

## Effect of (Changes in) Air Humidity on Transpiration and (Adaptation of) Stomatal Closure of *Tradescantia* Leaves during Water Stress

U. van Meeteren<sup>1, a</sup>, A. Rezaei Nejad<sup>1, 2</sup> and J. Harbinson<sup>1</sup>

<sup>1</sup>Horticultural Supply Chains Group, Dept. of Plant Sciences, Wageningen University, Marijkeweg 22, 6709 PG, Wageningen, The Netherlands

<sup>2</sup>Department of Horticultural Sciences, Faculty of Agriculture, Lorestan University, P.O. Box 465, Korramabad, Iran

**Keywords:** abscisic acid, chlorophyll fluorescence imaging, cultivation, desiccation, PSII efficiency, vapour pressure deficit, relative humidity

### Abstract

This paper summarises our recent research on the physiological effects of prolonged high RH during growth on stomatal function and we discuss possibilities that arise from this work for reducing postharvest quality problems in cut flowers. Chlorophyll fluorescence imaging was used to measure stomatal closure in response to desiccation of *Tradescantia virginiana* leaves grown under high (90%) and moderate (55%) relative humidities, or transferred between these humidities. Stomata of plants transferred from moderate RH conditions to high RH showed the same diminished closure in response to desiccation, as did stomata that developed at high RH. This response was found both when the leaves were either fully expanded or still actively expanding during the moderate RH pre-treatment. However, when leaves were grown in high RH prior to a moderate RH treatment, the reduced stomatal closure response to desiccation was only reversed in leaves (regions) which were still actively expanding during moderate RH treatment. This indicates that with respect to stomatal responses to desiccation, high RH leaf regions have only a limited capacity to adapt after transfer to moderate RH conditions. It is suggested that the diminished stomatal closure in high RH-grown plants is the result of changes in the signalling pathway for ABA-related closure induced by a prolonged period (several days) at a low ABA level. A short increase of VPD (by decreasing RH or increasing temperature) once every 2 or 3 days is probably sufficient to overcome vase life problems of cut flowers grown at high RH. Testing the acclimation ability of stomata to desiccation by transferring high RH grown plants to low VPD for just a few days would be a simply and effective screening procedure for genotypes with more adaptable stomata.

### INTRODUCTION

Among the environmental conditions during the cultivation period that influence the vase life of cut flowers air humidity is one of the most important, as shown for roses (Mortensen and Fjeld, 1995, 1998; Mortensen and Gislerød, 1997, 1999, 2000; Torre et al., 2001; In et al., 2007). This effect of air humidity during the pre-harvest phase is mainly due to its effect on stomatal behaviour of the cut flowers. The closure of stomata of roses grown at high RH is less sensitive to water stress and darkness (Torre and Fjeld, 2001; Torre et al., 2001) and the cut stems show excessive water loss in indoor conditions (Mortensen and Gislerød, 1999). To obtain the optimal response to multi-factorial environmental changes, stomata sense many environmental factors and have the ability to integrate environmental and endogenous signals (Kearns and Assmann, 1993; Hetherington and Woodward, 2003). Besides the short-term effects of these factors, the history of growth conditions can affect the fine-tuning of the stomatal response. The reason why stomata of high RH-grown leaves are less hydrosensitive is not clear. Desiccation is one of the principal causes of plant death in rooted leafy cuttings and micropropagated plants transferred suddenly from a high to moderate or low RH.

---

<sup>a</sup> [uulke.vanmeeteren@wur.nl](mailto:uulke.vanmeeteren@wur.nl)

Therefore, it is common in leafy cuttings and micro propagated plants to wean the plants gradually from high to low RH. Beyond the fact that it occurs, little else is known about the phenomenon of stomatal acclimation to RH or vapour pressure deficit (VPD).

Ease of propagation and cultivation, and the large size of its stomata have made *Tradescantia virginiana* L. one of the commonest model plants for stomatal research. We used *T. virginiana* to study the physiological effects of prolonged high RH during growth on stomatal function. Special attention was given to (the possibility of) adaptation of stomata to changes in RH. In this paper we give a summary of our recent research (Rezaei Nejad and van Meeteren, 2005, 2007, 2008; Rezaei Nejad et al., 2006) and discuss it in relation to possibilities for the production of cut flowers.

## **MATERIAL AND METHODS**

### **Plant Material and Growth Conditions**

Young *Tradescantia virginiana* L. plants were grown in plastic pots filled with a commercial potting compost in two growth chambers each with a different relative air humidity (moderate: 55±5% and high: 90±5%). The temperature was 21±0.5°C, resulting in VPDs of 1.12 and 0.25 kPa for the moderate and high RH conditions, respectively. The light intensity in both growth chambers was 120±10 μmol m<sup>-2</sup> s<sup>-1</sup> from fluorescent tubes (TLD 33, Philips) with a photoperiod of 16 h/day.

### **Mapping of PSII Photochemical Yield Using Chlorophyll Fluorescence Imaging**

The non-destructive and non-invasive chlorophyll fluorescence imaging technique allows studying temporal dynamics of stomatal closure. In *Tradescantia* leaves subjected to water stress, the value of  $\Phi_{\text{PSII}}$  under low O<sub>2</sub> concentration is linearly related to stomatal aperture (Rezaei Nejad et al., 2006). Chlorophyll fluorescence images were made under an atmosphere of 20 mmol mol<sup>-1</sup> O<sub>2</sub>, 350 μmol mol<sup>-1</sup> CO<sub>2</sub> and remainder N<sub>2</sub> (a non-photorespiratory condition) as described elsewhere (Rezaei Nejad et al., 2006). During preliminary experiments we measured a control leaf (whose cut end remained in water) in parallel with the leaf being desiccated, but it was soon clear that the  $\Phi_{\text{PSII}}$  of the cut leaf in water showed no discernible changes during the course of the measurement. It was therefore decided to dispense with the parallel measurements on a control leaf and to include another treatment leaf instead. Under this regime the first image of  $\Phi_{\text{PSII}}$  that was taken from the cut leaves (which were still in water) served as a reference image. The desiccation process was begun by removing the leaves from water and images were then taken every 30 min for 150 min.

### **Adaptation of Different Parts of a Growing Leaf to Moderate or High RH**

Plants growing in a moderate RH climate room were transferred to a high RH climate room. Ten days after the transfer, marked leaves were used for the measurement of chlorophyll fluorescence in response to desiccation under non-photorespiratory conditions (described above). These leaves consisted of two parts: (1) the distal parts which were grown firstly at a moderate RH and then exposed to a high RH for 10 days, and (2) the basal parts which were produced during the 10 days exposure to a high RH. A similar experiment was carried out with leaves which were grown first at a high RH and then transferred to a moderate RH (for more details see Rezaei Nejad and van Meeteren, 2008).

### **Changes in RH around a Leaf of a Plant during Growth**

To investigate the long-term local effects of high RH on plants growing at moderate RH (55%), one emerging leaf from each plant growing at moderate RH was placed inside a glass tube (one leaf per tube). High RH (90%) was maintained inside half of the tubes by ventilating them with air that had passed through temperature controlled columns of Iron (II)-sulphate heptahydrate (Fluka). Air from the climate room (55% RH) was pumped directly into the remaining tubes with leaves. After three weeks, the leaf

grown inside the tube and an adjacent leaf grown outside the tube at moderate RH in each plant were used for measurements of  $\Phi_{\text{PSII}}$  (Rezaei Nejad and van Meeteren, 2007).

### **Stomatal Responses to Short-Term Application of ABA**

Stomatal aperture in response to exogenous ABA was determined using epidermal strips as described elsewhere (Rezaei Nejad and van Meeteren, 2007). Epidermal strips were removed from the margins and main-vein areas of the abaxial surfaces of leaves from plants grown at moderate or high RH. Stomatal aperture of 10 randomly selected stomata in each strip was measured from digitized video images ( $\times 800$  magnification) of stomata using a microscope connected to a Nikon digital imaging camera DXM-1200.

## **RESULTS AND DISCUSSION**

After desiccation of cut *Tradescantia* leaves, the transpiration rate of high (90%) RH-grown leaves was about 2.5 times that of moderate (55%) RH-grown leaves. Though the average stomatal aperture of both moderate (55%) and high (90%) RH-grown leaves responded to desiccation, exposure to darkness and abscisic acid (ABA) application, the high variability of stomatal closure in high RH-grown plants was striking (Rezaei Nejad and van Meeteren, 2005). Some stomata developed at high RH closed in response to darkness or to a decrease in relative water content (RWC) to the same extent as did stomata from moderate RH-grown plants, whereas others closed only partly or did not close at all. During desiccation, leaves grown at moderate RH showed a rapid decrease of PSII efficiency ( $\Phi_{\text{PSII}}$ ), especially during the second hour of desiccation (Figs. 1B to E). The low  $\Phi_{\text{PSII}}$  measured under low  $\text{O}_2$  concentration (our non-photorespiratory condition) is closely related to stomatal closure. In leaves grown at high RH  $\Phi_{\text{PSII}}$  decreased less during desiccation; after 2 h of desiccation the distribution of  $\Phi_{\text{PSII}}$  in high RH leaves was much more heterogeneous than in moderate RH-grown leaves with clear differences between different areas of a leaf (Figs. 1D and E). Direct measurements of stomatal aperture showed 77% stomatal closure in the leaf margins after 2 h desiccation compared to only 40% closure of stomata in the main-vein areas in high RH-grown leaves. Figure 2 indicates that besides a difference between moderate and high RH-grown leaves in the average rate of stomatal closure during desiccation, stomata of leaves of high RH plants were less hydrosensitive compared to stomata of moderate RH plants.

A high RH maintained around a single leaf from a moderate RH-grown plant resulted in its stomata having a diminished response to desiccation (Fig. 3). The stomata of this leaf reacted more slowly and some remained open, similar to the stomatal responses of the plants grown in a high RH climate room. This shows the importance of the microclimate around individual leaves. Heterogeneous humidity in a greenhouse, therefore, could be a possible cause for heterogeneous postharvest behaviour of cut flowers.

By imaging of  $\Phi_{\text{PSII}}$  (under low  $\text{O}_2$  concentration) it was possible to study stomatal closure of regions within one leaf formed under different environmental conditions when plants were transferred during their cultivation. This offers a simple means to monitor the adaptation of stomatal behaviour. Figure 4 shows the images of  $\Phi_{\text{PSII}}$  of leaves in water (A1 and B1) and after 150 min of desiccation (A2 and B2) under non-photorespiratory conditions. In Figures 4A1 and A2, the distal part of the leaf was first grown at a moderate RH followed by 10 d at a high RH (M $\rightarrow$ H). The base of the leaf was produced at the high RH (H) during the period of exposure of the distal part to the high RH. In Figures 4B1 and B2, the distal part of the leaf was first grown at a high RH followed by 10 d at a moderate RH (H $\rightarrow$ M). The base of the leaf was produced at the moderate RH (M) during the period of exposure of the distal part to the moderate RH. After 150 min of desiccation,  $\Phi_{\text{PSII}}$  had decreased in both parts of each leaf (Figs. 4A2, B2 as compared with 4A1, B1). The decrease of  $\Phi_{\text{PSII}}$  in both parts of the leaf in Figure 4A2 was to the same extent. Irrespective of the duration of desiccation, the mean  $\Phi_{\text{PSII}}$  in the distal parts of the leaves which were first grown at a moderate RH followed by 10 d at a high RH (M $\rightarrow$ H) was the same as that in the basal regions of the leaves, even though the bases of the leaves had grown exclusively at a high RH (H) (Rezaei Nejad and van Meeteren,

2008). The decrease of  $\Phi_{PSII}$  was different between the two parts of the leaf in Figure 4B2. This indicates that stomata grown at moderate RH can adapt to a high RH, resulting in poor closure response to desiccation. In the inverse experiment (leaves first grown at high RH and then transferred to moderate RH), the stomata of the two leaf regions still differed in their closure in response to desiccation 10 d after the transfer- which was the limit of the measurements. This indicated that the stomata of the distal region did not acclimate to the moderate RH to an extent that their closure response matched that of stomata from the basal region. By transferring complete plants, it was demonstrated that 4 days of exposure to high RH was the minimal exposure time to induce the diminished closure response (Rezaei Nejad and van Meeteren, 2008). After 10 d of exposure to a high RH, this loss of the closure response could only be partly reversed by returning the plants to a moderate RH. When similar adaptation dynamics are present in cut flower crops like rose, it implies that periods with high RH of 4 d or longer could induce postharvest quality problems, which can no longer be 'repaired' by subsequent periods of exposure to a moderate or low RH.

Abscisic acid (ABA) is a key component of the signal-transduction pathway for stomatal closure. A lower ABA concentration was found in leaves grown continuously at high RH compared to leaves grown at moderate RH (Rezaei Nejad et al., 2006). Within 1 day of transferring moderate RH-grown plants to a high RH, the ABA level of moderate RH grown leaves decreased to the same low level as found in high RH grown leaves (Rezaei Nejad and van Meeteren, 2008). These results suggest that a low ABA concentration during growth at high RH is related to the diminished stomatal closure in response to drought stress. However, leaves grown at moderate and high RH showed a different response to ABA (Fig. 5). The average stomatal aperture at the margins of the leaves grown at high RH before the application of ABA was higher than around the main veins. Stomatal aperture decreased after application of ABA to both parts of the leaf, but the stomatal aperture was much wider at the margins. In moderate RH-grown leaves, all stomata closed in the same extent after application of ABA (Fig. 5). Also in plants transferred from moderate to high RH, the closure response could not be fully restored by the application of ABA. Transferring plants from high to moderate RH resulted in increased ABA levels within 2 d without a recovery of the stomatal closing response. The loss of ABA response induced by high RH, the lag between the reduction of ABA (day 1) and the loss of stomatal responsiveness (day 4) in the plants transferred to high RH, as well as the lack of restoration of this responsiveness following the increase in ABA level subsequent to transfer back to moderate RH, all suggest that factors besides ABA are involved in the long-term RH effects on stomatal behaviour. However, a daily application of ABA to leaves for three weeks during growth at high RH resulted in stomata whose behaviour in response to desiccation was similar to that found in stomata from moderate RH-grown plants (Rezaei Nejad and van Meeteren, 2007). It appears that the ABA level must be below a certain level for a minimal period (several days) if the stomatal dysfunction is to develop.

Because of the lag time of 3 d between a decrease in ABA level and a loss of stomatal response to desiccation, it is likely that an increase in ABA level within this lag period can prevent the development of the stomatal dysfunction. If this is the case, a change from high to moderate RH within 2 or 3 days after the start of the high RH period will prevent the loss of stomatal responsiveness. A diurnal fluctuation in VPD by fluctuation of RH or temperature was effective for avoiding stomata malfunction of roses (Pettersen et al., 2007). According to our results with *Tradescantia*, it should be worthwhile to investigate if less frequent fluctuations would also prevent the development of stomatal dysfunction.

Mortensen and Gislérød (1999) showed that the magnitude of the responses to high RH differed significantly among rose cultivars. Screening the sensitivity of genotypes for the induction of stomatal malfunction by high RH can probably be done by testing the acclimation ability of stomata to desiccation after transferring plants for just a few days to high RH (low VPD). This would be an effective way of identifying genetic

variation for the development of stomatal dysfunction that could be used in breeding programmes. This selection strategy would remove the need to large greenhouse areas with differing VPDs in which to cultivate plants to the stage at which they produce flowering shoots.

### Literature Cited

- Hetherington, A.M. and Woodward, F.I. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424:901-908.
- In, B.-C., Motomura, S., Inamoto, K., Doi, M. and Mori, G. 2007. Multivariate analysis of relations between preharvest environmental factors, postharvest morphological and physiological factors, and vase life of cut 'Asami Red' roses. *Journal of the Japanese Society for Horticultural Science* 76:66-72.
- Kearns, E.V. and Assmann, S.M. 1993. The guard cell-environment connection. *Plant Physiol.* 102:711-715.
- Mortensen, L.M. and Fjeld, T. 1995. High air humidity reduces the keeping quality of cut roses. *Acta Hort.* 405:148-155.
- Mortensen, L.M. and Gislerød, H.R. 1997. Effects of air humidity and air movement on the growth and keeping quality of roses. *Gartenbauwissenschaft* 62:273-277.
- Mortensen, L.M. and Fjeld, T. 1998. Effects of air humidity, lighting period and lamp type on growth and vase life of roses. *Scientia Horticulturae* 73:229-237.
- Mortensen, L.M. and Gislerød, H.R. 1999. Influence of air humidity and lighting period on growth, vase life and water relations of 14 rose cultivars. *Scientia Horticulturae* 82:289-298.
- Mortensen, L.M. and Gislerød, H.R. 2000. Effect of air humidity on growth, keeping quality, water relations, and nutrient content of cut roses. *Gartenbauwissenschaft* 65:40-44.
- Pettersen, R.I., Moe, R. and Gislerød, H.R. 2007. Growth of pot roses and postharvest rate of water loss as affected by air humidity and temperature variations during growth under continuous light. *Scientia Horticulturae* 114:207-213.
- Rezaei Nejad, A. and van Meeteren, U. 2005. Stomatal response characteristics of *Tradescantia virginiana* grown at high relative air humidity. *Physiologia Plantarum* 125:324-332.
- Rezaei Nejad, A., Harbinson, J. and van Meeteren, U. 2006. Dynamics of spatial heterogeneity of stomatal closure in *Tradescantia virginiana* altered by growth at high relative air humidity. *Journal of Experimental Botany* 57:3669-3678.
- Rezaei Nejad, A. and van Meeteren, U. 2007. The role of abscisic acid in disturbed stomatal response characteristics of *Tradescantia virginiana* during growth at high relative air humidity. *Journal of Experimental Botany* 58:627-636.
- Rezaei Nejad, A. and van Meeteren, U. 2008. Dynamics of adaptation of stomatal behaviour to moderate or high relative air humidity in *Tradescantia virginiana*. *Journal of Experimental Botany* 59:289-301.
- Torre, S. and Fjeld, T. 2001. Water loss and postharvest characteristics of cut roses grown at high or moderate relative air humidity. *Scientia Horticulturae* 89:217-226.
- Torre, S., Fjeld, T. and Gislerød, H.R. 2001. Effects of air humidity and K/Ca ratio in the nutrient supply on growth and postharvest characteristics of cut roses. *Scientia Horticulturae* 90:291-304.

## Figures

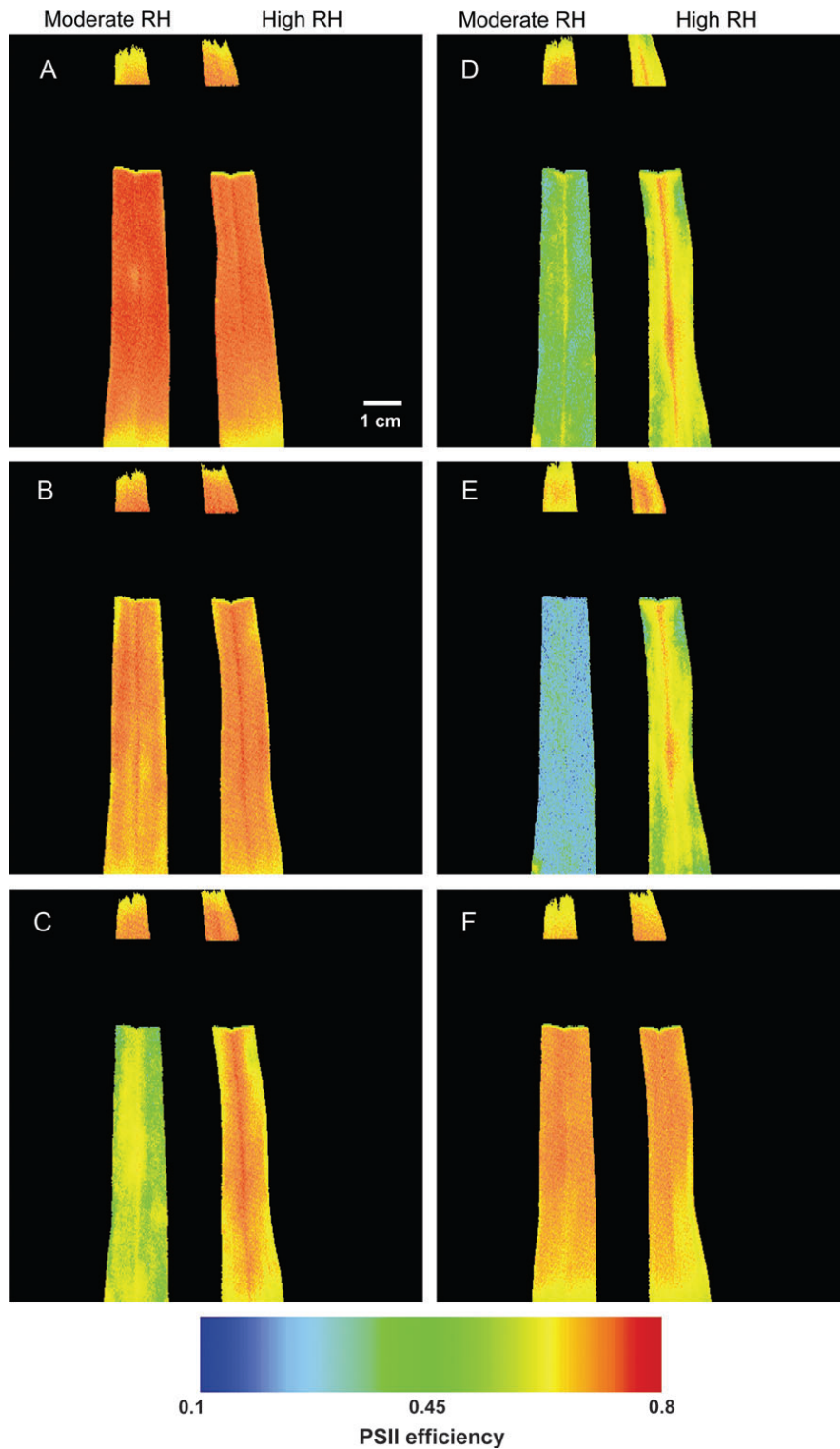


Fig. 1. Images of PSII efficiency ( $\Phi_{PSII}$ ) for control (A) and 60 min (B), 90 min (C), 120 min (D) and 150 min (E) desiccation after excision under  $20 \text{ mmol mol}^{-1} \text{ O}_2$ ,  $350 \text{ } \mu\text{mol mol}^{-1} \text{ CO}_2$  and 150 min desiccation under  $20 \text{ mmol mol}^{-1} \text{ O}_2$ ,  $5000 \text{ } \mu\text{mol mol}^{-1} \text{ CO}_2$  (F) in *Tradescantia virginiana* leaves grown at moderate (left leaf in each image) or high (right leaf in each image) RH (Rezaei Nejad et al., 2006).

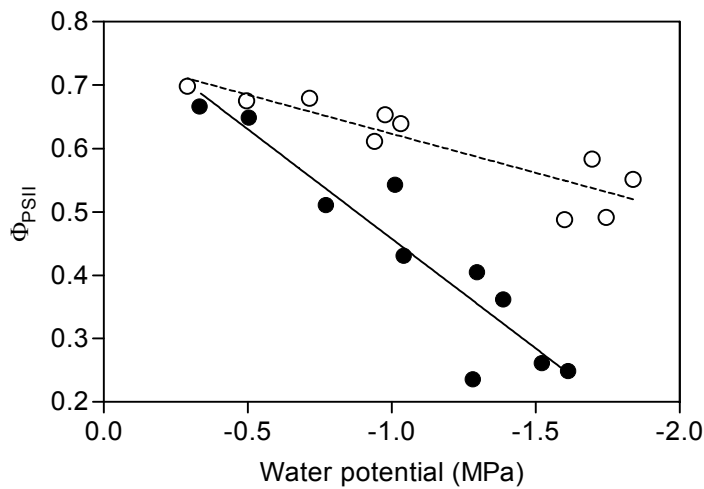


Fig. 2. Relationship between PSII efficiency ( $\Phi_{PSII}$ ) and leaf water potential in *T. virginiana* leaves grown at moderate (closed symbols) or high (open symbols) RH (Rezaei Nejad et al., 2006).

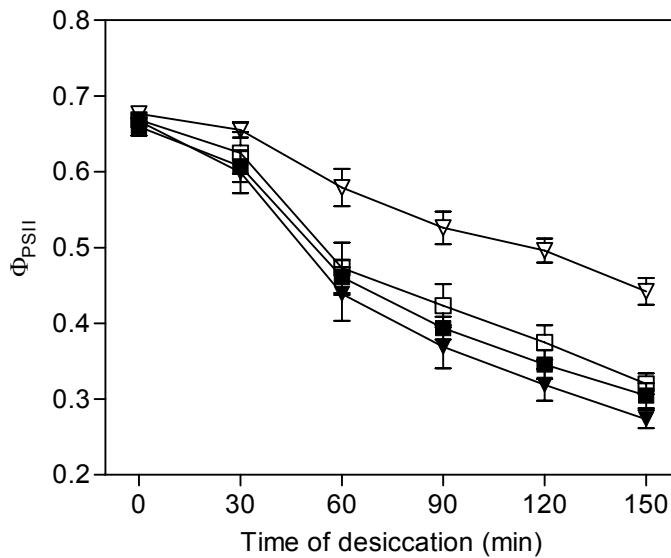


Fig. 3. PSII efficiency ( $\Phi_{PSII}$ ) of *T. virginiana* leaves over time of desiccation. Triangle symbols represent the  $\Phi_{PSII}$  of the mature leaves from plants of which one of their leaves was grown in a glass tube with high RH ( $90 \pm 5\%$ ). Square symbols represent the  $\Phi_{PSII}$  of the mature leaves from plants of which one of their leaves was grown in a tube with moderate RH ( $55 \pm 5\%$ ). From each plant the leaf grown inside the tube (open symbols) and an adjacent leaf grown outside the tube at moderate RH (closed symbols) were used for the measurements. Values are the mean of eight leaves  $\pm$  SEM (Rezaei Nejad and van Meeteren, 2007).

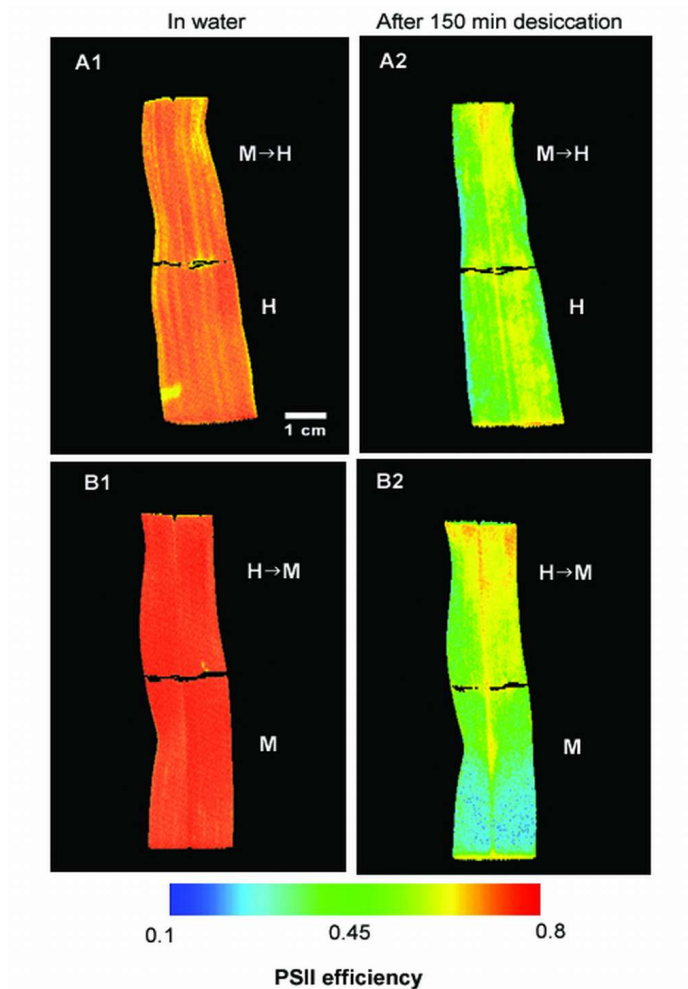


Fig. 4. Images of PSII efficiency ( $\Phi_{PSII}$ ) of *T. virginiana* leaves in water (A1 and B1) and after 150 min of desiccation (A2 and B2). In A, the distal part of the leaf (the part above the black line) was grown at a moderate RH (55%) followed by exposure to a high RH (90%) for 10 d (M→H). The base of the leaf was grown at the high RH during this 10 d period (H). In B, the distal part of the leaf was grown at a high RH followed by exposure to a moderate RH for 10 d (H→M). The base of the leaf was grown at the moderate RH during this period (M) (Rezaei Nejad and van Meeteren, 2008).

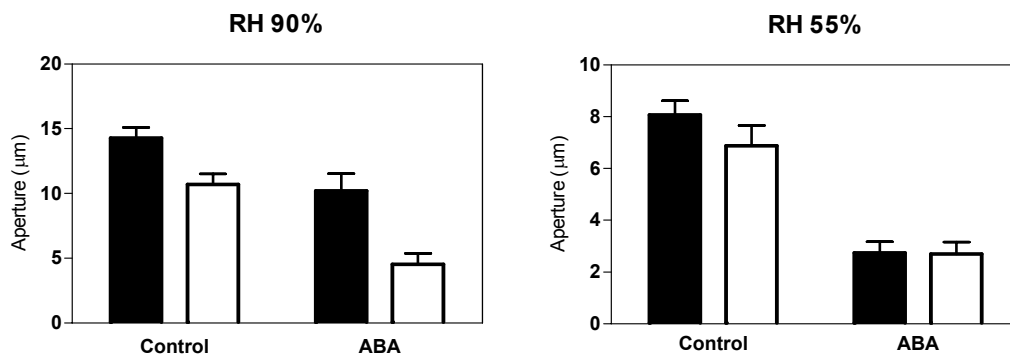


Fig. 5. Stomatal aperture at the margins (closed bars) and main-vein areas (open bars) of *T. virginiana* leaves grown at 90% (left) or 55% (right) RH in response to ABA (100  $\mu$ M) (from Rezaei Nejad and van Meeteren, 2007).