The Use of Imaging of the Efficiency of Photosystem II Electron Transport to Visualise the Effect of Dry Storage on the Photosynthesis and Stomatal Closure of Cut Rose Stems

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Abstract

Following their harvest, cut roses are generally stored dry prior to and during transport and only rewetted once they near the end of the chain. This treatment results in overall dehydration of the rose shoots and to the development of emboli within the xylem of the stems. A major consequence of this dehydration event will be stomatal closure as a result of the water stress that develops in the leaves. In addition to reducing water loss from the leaves, stomatal closure will also have major effects on leaf photosynthesis. Quantitative chlorophyll fluorescence imaging of leaves (or any other photosynthetic tissue) permits the visualisation of how efficiently light is being used to drive photosynthetic electron transport. Stomatal closure affects photosynthesis and thus photosynthetic electron transport. So, chlorophyll fluorescence imaging can be used to visualise the responses of leaves to the water stress imposed by cutting and the relief of water stress by rewetting. Results show that the degree of recovery of stomatal opening is generally only partial and that in addition to a persistent limitation of stomatal opening, there is an effect on photosynthetic electron transport due to processes acting at the level of the mesophyll. The results obtained illustrate the usefulness of chlorophyll fluorescence imaging to rapidly and effectively visualise and measure the effect of water stress on cut flowers and to quantify their recovery from this stress.

INTRODUCTION

In common with all other cut flowers, cut rose stems usually develop reduced relative water contents and water potentials, leading to water stress in the post-harvest phase. This is due to the combined effects of being severed from the vascular system of the parent plant on the one hand, and the vapour-pressure deficit between the leaf's internal gaseous phase and the external atmosphere on the other. The consequences of these changes in water status are various, with some, notably gas emboli in the xylem, being strongly associated with serious loss of post-harvest quality. The ability to detect the development and distribution of water stress and its alleviation by post-harvest treatments and procedures is necessary if water-stress induced loss of quality is to be better understood, detected and managed. To date, the techniques available for the detection of water-stress induced post-harvest problems are largely focussed upon the measurement of water uptake and transpiration by the stem as a whole. Though these techniques have proved their usefulness over many years and are simple to apply, they suffer from some drawbacks; as commonly applied they offer no means to describe spatial variability of the stress or its alleviation, they are relatively time-consuming in application, and they require climate-controlled rooms for their quantitative application. A significant consequence of water stress, and one that is intimately related to the control of transpiration, is an increase in stomatal resistance, leading ultimately to stomatal closure. This response has consequences for the processes of photosynthesis which offer a means of imaging stomatal closure and thus water stress. Using chlorophyll fluorescence it is possible to measure the quantum efficiency of light-use by photosystem II electron transport (Φ_{PSII}), which is also the quantum efficiency of linear photosynthetic electron transport (Genty

and Harbinson, 1996). It is possible to produce images of $\Phi_{PSII,}$ which should in principle allow images of stomatal resistance to be made (Genty and Meyer, 1994). The application of this technique requires, however, a detailed understanding of the relationship between linear photosynthetic electron transport and photosynthetic metabolism.

MATERIALS AND METHODS

Principles Underlying the Measurement Procedure

As a process, photosynthesis is based upon the coupling of photosynthetic electron transport with numerous metabolic processes. Of these, it is the processing of the products of the carboxylation of RuBP by CO_2 (the first step of CO_2 fixation) or of its oxygenation by O_2 (the first step of photorespiration) that are the most significant metabolic processes for photosynthetic tissues in air (Genty and Harbinson, 1996). The coupling of photosynthetic metabolism with photosynthetic electron transport is both intricate and incompletely understood, but it is clear that it is dependent upon the operating point of several fundamental photosynthetic processes. The hydrogen ion potential difference that develops across the thylakoid membrane, which provides the driving force for ATP synthesis, is comprised of both a transthylakoid voltage and proton concentration difference. The proton concentration difference is largely generated by a decrease in the intrathylakoid pH, and decreases in the intrathylakoid pH will restrict linear electron transport. The magnitude of the intrathylakoid pH is dependent on the ATP/ADP.Pi ratio, which is dependent upon the demand for ATP by photosynthetic metabolism. Additionally, the redox state of the stroma, a reflection of the ratio of reduced to oxidised ferredoxin, or the the NADPH/NADP ratio, also appears to be involved in the regulation of linear transport. The stromal and intrathylakoid pH changes associated with the development of the $\Delta\mu_{H+}$ are also important in the activation of enzymes in the stroma associated with photosynthetic metabolism. The combined roles of carboxylation and photorespiration in regenerating ADP, Pi, and oxidised ferredoxin has major implications for the response of photosynthetic electron transport to partial or complete stomatal closure. Though increases in stomatal resistance will produce a decrease in the intercellular CO_2 concentration and thus a decrease in the rate of carboxylation, this will not produce a proportional response in the rate of electron transport. This is because the normal atmospheric O_2 concentration of 20% results in an intercellular O_2 concentration that is not significantly affected by stomatal closure and the continued activity of photorespiration also supports electron transport. This interference from photorespiration can be almost completely eliminated by placing the photosynthetic tissue (normally a leaf) in an atmosphere with an O_2 concentration of 2% or less (Genty et al., 1990). Thus, a measurement of Φ_{PSII} made in a low CO_2 atmosphere may be used to reveal areas of closed stomata because of the effect that these will have on the movement of CO_2 into the leaf and because under low CO_2 conditions the potentially complicating effects of photorespiration are removed.

One possible problem with the use of Φ_{PSII} measurements used in conjunction with low O_2 atmospheres is the possible down-regulation of photosynthetic activity, and thus electron transport chain activity and Φ_{PSII}, that can occur in leaves subjected to non-photorespiratory conditions. This down-regulation is most likely due to a feedback limitation, or product inhibition of photosynthesis due to the potentially large increase in the rate of CO_2 fixation produced under the nonphotorespiratory conditions (Harbinson, 1994). A reduction of Φ_{PSII} due to this biochemically mediated regulatory process could easily be mistaken for a limitation due to a stomatal limitation. These two conditions may be distinguished by exposing the leaf to a CO_2 concentration sufficiently high to overcome any effect of stomatal limitation or closure, while using to low O_2 concentration to prevent any photorespiration. By this means the effect of stomatal restriction of photosynthesis and thus Φ_{PSII} can be distinguished from biochemical restrictions of photosynthesis.

In this article we shall illustrate the use of images of $\Phi_{PSII,}$ made by means of

chlorophyll fluorescence, in conjunction with changes in the composition of the gaseous phase, to map stomatal closure over the surface of rose leaves. The stems to which these leaves are attached will be subjected to drought treatment and then rewetted.

Treatment of the Rose Stems

Single stems of Rosa hybrida 'First Red' were obtained from a local grower. The stems were harvested at the normal stage for commercial harvesting, and immediately placed in ice-cold water prior to transport to the laboratory. There, the lowest 10cm of the stems were removed under water, and the stems placed in tap-water and stored in a cold-room at 4°C. The terminal leaflet of the youngest mature leaf was used for the measurements of Φ_{PSII}.

For the production of images of Φ_{PSII}, the leaflet was enclosed in a gas-tight cuvette that was placed in the imaging area of a commercial imaging fluorimiter (Fluorocam, Photon Systems International, Brno, Czech Republic). This instrument permitted the exposure of the leaf to a constant irradiance during which images of Φ_{PSII} could be made. During the imaging process the leaflet was exposed to an actinic irradiance of 100 μmol m^{-2} s^{-1} and allowed to reach steady-state efficiency by maintaining the leaf at this irradiance for 20 min in an atmosphere of normal air. That the leaf had in fact reached steady-state was verified by using a measuring protocol for Φ_{PSII} that made two consecutive images separated by 120s. If these two images differed from each other the leaf was considered not to have reached steady-state. Once the image of Φ_{PSII} had been made for the leaf in air, the gaseous phase was changed first to 2% O_2, 350 ppm CO_2, remainder N_2, and then to 2% O_2, 5 % CO_2, remainder N_2. After each change the leaf was allowed to reach steady-state before an image of Φ_{PSII} was made. The 2% O_2, 350 ppm CO_2 treatment was used to eliminate photorespiration and thus reveal areas of the leaf with possibly closed stomata, and the 2% O_2, 5 % CO_2, remainder N_2 treatment was used to overcome any stomatal limitation upon the rate of CO_2 fixation while at the same time preventing any significant photorespiration, thus allowing regions of a leaf with a down-regulation in the rate of CO_2 fixation due to reasons other than stomatal resistance to be identified. An area of about 1 cm diameter on the underside of each leaf was covered with petroleum jelly in order to block the stomata in this area and partly simulate stomatal closure.

RESULTS AND DISCUSSION

The images of the non-droughted leaf (Fig. 1) show little difference between the different gaseous treatments and little variation of Φ_{PSII} within each image. There are some small areas of low efficiency around the margins of the leaf, and these are evident in all three gaseous treatments, suggesting that they are due to a internal, cellular restriction of photosynthesis and not a stomatal effect. The lack of any significant differences between the air and 2% O_2, 5% CO_2 treatments implies that the leaf was not sink-limited - had this been so then the 2% O_2 treatment would have produced a large fall in Φ_{PSII}. In comparison to the image made in air and the image made in 2% O_2, 5% CO_2 treatments, the image made in 2% O_2, 350 ppm CO_2 showed a slightly lower Φ_{PSII} (more yellow and less orange in the image), implying a small non-cellular limitation on photosynthesis that could possibly be due to a stomatal limitation on gaseous diffusion into the leaf.

After 4 hours of dry storage the pattern of Φ_{PSII} distribution was radically altered (Fig. 2). When measured in air Φ_{PSII} was, if anything, slightly higher than in the control image (Fig. 1) implying a continued turnover of the photosynthetic electron transport chain at a rate that was slightly higher than in the control leaf. However, under conditions where photorespiration was prevented using 2% O_2, 350 ppm CO_2 it is evident that photosynthetic efficiency has been reduced, especially in the distal half of the leaf. That this decrease in efficiency is at least partly due to stomatal closure can be deduced by comparing the images made under 2% O_2, 350 ppm CO_2 and 2% O_2, 5 % CO_2. The increased CO_2 concentration largely restores photosynthetic efficiency over the leaf area, though not to the levels observed in the air treatment nor the control treatments. This

implies two things. First, there is a stomatal restriction acting upon photosynthetic electron transport, this accounts for the difference between the 2% O_2, 350 ppm CO_2 treatment and the 2% O_2, 5% CO_2 images. Second, in addition to the effect of drought on the stomata, there is also a non-stomatal restriction on carbohydrate metabolism that acts to restrict electron transport in the image obtained in a 2% O_2, 5% CO_2 atmosphere compared to the droughted leaf measured in air. Notably, drought has no negative effect on photosynthetic electron transport measured in air, at least not at the low irradiance employed in this measurement protocol. Though the difference between the 2% O_2, 5% CO_2 image and that made in air implies a limitation on photosynthesis acting at a metabolic level, the restriction may have its origins elsewhere, for example in the leaf carbohydrate transport pathway. Whatever its origins, the limitation evident in the 2% O_2, 5% CO_2 image indicates a non-stomatal drought-induced limitation of photosynthesis in this leaf.

After one day recovery in tap-water, the Φ_{PSII} images reveal a deterioration in the condition of the leaf (Fig. 3). This can be seen by comparing the 2% O_2, 350 ppm CO_2 image of (Fig. 2) with the 2% O_2, 350 ppm CO_2 of (Fig. 3). Since the end of the drought treatment on the previous day the area of depressed photosynthetic electron transport has increased and now covers the entire leaf. On the other hand, the images of Φ_{PSII} made in air and 2% O_2, 5%CO_2 are scarcely unaltered compared to the measurement made at the end the drought stress. The conclusion that may be drawn from these changes is that the stomatal restriction that developed during the drought stress has increased the area over which it exerts an effect, so there has been no recovery in the leaf's stomatal response to drought. Otherwise the photosynthetic function of the leaf appears unaltered compared to that at the end of the drought stress.

Finally, after 3 days recovery in tap-water it appears from the 2% O_2, 5% CO_2 image (Fig. 4) that the underlying photosynthetic integrity of the leaf is unaltered from day 2. The image made in 2% O_2, 350 ppm CO_2 is likewise very similar to that made on day 2, with a minor increase in the area of the leaf that lacks any stomatal restriction (the yellow areas - compare with the overall efficiency of the image made in 2% O_2, 5 % CO_2). The Φ_{PSII} image made in shows a slight decrease in the efficiency of PSII electron transport, but this change is minor. Even after 3 days, therefore, the leaf had not significantly recovered from the drought stress.

Literature Cited

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Figures

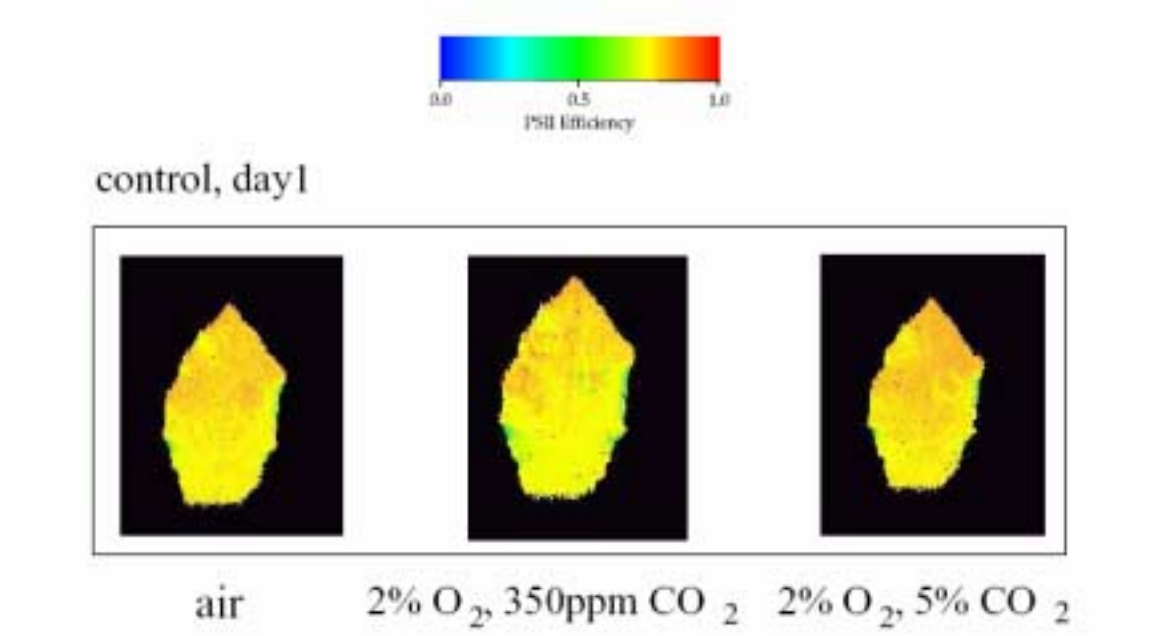

Fig. 1. Images of Φ_{PSII} of an attached rose leaf measured in different gaseous phases at an irradiance of 100 µ mol m^{-2} s^{-1}. Prior to the measurements the cut-rose stem was recut under water and stored in a cold room a 4 ºC. The colour bar above the images shows the colour mapping used for Φ_{PSII} (with this measurement technique the maximum relative efficiency obtained for PSII electron transport is about 0.82).

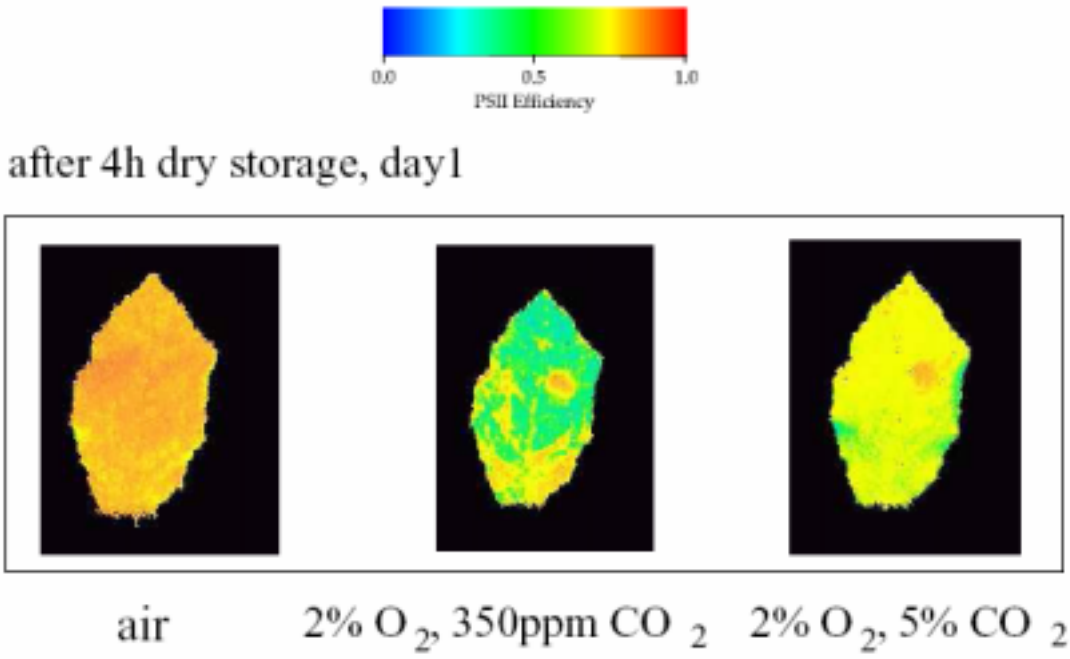

Fig. 2. Images of Φ_{PSII} of an attached rose leaf measured in different gaseous phases at an irradiance of 100 µ mol m^{-2} s^{-1}. Prior to the measurement the cut-rose stem (used to produce images in fig. 1) was droughted for 4 hours.

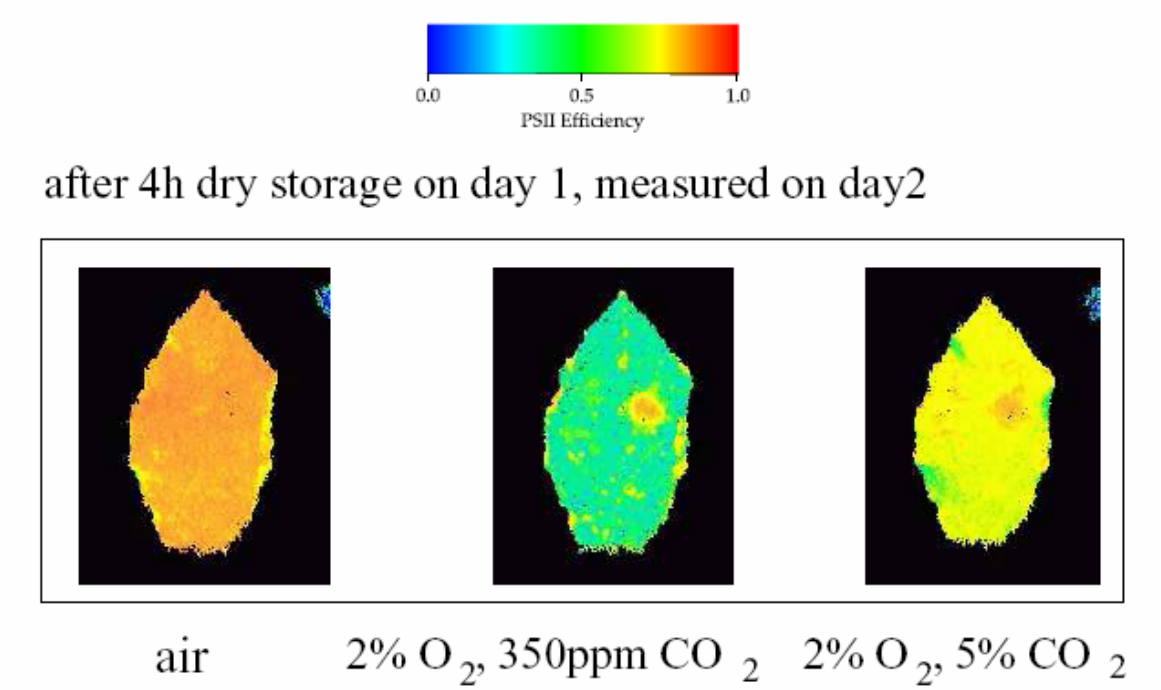

Fig. 3. Images of Φ_{PSII} of an attached rose leaf measured in different gaseous phases at an irradiance of 100 µ mol m^{-2} s^{-1}. Prior to the measurement the cut-rose stem (used to produce images in figs. 1 & 2) was allowed to rehydrate by being stored in tap-water for 24h.

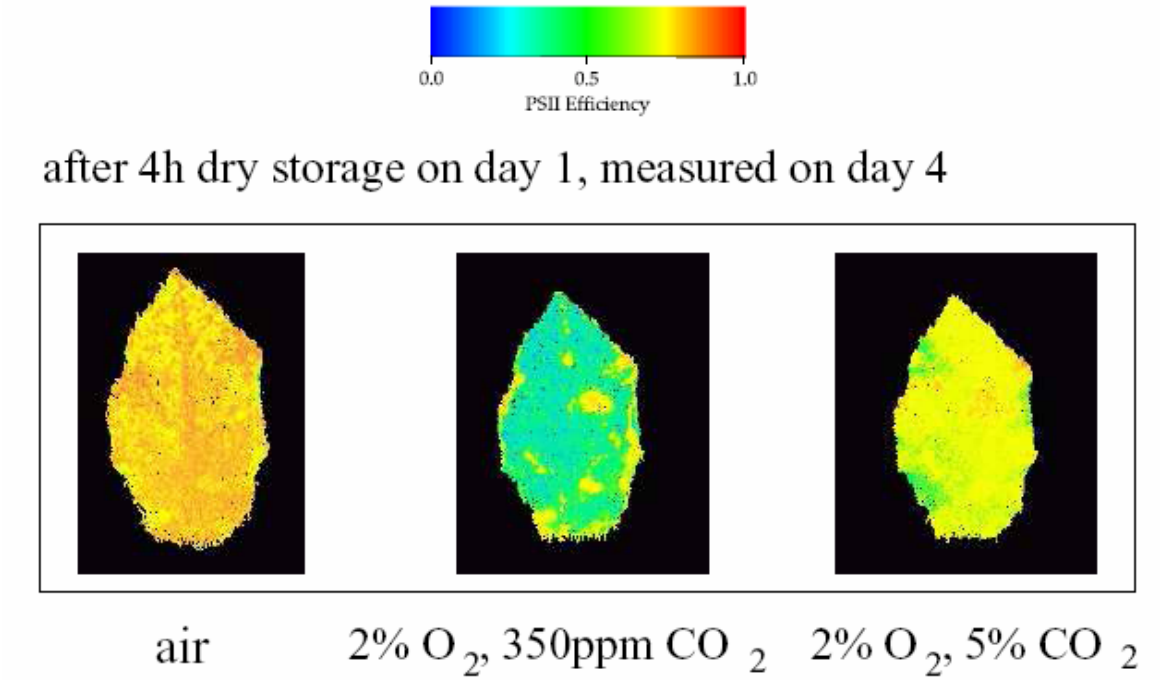

Fig. 4. Images of Φ_{PSII} of an attached rose leaf measured in different gaseous phases at an irradiance of 100 µ mol m^{-2} s^{-1}. Prior to the measurement the cut-rose stem (used to produce images in figs. 1, 2 & 3) was allowed to rehydrate by being stored in tap-water for 3 days.