Early Detection of Photosynthetic Dysfunction Caused by a Herbicide (Basta) Using Chlorophyll Fluorescence and Thermal Imaging System

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Abstract

The effects of Basta, a commercially available foliar-application type herbicide, on an *in situ Phaseolus vulgaris* L. leaf was analysed with the chlorophyll fluorescence and thermal imaging system. The images of photosynthetic electron transport activity in photosystem II (Φ_{PSII}) and leaf temperature across a leaf surface were obtained before and after the herbicide treatment. At 30 min after the treatment, Φ_{PSII} began to decrease in several sites within the treated region although the leaf temperature did not change. At 60 min after the treatment, the leaf temperature gradually increased at the sites where Φ_{PSII} had markedly decreased. Those results showed that the Φ_{PSII} primarily decreased and the leaf temperature increased after the Basta treatment. This suggests that Basta initially inhibited the photosynthetic electron transport activity and the inhibition caused the stomatal closure. This study demonstrated that the early detection and detailed analysis of the physiological damage caused by herbicide are possible using the chlorophyll fluorescence and thermal imaging system.

Key words: Chlorophyll fluorescence, Herbicide, Image diagnosis, Thermography

1. Introduction

The aerial application of pesticides including herbicides in farmland has become very common in the recent years. Although, aerial application of herbicides improves the efficiency of agricultural works, there is now a growing concern about the effects of the scattered herbicides on non-targeted areas. Hence, early detection of invisible herbicide damage is necessary to prevent the herbicide from effecting non-targeted plants.

Imaging techniques such as chlorophyll fluorescence imaging and thermal imaging are suitable for the early detection of plant damage because both techniques can detect invisible photosynthetic dysfunction across a plant leaf (Omasa and Takayama, 2003). Chlorophyll fluorescence imaging gives the relative maps of the photosynthetic electron transport activity in photosystem II (Φ_{PSII}) (Genty *et al.*, 1989; Genty and Meyer, 1995). On the other hand, thermal imaging provides information of the dynamic and heterogeneous distributions of leaf temperature that is an indicator of the extent of stomatal opening (Jones *et al.*, 2002; Jones and Leinonen, 2003).

In this study, the early detection of the effects of herbicide on attached plant leaf was performed with the chlorophyll fluorescence and thermal imaging system.

2. Materials and Methods

2.1 Chlorophyll fluorescence and thermal imaging system

Figure 1 shows a schematic diagram of the chlorophyll fluorescence and thermal imaging system developed from our previous works (Omasa and Takayama, 2001; 2003). Using the saturation pulse method, the image of Φ_{PSII} showing the distribution of photosynthetic electron transport activity was derived from the following equation: $\Phi_{PSII} = 1 - ({}^iF / {}^iF_m') \times (R_{SL} / R_{AL})$ (Genty *et al.*, 1989; Genty and Meyer, 1995). Where ${}^iF$ and ${}^iF_m'$ are the chlorophyll fluorescence intensity images captured under the continuous actinic light (photosynthetically active photon flux (PPF) of 400 μmol m^{-2} s^{-1}) and the saturation light pulse (PPF of 2500 μmol m^{-2} s^{-1}). The actinic light drives the photosynthetic reactions while the saturation pulse light causes a transient saturation of photochemical reactions. The light was provided by four 180W metal halide lamps (Sumita Optical Glass, Inc., LS-M180) equipped with short-pass filters (Corning, 4-96; $\lambda <$ 620 nm). R_{SL} and R_{AL} are the PPFs of the saturation light pulse and the actinic light, respectively. Images of ${}^iF$ and ${}^iF_m'$ were measured with a CCD camera (Hamamatsu Photonics, C5985-02) equipped with an interference filter (Optical Coatings Japan, IF-W, λ = 683 nm, half-band width = 10 nm). The images were recorded on the hard disk of a PC at 640 x 480 pixels with 8-bit resolution.

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Thermal images for the measurement of leaf temperature were captured with an optical-mechanical scanning thermographic system (JEOL, JTG-5200, thermal camera and controller) having a temperature resolution of 0.05 °C. The long-wavelength emissivity of the plant leaf was 0.98 ± 0.02. The air temperature and relative humidity were kept at 26.5 ± 0.1 °C and 48%. Other thermal environmental conditions were kept constant around the leaf area in the window. The detected signals were converted into exact-temperature images with 16-bit resolution and recorded on the hard disk of a PC at 512 x 480 pixels.

2.2 Plant material and herbicide treatment

The plant of *Phaseolus vulgaris* L. cv. Shin-edogawa was grown in a growth chamber for 4 weeks after sowing in a pot (12 cm diameter x 10 cm deep) that was filled with artificial soil (mixture of vermiculite and perlite, 1:1 v/v). The plant was illuminated for 10 h each day at a PPF of 300 μmol $m^{-2}s^{-1}$. Air temperature was 26.5 °C / 20.0 °C (Day / Night). Relative humidity was 45 % / 60 % (Day/Night). A mature leaf that remained attached to the plant was sandwiched between two opaque pieces of cardboard containing 5 cm x 5 cm windows which were aligned above and below the leaf and mounted horizontally on a fixing apparatus (Fig.1). After the leaf acclimated to the actinic light, the herbicide solution, 1:100 dilution of Basta (AgrEbo) with pure water, was sprayed in a 3 cm x 3 cm square region of the adaxial leaf surface. Basta is composed of 18.5 % Glufosinate-ammonium <Ammonium -DL-homoalanine-4-YL-(methyl)phosphinate> and 81.5 % mixture of water and detergents. The direct effects of the spray solution, such as temporal leaf temperature decline, disappeared within 10 min. The boundary-layer conductance within the measurement window was kept constant and uniform during the experiment according to the method of Omasa and Takayama (2003).

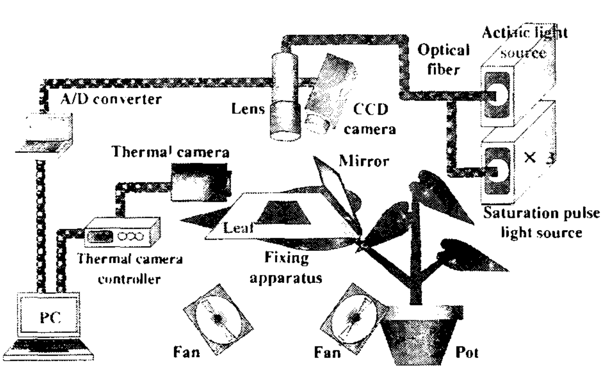

Fig. 1. Schematic diagram of chlorophyll fluorescence and thermal imaging system.

3. Results and Discussion

Figure 2 shows changes in the distribution of Φ_{PSII} and leaf temperature (T_L) on the leaf surface within the measurement window during 130 min after the Basta treatment. Before the treatment, Φ_{PSII} and leaf temperature were uniformly distributed on the leaf surface except for the vein with the mean values of 0.58 and 25.6°C, respectively. During the initial 30 min after the treatment, no change was observed in either image. At 30 min after the treatment, Φ_{PSII} started to decrease in several sites within the treated region, though the leaf temperature in those sites did not change. At 60 min after the treatment, Φ_{PSII} decreased all over the treated region and the low Φ_{PSII} sites expanded and they joined up each other. At that time, an increase at the leaf temperature in those sites was observed. At 130 min after the treatment, almost all areas in the treated region showed low Φ_{PSII} and high leaf temperature. The minimum value of Φ_{PSII} was 0.15 and the maximum value of leaf temperature was 28.3°C.

Glufosinate, the active ingredient of Basta, inhibits the activity of glutamine synthetase that is essential for the removal of toxic ammonia produced in the metabolism of living systems in plants (Lea and Ridley, 1989). Hence, the initial decrease in Φ_{PSII} indicated that the Basta treatment caused the ammonia level in plant tissue to increase, which subsequently inhibited the photosynthetic electron transport activity (Takayama *et al.*, 2003). This decrease in Φ_{PSII} was followed by an increase in the leaf temperature. This suggested that the stomatal closure was induced by the inhibition of photosynthetic electron transport activity

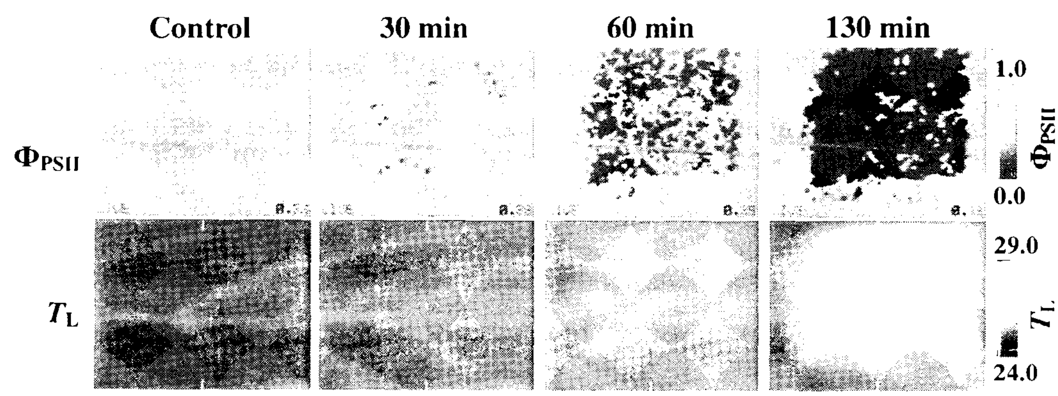

Fig. 2. Changes in the distribution of Φ_{PSII} and leaf temperature (T_L) during 130 min after the Basta treatment.

because stomata closed when the guard cells detected the high CO_2 concentration in the intercellular space. Those results showed that Basta primarily inhibited the photosynthetic electron transport, which induced the stomatal closure.

Consequently, this study concluded that both chlorophyll fluorescence imaging and thermal imaging were useful to detect the photosynthetic dysfunction caused by herbicide before visible symptoms appear. Moreover, this experiment showed that the concurrent use of these two imaging techniques enabled us to notice the subtle changes in plant's physiological status.

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References

Genty, B. and Meyer, S., 1995: Quantitative mapping of leaf photosynthesis using chlorophyll fluorescence imaging. *Aust. J. Plant Physiol.*, **22**, 277-284.

Genty, B., Briantais, J.M. and Baker, N.R., 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta.*, **990**, 87-92.

Jones, H.G. and Leinonen, I., 2003: Thermal imaging for the study of plant water relations. *J. Agric. Meteorol.*, **59**(3), 205-217.

Jones, H.G., Stoll, M., Santos, T., de Sousa, C., Chaves, M.M. and Grant, O.M., 2002: Use of infrared thermography for monitoring stomatal closure in the field: application to srapevine. *J. Exp. Bot.*, **55**, 2249-2260.

Lea, P.J. and Ridley, S.M., 1989: Glutamine synthetase and its inhibition. In *Herbicides and plant metabolism Seminar series / Society for Experimental Biology; 38* (ed. by Dodge, A.D.). Cambridge University Press, Cambridge, pp.137-170.

Omasa, K. and Takayama, K., 2001: Analysis of effects of abscisic acid on *Cucumis sativus* L. leaves using chlorophyll fluorescence imaging and thermal imaging. *PS2001 Proceedings: 12th International Congress on Photosynthesis.*, **S15–010**, 1-4.

Omasa, K. and Takayama, K., 2003: Simultaneous measurement of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. *Plant and Cell Physiol.*, **44**(12), 1290-1300.

Takayama, K., Konishi, A. and Omasa, K., 2003: Diagnosis of invisible photosynthetic injury caused by a herbicide (Basta) with chlorophyll fluorescence imaging system. *Agricultural Engineering International: the CIGR Journal of Scientific Research and Development.* **Manuscript IT 03 001**, 1-12.