be between 3.0 and 4.0. It is concluded that the OBD method could be used for studying effects of acid solutions on both the terrestial and aquatic plants.

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