**Reliability of leaf relative water content (RWC)
measurements after storage: consequences for in situ
measurements**

Journal:	<i>Botany</i>
Manuscript ID:	cjb-2015-0065.R1
Manuscript Type:	Methods
Date Submitted by the Author:	04-Jun-2015
Complete List of Authors:	Tanentzap, Fallon; Laurentian University, Biology Stempel, Alexandra; Laurentian University, Biology Ryser, Peter; Laurentian University, Biology
Keyword:	Field measurement, Plant Water Status, Relative Water Content

1 Reliability of leaf relative water content (RWC) measurements after storage:
2 consequences for in situ measurements

3

4 Fallon M. Tanentzap, Alexandra Stempel and Peter Ryser

5

6 Laurentian University, Department of Biology

7 935 Ramsey Lake Road, Sudbury, Ontario, Canada, P3E 2C6

8

9 Email addresses: FMT: FX_Kirkey@laurentian.ca

10 AS: AX_Stempel@laurentian.ca

11 PR: PRyser@laurentian.ca

12

13 Corresponding author:

14 P. Ryser, Laurentian University, Department of Biology, 935 Ramsey Lake Road,
15 Sudbury, Ontario, Canada, P3E 2C6

16 Phone: 1-705-675-1151 ext. 2353

17 Fax: 1-705-675-4859

18

19

Abstract

Relative Water Content (RWC) is widely used to describe plant water status, and is commonly measured gravimetrically. The ephemeral nature of leaf fresh mass poses severe constraints for such measurements in field-grown plants. These constraints can be overcome by transporting the leaves in waterproof containers into the lab. However, even then leaves lose water, and other changes may happen. The effects of a delay on the measurement of RWC have not been quantified so far. In this study the influence of duration of storage up to 96 hours and storage temperature on RWC and its components was investigated for four species. *Alnus incana*, *Impatiens capensis*, *Scirpus microcarpus* leaves were stored in plastic bags, those of *Comptonia peregrina* in plastic vials. RWC remained within 5% of the initial value during 24 h cool storage, but after that larger changes were observed. The effects of storage were species specific, being most pronounced in species poorly protected against desiccation, and under warm conditions. The effects of storage were not only limited to water loss, but also included cellular degradation. In general, storage at 10 °C for 24 h enables measurement of RWC for field-grown plants with accuracy of a few percent, but care has to be taken with species vulnerable to desiccation, possibly requiring faster measurement and a cooler storage temperature.

Key Words: Field measurement; Plant Water Status; Relative Water Content

Introduction

Leaf Relative Water Content (RWC) is commonly used to describe plant water status at a given time (Stocker 1929; Barrs 1968; Kramer 1969). It is a sensitive variable, which quickly responds to environmental conditions such as temperature, light, humidity and water supply (Slatyer 1962). RWC correlates closely with a plant's physiological activities and soil water status (Munne-Bosch and Penuelas 2004; Ozkur et al. 2009), and is a reliable trait, e.g., for screening for drought tolerance of different genotypes (Rachmilevitch et al. 2006). It is defined as the percentage of water present at the time of sampling, relative to the amount of water in a saturated leaf, calculated as

$$RWC (\%) = 100 \times (FM - DM)/(SM - DM) \qquad (Eq. 1)$$

where FM is leaf fresh mass at the time of collection, SM is leaf mass at saturated condition, and DM is leaf dry mass (Turner 1981). During recent years progress has been made in methods to measure plant water status non-invasively using foliar reflectance (e.g., Jörendens et al. 2009; Cheng et al. 2012), but such a method is currently not easily available for a wide use, especially for field investigations (Sancho-Knapik et al. 2013). The gravimetric method that has been described as optimal with respect to reliability and simplicity (Lösch 2001), remains widely used (Fernández-García et al. 2014; Jolly et al. 2014; Liu et al. 2014; Thameur et al. 2014; Chimungu et al. 2015, Gorai et al. 2015; Yang et al. 2015).

Accurate measurement of the different leaf masses is not trivial (Rachmilevitch et al. 2006). Measurement of the saturated mass, commonly achieved by floating leaf discs on water, has received considerable attention (Weatherley 1950; Barrs 1968; Smart and Bingham 1974; Gonzalez and Gonzalez-Vilar 2001) and protocols to saturate leaves in

large-scale comparative measurements of field-grown plants have recently been described (Garnier et al. 2001; Ryser et al. 2008). On the other hand, constraints influencing measurement of the initial fresh mass have received less attention, as in most of the studies leaf relative water content has been assessed in the laboratory or greenhouse, where fresh mass of the collected leaves can immediately be measured. In the field, however, the quick loss of water from excised leaves poses a problem, requiring protective measures against water loss. Tin cans (Stocker 1929; Jolly et al. 2014), plastic vials (Hadley and Smith 1983; Armas et al. 2010; Aref et al. 2013; Fernández-García et al. 2014) and sealed plastic bags (Smart and Bingham 1974; Gonzalez and Gonzalez-Vilar 2001; Davidson et al. 2006; Gonçalves et al. 2011; Ryser et al. 2011; Walter et al. 2011; Teszlák et al. 2013) have been used to protect the collected leaves against water loss during the transportation from field to laboratory. Govender et al. (2009) stress the importance of a proper sampling procedure to prevent water loss by keeping leaves in plastic bags and in a cool dark place. Possible changes in RWC during storage have been tested by Hadley and Smith (1983) and Breashers et al. (1997) for conifer needles, but sensitivity of less robust leaves than conifer needles has not been tested. Many publications do not even describe how and for how long the leaves are stored between excision and fresh mass measurement.

The purpose of this investigation is to determine constraints of accurate gravimetric measurements of RWC in the field, considering changes induced by the unavoidable delay in the measurement on leaf fresh mass, but also on other traits. The effects of the duration and conditions of the delay on water loss have never been quantified, neither other aspects potentially affecting the accuracy of the calculation of

RWC. Such aspects are cellular respiration (Gonzalez and Gonzalez-Vilar 2001; Rachmilevitch et al. 2006), which may decrease leaf dry mass during storage at warm temperatures, and membrane deterioration, which influences water content at saturation (Deschene et al. 1991).

For storage we used primarily re-sealable plastic bags. Plastic bags are frequently used for this purpose, but they are not impermeable to gases, and a slow diffusion of water molecules through plastic will lead to loss of water over time. However, compared to microcentrifuge tubes with lower diffusion rates, bags enable collection of large leaves with a minimal use of space. We assess changes caused by transportation delay by investigating the effects of both duration and temperature of storage on leaves of three species with contrasting vulnerability to water loss in order to establish a guideline for large-scale in situ RWC measurements. Additionally, we also investigate the effect of storage in microcentrifuge tubes on one species with small leaves. We will assess for how long leaves can be stored for the measured values of RWC remaining accurate, and how important a cool temperature during the storage is. It has been shown that cool storage of 1 h or 48 h does not influence the measured RWC of desiccation-resistant leaves such as conifer needles (Hadley and Smith 1983; Breashers et al. 1997), but the effects of duration and temperature of storage on leaves of a wider variety of species has not been investigated. Additionally to the effect of storage, we test the logistic feasibility of the method by measuring the daily course of leaf RWC of two species in a real field situation, so far away from the lab that the weight measurements could be conducted only the following day.

110 **Materials and methods**

111 *Leaf collection sites and species used*

112 Two experiments were conducted to test the reliability of RWC measurements when
113 immediate measurement of leaf fresh mass is not possible. In both experiments, leaf
114 samples were collected within Laurentian University Campus (46°27'59"N 80°58'23" W)
115 in Sudbury, Ontario, Canada. Three species with contrasting leaf characteristics and
116 vulnerability to desiccation were selected to be stored in plastic bags: *Impatiens capensis*
117 Meerb. (Balsaminaceae), *Scirpus microcarpus* J. Presl & C. Presl (Cyperaceae) and *Alnus*
118 *incana* ssp. *rugosa* (Du Roi) R. T. Clausen (Betulaceae). Additionally, storage effects in
119 microcentrifuge tubes were tested using a fourth species with small leaves, *Comptonia*
120 *peregrina* (L.) J. M. Coulter (Myricaceae). The annual herb *I. capensis* occurs under
121 shady and wet conditions and is extremely poorly protected against desiccation, whereas
122 *C. peregrina*, a dwarf shrub of exposed rocky sites is well protected against water loss. *A.*
123 *incana* is a small tree forming dense canopies in swamps, and the graminoid *S.*
124 *microcarpus* grows on open moist conditions. *I. capensis* and *C. peregrina* reflect
125 extremes of vulnerability to desiccation across the species at the collection site.
126 Desiccation speed for the leaves of the four species was tested by laying them for one
127 hour unprotected on a lab counter at about 27°C. During this hour, *I. capensis* lost 62±5%
128 of its leaf fresh mass, *A. incana* 13±0%, *S. microcarpus* 10±2%, and *C. peregrina* 5±1%
129 (mean±1SE; N=6).

130 *Experiment 1*

131 In this experiment the change in leaf fresh mass due to loss of water during storage was
132 documented over periods of up to 96 h. The storage containers should protect leaves

133 against desiccation, but also be light enough to avoid exceeding the capacity of high-
134 precision balances. For the species with the smallest leaves we used microcentrifuge
135 tubes, and for larger leaves small re-sealable plastic bags.

136 Branches or shoots from each of the four species were collected and placed in a
137 large plastic bag in the field on 23 July 2012, 9:00 a.m., and brought within 15 min to the
138 lab, where they were placed in water-filled beakers. For three species, twenty fresh leaves
139 were collected per species, weighed and placed into pre-weighed resealable 10 ×15 cm
140 0.05 mm (2 mil) plastic polybags (WAT supplies, Sudbury, ON, Canada). For *C.*
141 *peregrina*, the species with the smallest leaves, 1.5 mL Fisherbrand™ Premium
142 microcentrifuge tubes were used. All the bags and tubes with leaves were weighed and
143 the values recorded as values at time zero. The bags and tubes were then put in sealed
144 plastic containers for storage. Half the samples were stored at room temperature, average
145 temperatures during the 4 days of the experiment being 29, 28, 27, and 26 °C,
146 respectively (iButton dataloggers DS1921G; Maxim Integrated, San Jose, California).
147 The other half of the bags were placed in a refrigerator with a temperature of 10 °C. All
148 bags with leaves were re-weighed after 3, 6, 24, 48 and 96 h after the first measurement.
149 Leaf fresh mass at the time of measurement was calculated by deducting the bag mass
150 from the measured mass of the bag with leaf. This value was then normalized by dividing
151 it by the initial, pre-bagging leaf mass, enabling a direct comparison of relative fresh
152 mass changes of all the leaves.

153 *Experiment 2*

154 In this experiment changes in leaf RWC, leaf fresh mass to saturated mass ratio, and leaf
155 dry mass to saturated mass ratio were documented after storage in plastic bags (3 species)

or microcentrifuge tubes (1 species) after 24 h and after 96 h, by comparing the values to the initial measurements. RWC is the target variable, which is calculated using leaf fresh mass, leaf saturated mass and leaf dry mass (leaf dry matter content). The fresh mass to saturated mass, and the dry mass to saturated mass ratios indicate how the components of RWC contribute to its change.

As saturated mass and dry mass of the stored leaves could not be repeatedly measured, each measurement required a separate set of leaves. On 24 July 2012, 9:00 a.m. 240 leaves were harvested on campus. Sixty leaves from each of the four species were collected directly into resealable bags or microcentrifuge tubes (*C. peregrina*). The bagged samples were transported in a cooler to the lab within one hour. Two thirds of the bags containing the leaves were then stored in airtight plastic boxes either in a refrigerator (10.0 °C) or at room temperature (averages for the 4 days of the experiment: 28, 27, 26, and 25 °C, respectively). For one third of the leaves their fresh mass was immediately measured, for another third after 24 h storage, and for the last third after 96 h storage. After the measurement of fresh mass, the leaves were placed in a refrigerator between moist paper towels for 24 h to attain saturation (Ryser et al. 2008). Leaf saturated mass was measured, after which the leaves were dried at 75°C for 48 h, and their dry mass determined.

Field measurements of RWC

To test the practicality of the method under a real field situation, diurnal fluctuation of leaf RWC was measured for two species, *Bidens cernua* L. (Asteraceae) and *Carex retrosa* Schwein. (Cyperaceae) in a wetland 40 km from the lab. Leaves of these two species were collected on five occasions from dawn to dusk (6:30, 10:00, 14:00, 18:00,

20:00) on 9 September 2012, with 10 replicate leaves at a time into pre-weighed resealable plastic bags. The bagged samples were placed inside a cooler (7-10 °C) and brought to the laboratory the following day. Leaf fresh mass, saturated mass and dry mass were measured as described for Experiment 2.

Results

Experiment 1

Leaves of all species lost water during storage in plastic bags, and the loss was faster at room temperature than in the refrigerator (Fig. 1; Table 1). For *C. peregrina* leaves, the species best protected against desiccation and the only species stored in microcentrifuge tubes, there was a slight but non-significant decrease in fresh mass, especially under the warm storage temperature. After 24 h in the refrigerator, *I. capensis* leaves had lost 7% of their fresh mass. For the other three species the loss did not exceed 2%. At room temperature, fresh mass declined faster, but the loss during the first 24 h remained for all species except *I. capensis* below 6% of the original value. Even after 96 h in the refrigerator leaves of three of the four species lost at most 6% of their fresh mass. *I. capensis* was the most vulnerable with 17% loss. Under warm conditions, *I. capensis* leaves lost 62% of their fresh mass within 96 h.

Experiment 2

Calculated values for leaf relative water content decreased during prolonged storage for all species (Fig. 2; Table 1). The decline was faster under warm conditions. Under cool conditions, the RWC values lost after 24 h at most 5% of the initial values (*I. capensis*, *A. incana*), under warm conditions at most 12% (*I. capensis*, *A. rugosa*, *S. microcarpus*).

After 96 h, RWC had further declined, except for warm-stored *A. incana* and *C. peregrina* leaves, for which RWC increased between 24 and 96 h. In ANOVA's conducted for each species, time had a significant effect on RWC for all species but *C. peregrina*, the interaction with storage temperature being significant for *I. capensis* and *S. microcarpus* (Table 1). Changes in the fresh mass to saturated mass ratio were close to those of RWC, but dry mass to saturated mass ratio (leaf dry matter content) showed a different behavior (Fig. 2). In all species but *S. microcarpus* there was a significant Time $\times$ Temperature interaction (Table 1). In case of *A. incana*, the ratio increased in the cold and the warm treatment already after 24 h. For *C. peregrina* an increase was observed in warm treatment only, for *I. capensis* in the cold treatment after 96 h. We are not aware of any mechanism to explain an increase of dry mass of leaves stored in cool and dark, and consequently, the increased dry mass to saturated mass ratio seems to indicate a decreased ability of the leaves to absorb water at saturating conditions, probably due to changes at cellular level.

Field measurements of RWC

Both *B. cernua* and *C. retrorsa* showed a significant diurnal variation in their RWC, the values declining from the $99\pm 1\%$ and $96\pm 1\%$ at 6:30, respectively, down to $94\pm 1\%$ and $87\pm 2\%$ by 18:00, but rising again to $98\pm 1\%$ and $95\pm 2\%$ by 20:00 (mean values ± 1 SE). *C. retrorsa* had throughout lower values of RWC than *B. cernua*. Compared to the diurnal variation and interspecific differences the standard error was small. Error variation in *C. retrorsa*, the species with a larger diurnal variation, increased during the day, indicating that the variation in RWC values was mainly caused by local variation rather than changes during storage. The effects of species ($p<0.001$) and time ($p<0.001$)

were highly significant, their interaction not ($R^2=0.467$, ANOVA, $N=10$, one outlier with a studentized residual >5 removed).

Discussion

The method of maintaining leaf fresh mass until measurement has to be functional allowing a large number of replicates of field-collected samples to be measured over a short time period. Re-sealable plastic bags are frequently used to store leaves of field-grown plants until leaf water content can be assessed, but potential measurement errors caused by water loss from leaves stored in such bags have not previously been quantified. Our laboratory results show that in cool storage, RWC was maintained within 5% of the initial value for at least 24 h, indicating that use of such bags to transport leaves from the field to the laboratory allows determination of leaf RWC of field-grown plants with reasonable accuracy. Our field test complemented the laboratory experiments by showing that the method is feasible for use in remote locations, and the results are precise enough to distinguish diurnal and interspecific differences.

However, our results also clearly show that caution has to be exerted when measurement of vulnerable species is delayed. Leaves of sensitive species, such as *I. capensis*, lost 7% of their fresh mass within 24 h at 10°C, and *A. incana* leaves showed after similar storage an increased dry mass to fresh mass ratio. This shows that during storage the leaves not only lost water, but the ability of some species to gain mass under saturating conditions declined as well. This is an indication of changes at cellular level, e.g., membrane degradation (Deschene et al. 1991), and the pronounced change in *A. incana* indicates that this effect is species specific as well. Previously, the effect of delay

in fresh mass measurement has been found to be non-significant for conifer needles (Hadley and Smith 1983; Breashers et al. 1997), but our data shows that this is not necessarily the case for all species. Hence, the constraints for the accuracy of the RWC measurement in the field depends on type of the investigated habitat, species of moist shady environments, for example, being more vulnerable to changes. Caution is also needed when there is a delay in measurement of variables other than RWC, for example leaf dry matter content.

Plastic bags will lose water through slow diffusion. Using less permeable containers, such as metal and glass jars, or more robust plastic tubes, avoids or reduces this problem, but large leaves require large containers and the required volume may become difficult to keep cool. The mass of such containers may exceed the capacity of the high-resolution balances needed to measure the leaf mass accurately. Removing leaves from the container at weighing may lead to further loss of water, especially if any has condensed in the walls of the container. Our data indicate hardly any water loss from microcentrifuge tubes, but they are not conclusive as the species used in the tubes was the one best protected against desiccation.

Temperatures in the cool storage in our experiment were around 10°C, considered to be realistic to achieve in the field to store a large amount of leaves. That temperature was sufficient to achieve a reliable measurement of leaf RWC within 24 hours, but our data indicates that for sensitive species lower temperatures would be preferable. The increasing FM/DM ratio in *C. peregrina* after 96 hours indicates that at prolonged storage a lower temperature would be preferable even when the leaves are stored in less permeable containers, in order to avoid cellular degradation.

We conclude emphasizing the well-known importance of a fast measurement of leaf fresh mass, and if a delay is unavoidable, making sure to store the leaves in a cool and well-protected container. In such a case it is important to note that the effects of duration and temperature of the storage are species specific. For sensitive species, e.g., herbs from shady and moist environments, special attention should be given to minimize the duration and temperature of the storage, and to storage container permeability. The effect of storage should possibly be quantified for such species. But nevertheless, storage up to 24 h in re-sealable plastic bags under temperatures up to 10°C does not compromise the data quality, resulting for most species in changes of the obtained RWC values of only a few percentages.

Acknowledgements

This research was funded by the Collaborative Research and Development grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) with Vale Canada Ltd. and the Centre for Excellence in Mining Innovation (CRDPJ 372568-08).

References

Aref, I., El Atta H., El Obeid M., Ahmed A., Khan P., and Iqbal, M. 2013. Effect of water stress on relative water and chlorophyll contents of *Juniperus procera* Hochst. ex Endlicher in Saudi Arabia. Life Sci. J. **10**(4): 681-685. doi:10.7537/j.issn.1097-8135.

- 292 Armas, C., Padilla F.M., Pugnaire F.I., and Jackson, R.B. 2010. Hydraulic lift and
293 tolerance to salinity of semiarid species: consequences for species interactions.
294 *Oecologia*. **162**(1): 11-21. doi: 10.1007/s00442-009-1447-1.
- 295 Barrs, H.D. 1968. Determination of water deficits in plant tissue. *In* Water Deficits and
296 plant growth. *Edited by* T.T. Kozlowski. Academic Press, New York. pp. 235–
297 368.
- 298 Breashers, D.D., Myers O.B., Johnson S.R., Meyer C.W., and Martens, S.N. 1997.
299 Differential use of spatially heterogeneous soil moisture by two semiarid woody
300 species: *Pinus edulis* and *Juniperus monosperma*. *J.Ecol.* **85**(3): 289-299. doi:
301 10.2307/2960502.
- 302 Cheng, T., Rivard B., Sánchez-Azofeifa A. G., Féret J. B., Jacquemoud S., and Ustin S.L.
303 2012. Predicting leaf gravimetric water content from foliar reflectance across a
304 range of plant species using continuous wavelet analysis. *J. Plant Phys.* **169**(12)
305 1134-1142. dx.doi.org/10.1016/j.jplph.2012.04.006.
- 306 Chimungu, J.G., Maliro, M.F., Nalivata, P.C., Kanyama-Phiri, G., Brown, K.M., and
307 Lynch, J.P. 2015. Utility of root cortical aerenchyma under water limited
308 conditions in tropical maize (*Zea mays* L.). *Field Crop. Res.* **171**(1), 86-98. doi:
309 dx.doi.org/10.1016/j.fcr.2014.10.009.
- 310 Davidson, A., Wang, S., and Wilmshurst, J. 2006. Remote sensing of grassland–
311 shrubland vegetation water content in the shortwave domain. *Int. J. Appl. Earth*
312 *Obs.* **8**(4), 225-236. doi:10.1016/j.jag.2005.10.002.
- 313 Deschene, A., Paliyath G., Loughheed E.C., Dumbroff E.B., and Thompson, J.E. 1991.
314 Membrane deterioration during postharvest senescence of broccoli florets:

- modulation by temperature and controlled atmosphere storage. *Postharvest Biol. Tec.* **1**(1): 19-31. doi: 10.1016/0925-5214(91)90016-5.
- Fernández-García, N., Olmos, E., Bardisi, E., García-De la Garma, J., López-Berenguer, C., and Rubio-Asensio, J.S. 2014. Intrinsic water use efficiency controls the adaptation to high salinity in a semi-arid adapted plant, henna (*Lawsonia inermis* L.). *J. Plant Phys.* **171**(5), 64-75. <http://dx.doi.org/10.1016/j.jplph.2013.11.004>.
- Garnier, E., Shipley B., Roumet C., and Laurent, G. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* **15**(5): 688–695. doi:10.1046/j.0269-8463.2001.00563.x.
- Gonçalves, W.G., Costa, A.C., Lima, D.P., Silva, A.A., and Megguer, C.A. 2011. Membrane permeability and relative water content in physic nuts plants submitted to fast water deficit. *Gl. Sci. Technol.* **4**(1). Available from <http://rioverde.ifgoiano.edu.br/periodicos/index.php/gst/article/view/258/245> [accessed 26 February 2015].
- Gonzalez, L., and Gonzalez-Vilar, M. 2001. Determination of relative water content. In *Handbook of plant ecophysiology techniques*. Edited by M. J. Reigosa Roger. Kluwer Academic Publishers, New York. pp. 207–212.
- Gorai, M., Laajili, W., Santiago, L. S., and Neffati, M. 2015. Rapid recovery of photosynthesis and water relations following soil drying and re-watering is related to the adaptation of desert shrub *Ephedra alata* subsp. *alenda* (Ephedraceae) to arid environments. *Environ. Exp. Bot.* **109**, 113-121. doi:10.1016/j.envexpbot.2014.08.011.

- 337 Govender, M., Weiersbye I.M., Witkowski E.T.F., and Ahmed, F. 2009. Review of
338 commonly used remote sensing and ground-based technologies to measure plant
339 water stress. *Water SA*. **35**: 741-752. Available from
340 <http://www.ajol.info/index.php/wsa/article/view/76861/67333> [accessed 26
341 February 2015].
- 342 Hadley, J.L., and Smith, W.K. 1983. Influence of wind exposure on needle desiccation
343 and mortality for timberline conifers in Wyoming, USA. *Arctic Alpine Res.* **15**:
344 127-135. Available from <http://www.jstor.org/stable/1550988>.
- 345 Jolly, W.M., Hadlow, A.M., and Huguet, K. 2014. De-coupling seasonal changes in
346 water content and dry matter to predict live conifer foliar moisture content. *Int. J.*
347 *Wildland Fire*, **23**(4), 480-489. doi:10.1071/WF13127
- 348 Jördens, C., Scheller M., Breitenstein B., Selmar D., and Koch, M. 2009. Evaluation of
349 leaf water status by means of permittivity at terahertz frequencies. *J. Biol. Phys.*
350 **35**: 255-264. doi: 10.1007/s10867-009-9161-0
- 351 Kramer, P.J. 1969. *Plant and Soil Water Relationships: A Modern Synthesis*. McGraw-
352 Hill, New York.
- 353 Liu, P., Yin, L., Deng, X., Wang, S., Tanaka, K., and Zhang, S. 2014. Aquaporin-
354 mediated increase in root hydraulic conductance is involved in silicon-induced
355 improved root water uptake under osmotic stress in *Sorghum bicolor* L. *J. Exp.*
356 *Bot.* **65**(17): 4747-4756. doi:10.1093/jxb/eru220.
- 357 Lösch, R. 2001. *Wasserhaushalt der Pflanzen*. Quelle & Meyer, Wiebelsheim, Germany.

- 358 Munné-Bosch, S., and Peñuelas, J. (2004). Drought-induced oxidative stress in
359 strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions.
360 Plant Sci., 166(4), 1105-1110. doi:10.1016/j.plantsci.2003.12.034.
- 361 Ozkur, O., Ozdemir, F., Bor, M., and Turkan, I. 2009. Physiochemical and antioxidant
362 responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environ.
363 Exp. Bot. 66(3), 487-492. doi:10.1016/j.envexpbot.2009.04.003.
- 364 Rachmilevitch, S., DaCosta, M., and Huang, B. 2006. Physiological and biochemical
365 indicators for stress tolerance. In Plant-environment interactions, 3rd ed. Edited by
366 B. Huang. CRC Press, Boca Raton, FL. pp. 321–356.
- 367 Ryser, P., Bernardi J., and Merla, A. 2008. Determination of leaf fresh mass after storage
368 between moist paper towels: constraints and reliability of the method. J. Exp. Bot.
369 **59**(9): 2461-2467. doi:10.1093/jxb/ern120.
- 370 Ryser, P., Gill H.K., and Byrne, C.J. 2011. Constraints of root response to waterlogging
371 in *Alisma triviale*. Plant Soil **343**(1-2): 247-260. doi: 10.1007/s11104-011-0715-0.
- 372 Sancho-Knapik, D., Peguero-Pina J.J., Medrano H., Fariñas M.D., Álvarez-Arenas T.G.,
373 and Gil-Pelegrín, E. 2013. The reflectivity in the S-band and the broadband
374 ultrasonic spectroscopy as new tools for the study of water relations in *Vitis*
375 *vinifera* L. Physiol. Plantarum **148**(4): 512-521. doi:10.1111/ppl.12007.
- 376 Slatyer, R.O. 1962. Internal water balance of *Acacia aneura* F. Muell in relation to
377 environmental conditions. UNESCO Arid Zone Research **16**: 137-146.
- 378 Smart, R.E., and Bingham, G.E. 1974. Rapid estimates of relative water content. Plant
379 Physiol. **53**(2): 258-260. Available from

- 380 <http://www.plantphysiology.org/content/53/2/258.full.pdf+html> [accessed 26
381 February 2015].
- 382 Stocker, O. 1929. Das Wasserdefizit von Gefäßpflanzen in verschiedenen Klimazonen.
383 *Planta* **7**: 382-387. Available from
384 <http://link.springer.com/article/10.1007%2FBF01916035> [accessed 26 February
385 2015].
- 386 Teszlák, P., Kocsis M., Gaál K., and Nikfardjam, M.P. 2013. Regulatory effects of
387 exogenous gibberellic acid (GA₃) on water relations and CO₂ assimilation among
388 grapevine (*Vitis vinifera* L.) cultivars. *Sci. Hortic.-Amsterdam* **159**:41-51. doi:
389 [dx.doi.org/10.1016/j.scienta.2013.04.037](https://doi.org/10.1016/j.scienta.2013.04.037).
- 390 Thameur, A., Ferchichi, A., and López-Carbonell, M. 2014. Involvement of abscisic acid
391 metabolites and the oxidative status of barley genotypes in response to drought.
392 *Can J. Plant Sci.* **94**(8), 1481-1490. doi:10.4141/CJPS-2014-069.
- 393 Turner, N.C. 1981. Techniques and experimental approaches for the measurement of
394 plant water status. *Plant Soil* **58**(1-3): 339-366. Available from
395 <http://link.springer.com/article/10.1007/BF02180062> [accessed 26 February
396 2015].
- 397 Walter, J., Nagy L., Hein R., Rascher U., Beierkuhnlein C., Willner E., and Jentsch, A.
398 2001. Do plants remember drought? Hints towards a drought-memory in grasses.
399 *Environ. Exp. Bot.* **71**: 34-40. doi:10.1016/j.envexpbot.2010.10.020.
- 400 Weatherley, P.E. 1950. Studies in the water relations of the cotton plant. I. The field
401 measurement of water deficits in leaves. *New Phytol.* **49**(1): 81-97. Available

402 from <http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.1950.tb05146.x/epdf>
403 [accessed 26 February 2015].
404 Yang, S., Li, X., Ma, Y., Sun, X., Yang, Y., and Yang, Y. 2014. Proteome response of
405 wild wheat relative *Kengyilia thoroldiana* to drought stress. Can. J. Plant Sci.
406 **95**(1): 1-13. doi:10.4141/CJPS-2014-294.
407

Draft

Table 1. Results of repeated measures ANOVA on leaf fresh mass (FM) with 6 measurements after storage of 0 h, 3 h, 6 h, 24 h, 48 h, and 96 h (Experiment 1, data arcsine transformed, one outlier removed) and results of ANOVAs of leaf relative water content (RWC), leaf fresh mass to saturated mass ratio (FM/SM), and leaf dry mass to saturated mass ratio (DM/SM) measured after 0h, 24 h and 96 h storage in a refrigerator or at room-temperature with time of storage and storage temperature as factorial variables (Experiment 2, seven outliers removed). ANOVAs were conducted for each species separately. F values and the level of significance are given (* $p < 0.01$; ** $p < 0.01$; *** $p < 0.001$, n.s. not significant).

	N	R2	Temperature	Time	Temp x Time
FM, Experiment 1					
<i>Impatiens capensis</i>	20		65.7***	273***	57.3***
<i>Alnus incana</i>	19		142***	739***	131***
<i>Comptonia peregrina</i>	20		0.0 n.s.	1.2 n.s.	1.6 n.s.
<i>Scirpus microcarpus</i>	20		60.3***	159***	28.5***
RWC, Experiment 2					
<i>Impatiens capensis</i>	58	0.664	1.1 n.s.	73.4 ***	10.4 **
<i>Alnus incana</i>	59	0.179	0.8 n.s.	10.3. **	0.2 n.s.
<i>Comptonia peregrina</i>	58	0.029	1.0 n.s.	0.6 n.s.	0.9 n.s.
<i>Scirpus microcarpus</i>	58	0.561	4.9 *	40.9 ***	15.0 ***
FM/SM, Experiment 2					
<i>Impatiens capensis</i>	58	0.67	1.3 n.s.	73.9 ***	11.2 **
<i>Alnus incana</i>	59	0.141	1.0 n.s.	7.5 **	0.4 n.s.
<i>Comptonia peregrina</i>	58	0.04	1.6 n.s.	0.6 n.s.	1.6 n.s.
<i>Scirpus microcarpus</i>	58	0.552	5.0 *	40.3 ***	14.6 ***
DM/SM, Experiment 2					
<i>Impatiens capensis</i>	58	0.154	2.9 n.s.	2.9 n.s.	5.6 *
<i>Alnus incana</i>	59	0.286	2.6 n.s.	16.1 ***	4.5 *
<i>Comptonia peregrina</i>	58	0.159	7.6 **	0.2 n.s.	9.8 **
<i>Scirpus microcarpus</i>	58	0.055	1.1 n.s.	0.1 n.s.	0.2 n.s.

Fig. 1. Leaf fresh mass of the four investigated species after storage times from 3 to 96 h, stored in a refrigerator (light symbols) or at room temperature (dark symbols). The values are normalized to the directly measured initial mass. The error bars indicate ± 1 SE.

Fig. 2. Leaf relative water content (RWC), leaf fresh mass to saturated mass ratio (FM/SM), and leaf dry mass to saturated mass ratio (leaf dry matter content; LDMC) for the four investigated species, measured after 0h, 24 h and 96 h storage in a refrigerator (light grey bars) or at room-temperature (dark grey bars).

Draft

Fig. 1. Leaf fresh mass of the four investigated species after storage times from 3 to 96 h, stored in a refrigerator (light symbols) or at room temperature (dark symbols). The values are normalized to the directly measured initial mass. The error bars indicate ± 1 SE.

257x185mm (300 x 300 DPI)