Leaf Quality of *Aechmea* (Bromeliaceae) under Non-Adapted Greenhouse Conditions

E. Londers¹, I. Vervaeke¹, R. Deroose² and M.P. De Proft¹  
¹Laboratory of Plant Culture, Catholic University Leuven, W. De Croylaan 42  
B-3001 Leuven, Belgium  
²Deroose Plants, Droogte 139, B-9940 Evergem, Belgium

Keywords: Necrosis, Leaf Anatomy, Water Household, Turgor Pressure, Humidity

Abstract  
Bromeliad growers are getting necrosis in the foliage of diverse *Aechmea* cultivars, mainly under climate conditions characterised by high air humidity during the night period. These necrotic areas both occur in greenhouse cultivation and after transport. Spineless *Aechmea* hybrids seem to be very sensitive in this respect (Londers, 2001). The leaf damage consists of localised ruptured cells in the chlorenchym. The occurrence of necrosis on specific leaf locations is linked with the local plant water status, more specific with a higher leaf turgor pressure. Plants placed under high air humidity (95-100 % RH), show increased leaf turgor pressure. Based on the results obtained so far, high turgor pressure is considered to be the initial condition for the occurrence of the observed leaf damage.

INTRODUCTION  
The family of the Bromeliaceae comprises a diversity of species and genera, native to Central and South America. As ornamentals, their inflorescence and long flowering period are very much appreciated by growers and consumers. Bromeliads occur from sea level up to 4000 m altitude, resulting in a wide range of environmental adaptations (Leme and Marigo, 1993), such as CAM (Crassulacean Acid Metabolism), which is an adaptation to limits in water availability.
  
For commercial purposes though, bromeliads are mostly grown out of their natural habitat, under greenhouse conditions that are not adapted to their specific needs, e.g. high relative humidity during the night. These non-adapted conditions may cause plant quality problems. One documented problem is the occurrence of necrotic spots in the foliage of diverse *Aechmea* cultivars, mainly during summertime. Necrosis mostly occurs at the bend of the leaf blade, with run outs towards the leaf top (Fig. 1B and 1C). This leaf quality problem both occurs in cultivation and after transport (Poole and Henley, 1992; Poole and Conover, 1992). Spineless *Aechmea* hybrids seem to be very sensitive in this respect (Londers, 2001).

In this paper, an anatomical description of the documented leaf damage (Poole and Henley, 1992; Poole and Conover, 1992) is presented, by comparison with healthy leaf tissue. Water household, a key element for good leaf quality, was investigated by water potential, osmotic potential and turgor pressure measurements. Our first objective was to investigate the above parameters along the leaf length axis. Later on, these parameters were investigated under high air humidity (95-100 % RH), as the documented leaf damage often occurs under high air humidity (Londers, 2001). The results are discussed in the broader context of the commercial growth of *Aechmea* cultivars under greenhouse conditions, more specific, a hypothesis for the occurrence of necrosis on specific leaf locations will be given.

MATERIALS AND METHODS

Plant Material and Culture  
A leaf damage sensitive, spineless *Aechmea* hybrid was selected (Fig. 1A). *Aechmea* belongs to the subfamily of the Bromelioidae and the subgenus *Platyaechmea* of *Aechmea* (Smith and Downs, 1979). This subgenus is now elevated to the genus level.
and is called *Hoplophytum* (Till, 1999). The broad leaves of *Aechmea* plants are wider at the base and form a funnel-shaped rosette. This rosette functions as a tank from which the pink inflorescence appears (Oliva-Esteva and Steyermark, 1987). The powdery character of their leaves and the flowering stem are due to many white, mealy trichomes that cover it (Oliva-Esteva and Steyermark, 1987). The *A. hybrida* used is the result of a breeding program for spineless *Aechmea* cultivars. Plants used did not flower and were obtained at young stage from N.V. Deroose Plants; they showed 23 (± 4) leaves (n=10) with the longest leaf being 48 (± 6) cm long and 4.5 (± 0.4) cm wide.

Plants were grown in a greenhouse at the Catholic University of Leuven, Belgium, with light (Sodium vapour lamps, 400 W), temperature and humidity control. Greenhouse conditions were set for bromeliad production as it is used in Belgium. Pest control and fertilization were performed to reach an optimal plant quality.

**Microscopy**

To study plant leaf anatomy paraffin slides were made. Firstly, plant leaf samples were preserved in ethanol 70 %. Next, plant material was gradually dehydrated using a series of different ethanol, butanol and xylol solutions, based on Van Cotthem and Fyns-Claessens (1972). Paraffin slides were cut with a rotation microtome (Euromex Mic 540) and stained with fast green, safranin and haematoxylin. Slides were evaluated with a light microscope (Olympus BX40), which was connected to a JVC (TK-C1381) video camera on a computer using the MicroImage™ analysis software.

**Water Potential, Osmotic Potential and Turgor Pressure**

Leaf water potential measurements were conducted on fully expanded leaves using a WP4 psychrometer (Decagon Devices Inc.). Five discs from the same leaf part were punched out with a cork-bore of 1.2 cm diameter. After a quick abrasion of the leaf cuticle the samples were sealed in the WP4 chamber and equilibrated for 45 min. For further osmotic potential measurements the same discs were sealed in plastic cups and frozen (-20°C) for at least 24 h. After thawing, discs were placed a second time in the WP4 chamber for equilibration. The turgor pressure was calculated from the water and the osmotic potential data. The matrix potential was assumed to be zero.

**Climate Registration**

The greenhouse climate was registered, using a Testo 400. Probes for air temperature and relative air humidity were connected. Measurements were analysed with Testo Comfort Analysis Software.

**Statistical Analysis**

For all measurements mean and standard error (SE) are given. Sample size is measurement dependent. Results were compared with Student’s t-Test. For each analysis a significance level of 5 % was maintained.

**RESULTS**

**Leaf Anatomy**

1. **Healthy Leaf.** When a leaf is viewed in cross section, the adaxial part consists of two or three water-storing hypodermal layers of varying thickness made up of large chloroplasts-free parenchyma cells, called the hydrenchym (Fig. 2A). The abaxial leaf part is build up by dense layers of chlorenchym, a tissue comprised of green parenchyma cells (Fig. 2B and 2C). In this mesophyl, foliar veins are embedded (Fig. 2B). Phloem fills the lower portion of the vascular core, xylem the upper half; a sheath of fibrous strengthening cells surrounds both. Intercostal air lacunae are embedded in the chlorenchym, build up by stellate-lobbed cells (Fig. 2C).

2. **Damaged Leaf.** Microscopically seen, the observed leaf damage occurs in the dense chlorenchym tissue. Several adjacent cells are ruptured, resulting in holes in these
chlorenchym layers (Fig. 2D).

3. Leaf Thickness along Leaf Axis. Fig. 3 presents leaf thickness and hydrenchym fraction along the leaf axis, from leaf base to leaf top. Samples were taken on fully expanded leaves at leaf base, half leaf length and at leaf top (n=10). Leaf thickness decreases from leaf base (883.4 ± 24.7 µm) to half leaf length (691.7 ± 23.1 µm). From half leaf length to leaf top (691.6 ± 18.0 µm) leaf thickness stays rather constant. In addition, hydrenchym thickness was measured and hydrenchym fraction of total leaf thickness was calculated. Absolute hydrenchym thickness shows a pattern similar to total leaf thickness: decrease from leaf base (188.1 ± 15.4) to half leaf length (54.9 ± 9.9 µm) and constant from half leaf length to leaf top (54.5 ± 9.0 µm). Although both total leaf and hydrenchym thickness decrease from base to top, hydrenchym decrease is sharper, resulting in lower hydrenchym fraction at half leaf length and leaf top.

Water Potential, Osmotic Potential and Turgor Pressure

1. Along Leaf Axis. WP4 measurements along Aechmea hybrid leaf axis are presented in Fig. 4. Samples were taken on fully expanded leaves around 8:00 am at leaf base, half leaf length and at leaf top (n=30). Both water and osmotic potential gradually decreases from leaf base to leaf top, with measurements at leaf base being significantly different from measurements at half leaf length and leaf top (P ≤ 0.05). Moreover, water potential measurements at half leaf length and leaf top, are also significantly different (P ≤ 0.05). Turgor pressure is slightly higher at half leaf length (0.60 ± 0.26 MPa) than at leaf base (0.45 ± 0.44 MPa) and at leaf top (0.42 ± 0.28 MPa). Only turgor pressure at half leaf length and at leaf top was significantly different (P ≤ 0.05).

2. Under High Air Humidity. WP4 measurements of plants placed under high air humidity are showed in Fig. 5. High air humidity was created by placing the plants under a plastic cap. Air humidity control was set between 95 and 100%. Climate was controlled with Testo 400 climate registration and found to be stable between set parameters (results not shown). Plants were followed from the first day till four days after the start of the high air humidity treatment. Samples were taken on fully expanded leaves around 8:00 am between half leaf length and leaf top (n=30). Osmotic potential stays constant during the treatment. Turgor pressure has elevated significantly (P ≤ 0.05) during the first day of treatment with 0.23 MPa and stays constant during the rest of the week. Water potential follows the same pattern as turgor pressure.

DISCUSSION

The documented leaf damage is known to occur mostly on the leaf bend, with run outs to the leaf top. The damage can be firstly seen at both leaf sides as collapsed leaf tissue, the affected spot lying lower than healthy leaf tissue surface. After some hours, the spot turns yellow and finally ends in necrotic leaf tissue. The macroscopic leaf damage documented, this paper reveals the microscopic leaf damage origin in the dense chlorenchym tissue layer. A hypothesis for the occurrence of ruptured chlorenchym tissue cells is leaf water status disturbance.

In order to comprehend the damage occurrence on specific leaf spots, water and osmotic leaf potential together with leaf turgor pressure were measured along leaf length axis, being key elements in leaf water status. Measurements indicated higher turgor pressure at half leaf length (0.60 MPa), than at leaf base (0.45 MPa) and leaf top (0.42 MPa). Only turgor pressure at half leaf length and leaf top were significantly different (P ≤ 0.05); the lack of statistical significance between leaf base and half leaf length is probably a consequence of the experimental design (n=30). Possible explanations for higher leaf turgor pressure at half leaf length, which is usually the leaf bend, can be hydrenchym layer thickness or osmotic potential.

Hydrenchym, the accordion-pleated water-storage parenchyma should be seen as water-buffering system under arid climate conditions (Benzing, 2000). As shown in the anatomical study results, both absolute hydrenchym layer thickness and hydrenchym fraction of total leaf thickness are highest at leaf base. Flores (1975) considers negative
geotropic to be responsible for these basal ‘water depots’. Smaller hydrenchym layers, which include lower ‘buffer capacities’, could probably result in higher turgor pressures.

Osmotic potential can also generate higher turgor pressure (Lüttge, 1987). Difference in osmotic potential in this Aechmea hybrid can be explained by higher photosynthetic activity at half leaf length, even more at leaf top, resulting in higher organic acid concentrations (Lüttge, 1987; own registration). Schmidt and Kaiser (1987) described a water passing mechanism from the hydrenchym to the chlorenchym for Peperomia magnoliifoliae. Barcikowski and Nobel (1984) found chlorenchym water retention to be much higher than hydrenchym water retention. Above statements in mind, it is not unthinkable that high absolute osmotic potential, generated by organic acid in the chlorenchym, results in high turgor pressure, more specific in the chlorenchym, which is exactly the tissue where the documented damage originates.

Both discussed parameters though, do not account for turgor pressure being significant lower at leaf top than at half leaf length, as hydrenchym thickness is comparable at both locations and osmotic potential is even more negative at leaf top than at half leaf length. A high transpiration rate at leaf top is a possible explanation for lower leaf turgor pressure at this location. To the authors, a combination of all three parameters, namely hydrenchym thickness, osmotic potential and transpiration, causes very probably the highest leaf turgor pressure at half leaf length, which is about the leaf bend.

Aechmea hybrid plants placed under continuous high air humidity (95-100 %RH) showed increased turgor pressure. This implicates that plants placed under high humidity are more endangered by the documented leaf damage, as more water is available to build up a high turgor pressure. For Aechmea, known as CAM plants (Till, 1999), especially high air humidity during the night should be considered leaf quality threatening, as organic acid build up results in high osmotic potential.

The authors consider turgor pressure build up as an initial condition for the occurrence of the leaf damage, though not sufficient on itself to cause the damage. The measured increase of the turgor pressure under high air humidity, most probably generates a maximum, but non cell wall rupturing, turgor pressure in the chlorenchym. A leaf cell at its maximum turgor pressure though, can’t take any extra pressure, caused by leaf temperature or mechanical stress, without causing damage. High light intensities result in a leaf temperature increase, especially at the leaf bend. Temperature measurements (results not shown) revealed a higher leaf temperature increase at the chlorenchym side than at the hydrenchym side. Plant sleeving causes mechanical stress to the plants exposed. By sleeving the plants for transport, the highest mechanical stress exists at the leaf bend, exactly the place where leaf damage occurs.

Growers should be aware that high air humidity not only endangers leaf quality in cultivation, but also during transport. This implicates also that plastic packing is not recommended.

Further investigation on the leaf damage documented, should result in a better greenhouse management for plants exposed to the damage.

ACKNOWLEDGMENTS
This research was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). The authors wish to thank Reginald Deroose NV for supplying plant material.

Literature Cited
communicação visual, Brazil.
Till, W. 1999. Alphabetical list of currently accepted taxa.

**Figures**

![Aechmea hybrid](image)

**Fig. 1.** Spineless, leaf damage sensitive Aechmea hybrid used. A. Funnel-shaped rosette with inflorescence. B. Documented leaf damage: from leaf bend to leaf top. C. Documented leaf damage: close-up.
Fig. 2. Light microscopic images of *Aechmea* hybrid leaf cross sections at half leaf length. A. Hydrenchym: water-storing hypodermal layers at adaxial leaf side (40x10). B. Foliar vein: filled with phloem (adaxial) and xylem (abaxial), surrounded by a sheath of fibrous strengthening cells (40x10). C. Intercostal air lacunae, embedded in chlorenchym, build up by stellate-lobbed cells (40x10). D. Ruptured cells in dense chlorenchym (20x10).

Fig. 3. Transversal *Aechmea* hybrid leaf anatomy: total leaf (■-µm) and hydrenchym layer (□-µm) thickness; hydrenchym fraction of total leaf thickness (■-%). LB: Leaf Base; HLL: Half Leaf Length; LT: Leaf Top. SE ± is given by Y-error bars (n=10).
Fig. 4. Water Potential (■-MPa), Osmotic Potential (■-MPa) and Turgor Pressure (□-MPa) through *Aechmea* hybrid leaf. LB: Leaf Base; HLL: Half Leaf Length; LT: Leaf Top. SE ± is given by Y-error bars (n=30). Samples are taken around 8:00 am.

Fig. 5. Water Potential (■-MPa), Osmotic Potential (■-MPa) and Turgor Pressure (□-MPa) between half leaf length and leaf top of *Aechmea* hybrid under 95-100 % RH. SE ± is given by Y-error bars (n=30). Samples are taken around 8:00 am.