

THE INFLUENCE OF SIMULATED ACIDIC RAIN ON PLANTS VOLATILE ORGANIC COMPOUNDS EMISSION AND PHOTOSYNTHETIC PARAMETERS

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Abstract

Acidic rain has received increasing interest due to the raising emissions of SO_2 and NO_x which determine a decrease of rain water pH under 5. The influence of such water deposition on plants leaf includes necrosis and nutrient losses. In this study the influence of simulated acidic rain on *Phaseolus vulgaris* L plants has been studied. In order to simulate the acidic rain, we used three different pH values (4, 4.5 and 5) of sulphuric acid solution which could occurred in the nature. The photosynthetic parameters decrease in the first 24 hours after stress application but recovered at the initial values after but only in the case of an acidic rain with pH higher than 5. The green leaf volatiles emission scale with strength stress and terpenes emission has been shown to have some protective effect. The visible symptoms of damage (chlorosis and necrosis) have been highlighted using light microscopy.

Key words: acidic rain, terpenes, photosynthesis, *Phaseolus vulgaris* L.

INTRODUCTION

Acidic rain is one of the severest problems which affect the ecosystems which occur in Europa, North America and China (Rodhe et al., 2002). Burton and Aherne (2012) have been shown a high sulphate deposition in small lakes from Ireland. Furthermore, due to the protocol of Convention on Long-range Transboundary Air Pollution the concentrations of different acidic ions decrease in the US and all European country (see Lajtha and Jones, 2013, for an overview). On the other hand, acidic deposition in East Asia increased in the last years, large regions from South and East China being strong affected since 2010 (Duan et al., 2011). In Taiwan, a survey from 1991 to 2006 indicated that the rain water pH had been around 4.5 (Lu, 2007).

Plants suffer from acidic rain not only visible damage as necrosis or chlorosis but also invisible ones as nutrient loss from leaves epicuticular wax destruction or photosynthesis alteration (Oguntimohin et al., 2010). Low value of the soil pH could decrease the plants grow (Reid and Watmough, 2014) and lowers evapotranspiration of different trees (Green et al., 2013).

Even more it have been demonstrated that two species *Q. alba* and *A. rubrum* react different to acidic pH but acidic deposition could also play an important role in determining future species ranges (Medeiros et al., 2016). In a previous paper, it has been demonstrated that acidic rain induced oxidative stress related to membrane damage but did not cause irreversible changes (Velikova et al., 2000). Oxidative stress could induce emission of volatile organic compounds as green leaf volatiles which are formed in a process where free octadecanoid fatty acids are released from plant membranes by phospholipases (Matsui, 2006) and terpenes formed in 2-C-methyl-D-erythritol 4-phosphate pathway (Baldwin et al., 2006). Such emission has been shown for other oxidative stress as ozone, drought or flooding (Copolovici and Niinemets, 2016; Mochizuki et al., 2017; Yuan et al., 2016).

In the same direction oxidative stress decrease photosynthetic parameters including assimilation rate and stomatal conductance (Copolovici and Niinemets, 2010)

The aim of the present paper was to investigate the effect of simulated acidic rain with different values of pH on volatile organic compounds and photosynthetic parameters.

MATERIALS AND METHODS

For measurements bean plants *Phaseolus vulgaris* L. (Minodor, Agrosel Campia Turzii) has been grown in the laboratory for 3 weeks under artificial light intensity of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Period light / dark was 12-12 hours and the temperature 25°C . To simulate acid rain, three solutions of H_2SO_4 were prepared at pH 4; 4.5 and 5 respectively.

Every plant has been sprayed with corresponded acidic solution one time and the controls plants have been sprayed with distill water.

Photosynthetic characteristics were determined with a portable gas exchange system GFS-3000 (Waltz, Effeltrich, Germany) as described in (Niinemets et al., 2010). Volatile organic compounds (VOC) were sampled via the outlet of the gas-exchange cuvette in a multibead stainless steel cartridge filled with different carbotraps.

The adsorbent cartridges were analyzed for green leaf volatiles, (GLV) and terpenes using a Shimadzu TD20 automated cartridge desorber integrated with a Shimadzu 2010 Plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan) using a method described in (Kannaste et al., 2014).

Fresh leaves from several plants were visualized with methylene blue staining by using an Axio Scope A1 light microscope equipped with Axio Cam MRc 5, ZEN lite 2012 software (Zeiss, Germany). at magnification 5x.

RESULTS AND DISCUSSIONS

Germination tests

The tests were carried out on the seed germination of *Phaseolus vulgaris* L. in both water and solutions with various pH values. It could be observe that the number of seeds that germinate decreases with the increasing of acidity (Tabel 1).

Table 1. The seeds of *Phaseolus vulgaris* L. germinated in environments with various pHs

Treatments	% of seeds germination
Control	100
pH 5	100
pH 4.5	10%
pH 4	0%

Photosynthetic parameters

The photosynthetic parameters decreased drastically after plants have been sprayed with acidic solutions of pH 4 and 4.5. After 2 hours assimilation rates recovered at the initial values (see Figure 1 for plants sprayed with acidic water at pH 4) while stomata conductance increased at higher values.

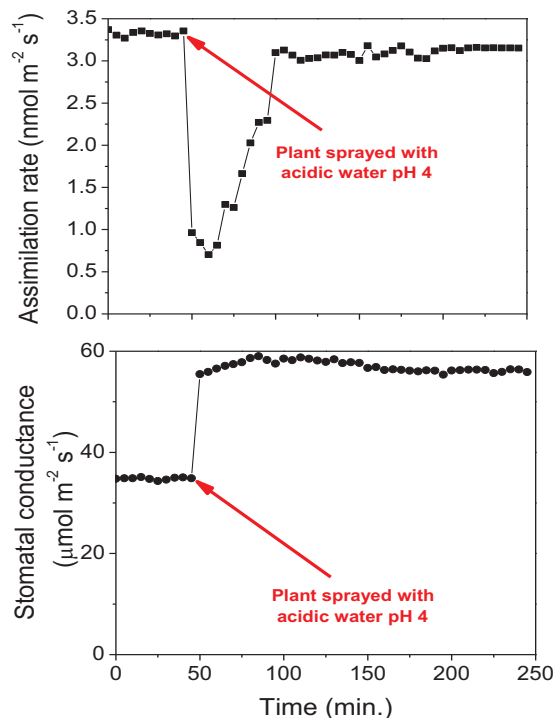
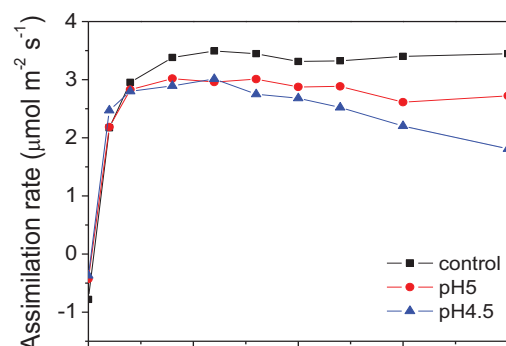


Figure 1. Evolution of photosynthetic parameters of plants sprayed with acidic water at pH 4

Even more, assimilation rate and stomatal conductance at 48 hours and 7 days after treatment with pH 4.5 and 5 recovered at the initial values.

The influence of light on stomatal conductance and assimilation rate have been show that plants which suffer from an episode of acidic rain are more sensitive to light than control (Figure 2).



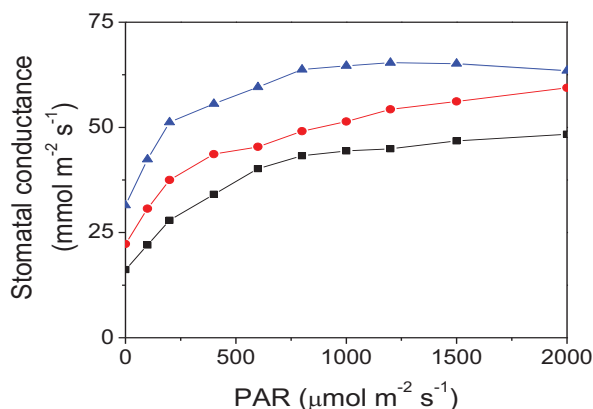


Figure 2. The influence of light on photosynthetic parameters

Volatile organic compounds emission

Sprayed plants with acidic water have been monitored for 168 hours. The emission of volatile organic compounds depends on the time with a maximum at 1 hour (Figure 3). The emission decreased after 2 days those we could presume that the formation of volatile compounds could be associate with a protection response of the plants.

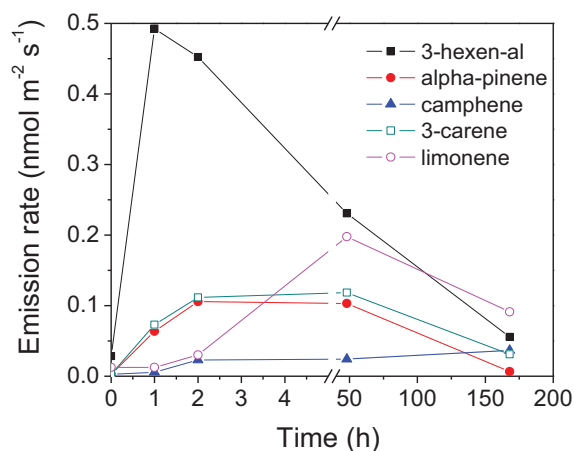


Figure 3. Emission rate of different volatile organic compounds from *Phaseolus vulgaris* L. leaves after spraying with acidic water at pH 4.5

The relatively high concentrations of 3-hexenal appeared at the beginning of the period of stress could be due to the cell membrane destruction associated with activation of lipoxygenase pathway.

Furthermore, the emission of different volatiles could be associated with a stress signal (see Copolovici and Niinemets, 2016).

The emission of green leaf compounds increases with increasing of plant stress strength (pH becomes more acid) while total

emissions of monoterpenes do not depend by pH being significantly increased for all plants under stress (Figure 4).

Plants microscopy

All leaves have been inoculated with methylene blue for a better visualisation of necrosis (Figure 5).

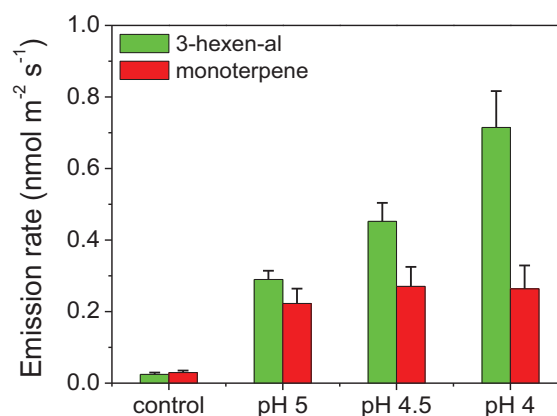


Figure 4. The emission of volatile organic compounds after 2 hours of acidic rain sprayed

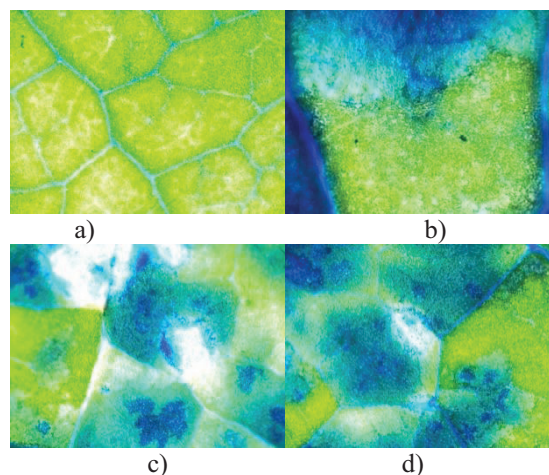


Figure 5. Microscopically samples for *Phaseolus vulgaris* L. after treatment with a) water (control); b) pH = 5; c) pH = 4.5; d) pH = 4

The blue area (which corresponds to dead cells) is bigger for plants which have been treated with solution with pH 4 but even at pH 5 there are spots with necrosis.

CONCLUSIONS

It have been showed that photosynthesis parameter values decreased in the first hours after applying the stress then recovered completely after 24 hours.

The emission of green leaf volatiles increases with the intensity of stress (a lower value of pH) while total monoterpenes are not affected by stress strength.

Microscopic evidence shows that there are a number of cells in areas where acidic rain drops have fallen even with pH 5.

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