Aspects of stress tolerance in bryophytes

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Abstract. To understand the various physiological processes and stress responses of bryophytes comparing with higher plants' reactions it is essential to know the actual water status of the bryophyte tissue. Cell water relations in bryophytes essentially are the same as those of other plant cells and can be described by the 'Höfler diagram'. Surface water amount can vary widely without affecting cell water status, which can result in difficulties in expressing precise actual water content (WC). The knowledge of WC at full turgor is principal to calculate RWC. The different adaptive types of bryophytes and higher plant cells respond similarly to water deficit. Bryophytes include but are not inherently shade plants. Shade-loving bryophytes saturate at a PPFD of 100-300 μmol m^{-2} s^{-1}, responses are similar to those of the shade-loving vascular plants. Sun-exposed bryophytes saturate at a PPFD of 1000 μmol m^{-2} s^{-1}. In this species REFR rises almost linearly with increasing irradiance and they show extraordinary high levels of NPQ, which can be suppressed by DTT. 1-qP generally stabilises at around 0.3 to 0.4. Responses of this kind are found in a taxonomically and ecologically diverse range of bryophytes. PPFD response patterns in bryophytes having complex ventilated photosynthetic systems are similar to vascular plants'ones. In sun-exposed bryophytes O_2 and CO_2 are largely interchangeable as electron sinks and CO_2-uptake accounts for ~ 60% of the low PPFD saturation value. Shade-adapted species appears less able to use O_2 as electron sink, or to generate high NPQ at high irradiance. In bryophytes the strongest limiting stress factors are desiccation and high temperature, and the last one can be lethal if the tissue is metabolically

active. It is important to determine the constitutive and inducible mechanisms of desiccation tolerance in bryophytes.

Keywords: desiccation tolerance, stress responses, bryophytes, PPFD-response curves, chlorophyll fluorescence

Abbreviations used

Chl: chlorophyll; DTT: dithiothreitol; Ψ: water potential; NPQ: non-photochemical quenching; PPFD: photosynthetic photon flux density; pv: pressure-volume; qP: photochemical quenching; REFR: relative electron flow; UV-B: ultraviolet-B; RWC: relative water content; WC: water content.

1. Introduction

Bryophytes share most of their physiology with other green land plants, but there are also important differences; the similarities and differences do not necessarily fall in line with simple expectations (Proctor 2000). Because most bryophytes have simple 'stems' and 'leaves', therefore tradition has regarded them 'lower plants' or underdeveloped miniatures of vascular plants, which organisms that have evolutionarily not yet made the grade. The divergence of bryophytes and the various vascular plant groups happened 400 million years ago or earlier. 400 million years were enough for the development of evolutionary independent lines (phylum), as we call them hornworts, liverworts and mosses. Also contrast with expectations bryophytes physiologically are not primitive. With their succesful strategy they are making up a prominent part of the vegetation in oceanic temperate forests, tropical cloud forests, bogs and fens, polar and alpine fellfields and tundras. Their poikilohydric habit means they are taking up water and nutrients over the whole surface of the shoots *via* direct absorption from dry and wet deposition (Table 1). On the one hand they are limited by their lack of roots, but they can colonize hard and impermeable surfaces, like tree trunks, rock outcrops, roof surfaces, from which vascular plants are excluded. So they are successful in many nutrient-limited habitat and many of them are vulnerable to tolerate atmospheric pollutants.

2. Water relations in bryophytes

To understand the various physiological processes and stress responses of bryophytes comparing with higher plants reactions it is essential to know the actual water status of the bryophyte tissue.

Conflicting requirements of water conduction and storage and free gas exchange for photosynthesis (molecular diffusion is slower in water than in air by a factor of about 10^4) are achieved in various ways in bryophytes:
- water-repellent cuticular material on leaf surfaces;
- granular or crystalline epicuticular vax (glaucous-looking endohydric species) on surfaces;
- shoots with closely overlapping concave leaves: inner faces for water storage, outer ones for free gas exchange (kept in dry);
- papilla/mamilla covered leaf surfaces, apices remaining dry, interstices for water (interstices between them provide a countinuous network of water-conducting channels);
- complex ventilated photosynthetic tissue (Polytrichales leaves, Marchantiales thalli); the leaves of Polytrichales and thalli of Marchantiales have complex ventilated photosynthetic tissues paralleling leaves of vascular plants (pores and chambers) with preventing water loss (surface vaxes, water-repellent edges of pores and stomatas); increased area for CO2-uptake.

Table 1. *Comparison of characteristics are important in water relations in bryophytes and vascular plants*

Bryophytes	Vascular plants
lack of roots, rhizoids	water uptake in roots
ectohydry: external water movement in capillary spaces endohydry: some bryophytes have well-developed internal conducting structures (in a limited number of large acrocarpous mosses), that is not approaching vascular plant transpiration stream myxohydry: some combination of the two, balance between them, none of them is predominant poikilohydry,	internal water conduction (xylem)
	root pressure
	waterproof and water-repellent cuticle in leaves and young stems
lack of complex water movement, relatively diffuse water movement (there is no unified stream)	cuticular and stomatal transpiration streams
stomata in a few cases, their role is not relevant	complex water movement

Cell water relations in bryophytes essentially are the same as those of other plant cells and described by the 'Höfler diagram' (Figure 1a, b): the relation between cell osmotic potential and water content can be described as a rectangular hyberbola. The relation of cell water potential to cell water content follows this hyperbola up to the turgor-loss point. It then breaks away to follow a line to the full-turgor (where RWC=1.0 and Ψ=0). When the axes of the graph relating water potential to water content is plotted on a reciprocal scale, the hyperbola becomes a straight line (Figure 2a). The graph of $1/\psi$ against (1-RWC) is referred as a pressure-volume (pv) curve. The horizontal dotted line indicates the turgor-loss point. After psychrometric measurements from the pv-curves $\psi_{full\ turgor}$, ε_B can be read (bryophyte cell walls are rather extensible $\Rightarrow$low ε_B).

Surface water amount can vary widely without affecting cell water status which can result in difficulties in expressing precise actual water content (3 types of water: capillary, apoplastic, symplastic). The knowledge of WC at full turgor is principal to calculate RWC. This value is physiologically comparable with those for vascular plants. RWC values based on „saturated" water content can be wholly misleading. Full turgor water content can often be obtained by carefully blotting samples. (Actual WC/ WC at full turgor)*100 is expressed on fresh weight base or dry weight base.
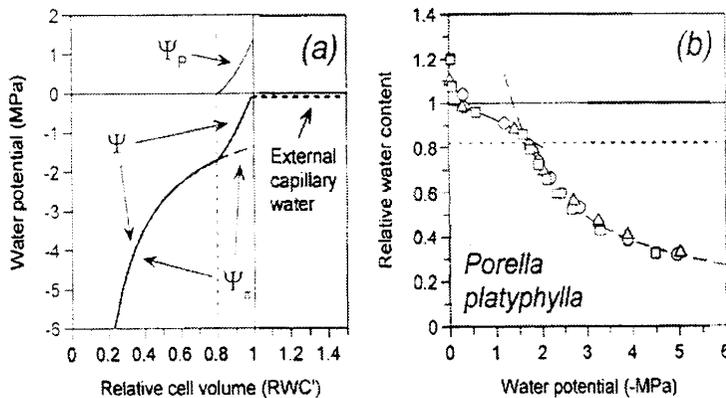
Figure 1a, b: (a) Höfler diagram for a bryophyte illustrating the relationship of cell water potential (ψ) and its components to cell water content and external capillary water. (b) The relation of relative water content to water potential for the leafy liverwort Porella platyphylla, from thermocouple measurements. Water content was originally plotted as% dry weight, and the full-turgor point estimated by inspection from the graph, as described by Proctor et al. (1998). The horizontal dotted line indicates the turgor-loss point. A rectangular hyperbola has been fitted to the data point below this, and the a polynomial regression to the points between full turgor and turgor loss. Original figures from Proctor 2000.
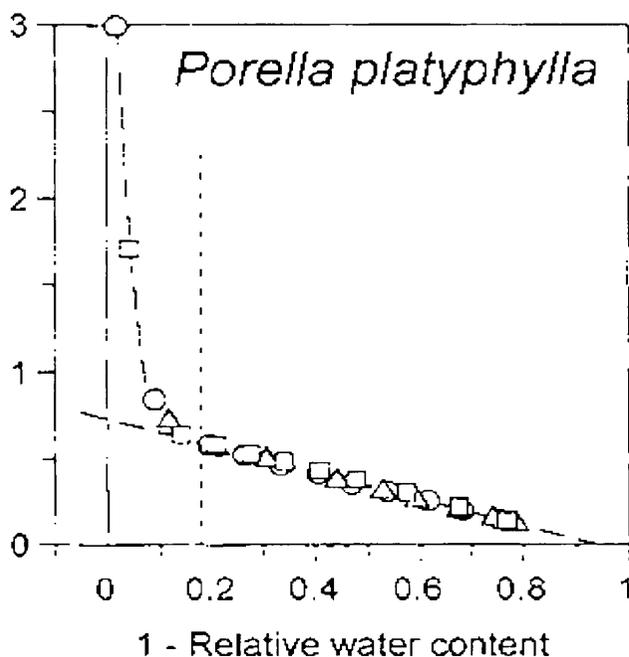
Figure 2: Pressure-volume graph from the same data as Fig. 1b. Original figure from Proctor 2000.

2. Stress factors and stress responses in bryophytes

In bryophytes the strongest limiting stress factors are desiccation and high temperature or the combination of the two or the combination of high water content and high temperature, but the last version is not too common, according to the morphological structure of these plants.

3.1 Water deficit and responses

The different adaptive types of bryophytes and higher plant cells respond similarly to water deficit. The comparison was made in terms of true relative water content (true relative water content = cell water content relative to that at full turgor) (Figure 3).
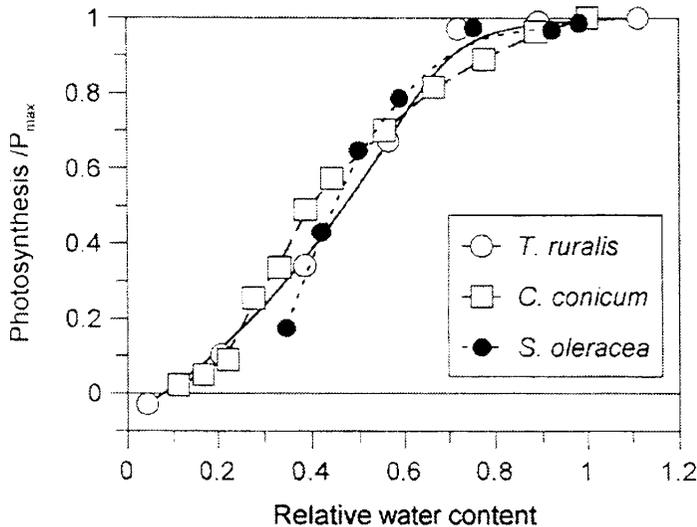
Figure 3: *Response of net photosynthesis to cell water deficit in two contrasting bryophytes (Tortula ruralis, Conocephalum conicum) and in spinach (Spinacia oleracea), a mesophytic vascular plant, from gas-exchange measurements. Original figure from Proctor 2000.*

3.2 Different light conditions and light responses

Bryophytes have been regarded as showing unified shade plant-like characteristics on evidence including the fine structural features of the chloroplasts, and the typically low chl a/b ratios (of course in the range of shade-adapted vascular plants), although they are living between very different light conditions. Median chl a/b ratio for 45 mosses was 2.33, and for 18 liverworts 1.99 (Figure 4).

There is wide variation in their light responses. The shade-loving species (exemplified by *Plagiomnium undulatum*) saturate at a PPFD of 100–300 μmol m^{-2} s^{-1} (corresponding to 5-10% of full sunlight), the response curves of this species are generally similar to those of the shade-loving vascular plants (left graph of Figure 5). The sun-exposed species (exemplified by *Racomitrium lanuginosum*) saturate at a PPFD of 1000 μmol m^{-2} s^{-1}.(right graph of Figure 5). The sun-exposed bryophytes show two remarkable features in their PPFD response curves. First, REFR does not saturate, but continues to rise almost linearly with increasing irradiance. Second, these species show extraordinary high levels of NPQ, which often also continues to rise almost linearly to irradiances equivalent to full sunlight. At the same time 1-qP generally stabilises at around 0.3 to 0.4. Responses of this kind are found in a taxonomically and ecologically diverse range of bryophytes

(Marschall *et al.,* 2000).High NPQ is suppressed by DTT. This suggests that high levels of xanthophyll-cycle-mediated photoprotection similar to that of higher plants, extra excitation energy dissipating as heat.

PPFD response patterns in bryophytes having complex ventilated photosynthetic systems are similar to vascular plants (Figure 6).

Relative electron flow rate (REFR) and CO_2-uptake curves for sun-exposed *Tortula ruralis* show that CO_2-uptake accounts for ~ 60% of the low PPFD saturation value. This means the rest of the energy goes to other electron sinks (Figure 7).

REFR responses for two contrasting species in various gas mixtures were the followings: the sun-exposed *Schistidium apocarpum* shows little difference in REFR between normal and CO_2-free air and $N_2+1\%$ CO_2. It means O_2 and CO_2 are largely interchangeable as electron sinks. REFR is very low in pure N_2. 1-qP saturates at ~ 0.5 in the treatments that provide an electron sink, but rapidly rises to ~ 1.0 in pure N_2. NPQ quickly reaches a steady value of ~ 6.0 in pure N_2, but continues to rise with PPFD in the other treatments. The shade-adapted *Plagiomnium undulatum* appears less able to use O_2 as electron sink, or to generate high NPQ at high irradiance. The highest NPQ (~ 4.0) developed in N_2. REFR is markedly depressed in CO_2-free air.

3.3 Desiccation tolerance, as an adaptive strategy

Desiccation tolerance is a very widespread phenomenon among living organisms ((occurs among microorganisms, fungi, algae, lichens, bryophytes, vascular plants (it is uncommon in vegetative tissues, but characteristics in spores and seeds), in animal groups: invertebrates)). It presents independently from geological times in the plant kingdom, common and characteristics, but not universal in bryophytes. The origin of desiccation tolerance can be explained from the intermittent availability of water to the plant. Between two periods of precipitation, bryophytes can reach full turgor in many ways: storing sufficient water to extend to moist periods with the help of their morphological features; from dewfall, cloudmist, interception, precipitation.

Bryophytes are much more tolerant of high (or very low) temperatures dry than wet. Species of constant moist and shade are the most sensitive to desiccation. The rate, the speed of desiccation and the light condition is very important in the recovery of photosynthesis upon rehydration.

3.4 UV-B-tolerance in bryophytes

The effect of UV-B is heavier with desiccation. An effective antioxidant system, different anatomical and morphological features of the species and

the presence of UV-B absorbing materials in the cell wall represent the protective mechanism against UV-B in bryophytes.

3.5 Temperature stress and tolerance in bryophytes

There is a close relationship between temperature tolerance and the water content of the cells. The high and low temperatures are less harmful in dry state of the bryophyte tissue. The lethal temperature for moist, metabolically active bryophytes are the same as in vascular plants (40-50°C).
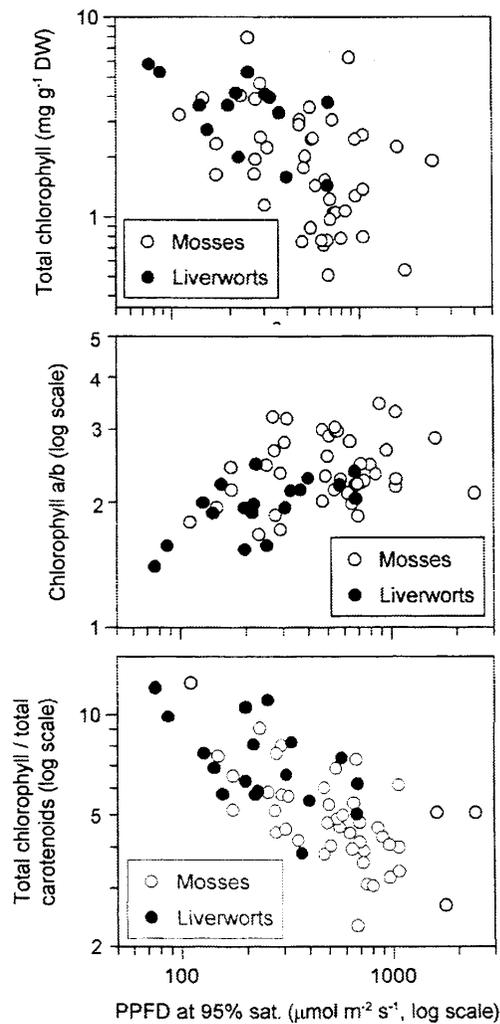
Figure 4: Logarithmic plots of pigment parameters and PPFD$_{95\%}$ ('95% saturation' PPFD was calculated from fitted REFR curves).The figure shows the result of a survey of 63 bryophytes.

3.6 Desiccation tolerance mechanisms in bryophytes

What are the factors that enable a plant to be tolerant of desiccation? Cell structure that can lose most of its water without disruption, and membranes that retain the essentials of their structure in the dry state or are readily and quickly reconstituted on remoistening.

In vascular plants under desiccation or/and extreme temperature macromolecules of cytoplasm stabilized by compatible solutes (carbohydrates, polyols, proteins) (= 'molecular packaging') producing a vitreous phase. Soluble carbohydrate pool, including sucrose, fructan and polyols, have a great importance in certain bryophyte genera, and a similar role has been proposed in desiccation tolerance (Marschall *et al.,* 1998). The storage carbohydrates are 'membrane compatible' (Marschall *et al.,* 1998). The soluble carbohydrates did not show large responses to changes in the environment suggesting that they are well-buffered and available to act as desiccation protectants.

Photosynthetic apparatus is homoiochlorophyllous, does not disintegrate, recovery is quick and complete upon rehydration (Tuba *et al.,* 1998). Protein synthesis also recover quickly (Oliver *et al.,* 1998) in desiccation-tolerant species. Their antioxidant system protects them against ROS during desiccation and following rehydration.

4. Summary

Intermittent availability of water keeps up photosynthesis and growing in bryophytes. The amount of external water can vary widely without affecting the water status of the cells. In dry period they are metabolically inactive. Their desiccation tolerance represents an 'escaping drought' strategy, based on moistly constitutive mechanisms. The preservation of photosynthetic system and chloroplast structure wholly during desiccation, instead of *de novo* synthesis, seems to be an effective mechanism with using repair systems as well. The newest research line focusing on the inducible mechanisms can enrich our knowledge on desiccation tolerance.

It can be stated that bryophytes are not a primitive precursors of vascular plants, but the diverse and highly evolved representatives of an alternative adaptation strategy.
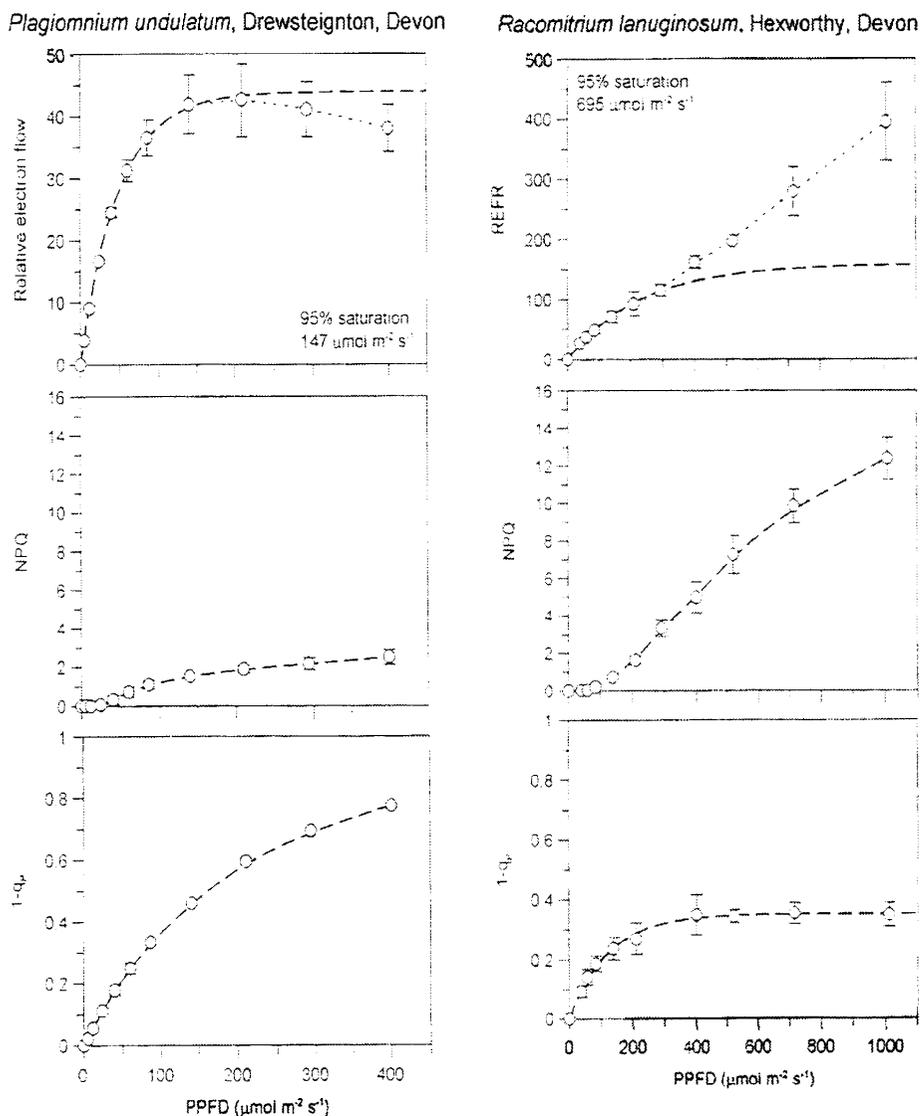
Figure 5: *PPFD response curves of relative electron flow rate (REFR), NPQ and 1–qP, from chlorophyll-fluorescence measurements for two representative species. Plagiomnium undulatum is a shade-loving species, while Racomitrium lanuginosum is a species of sun-exposed habitats. Note the different x-axis scales of the curves, and the varying y-axis scales of the REFR graphs. The y-axis scales for NPQ and 1–qP are the same throughout.*
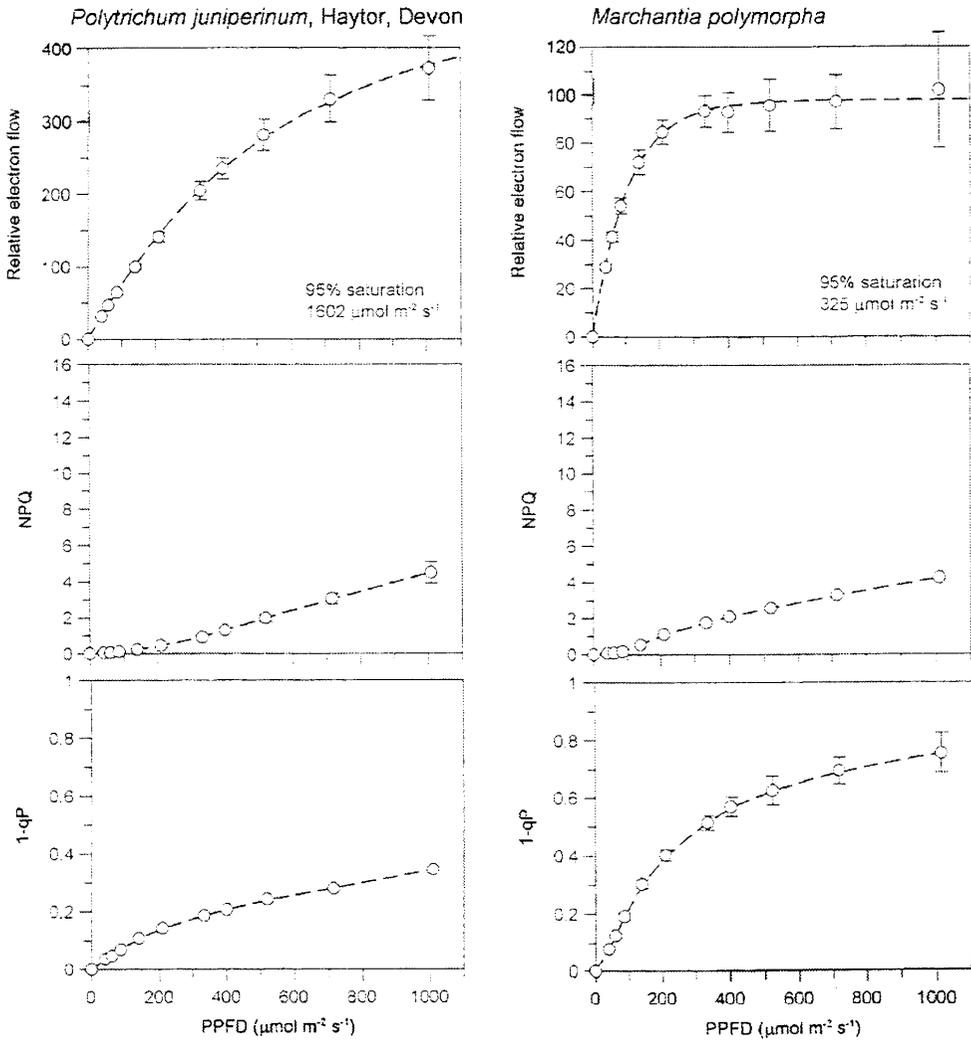
Figure 6: *PPFD response curves of relative electron flow rate (REFR), NPQ and 1–qP, from chlorophyll-fluorescence measurements for two representative species having complex ventilated photosynthetic systems.*
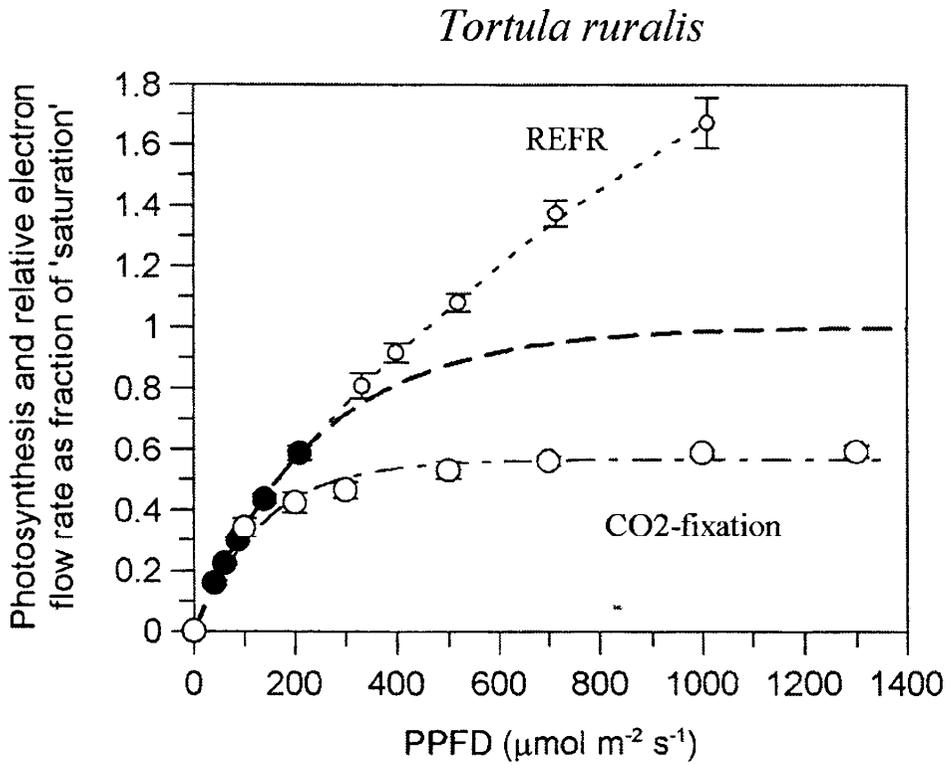
Tortula ruralis

Figure 7: *PPFD response curve of relative electron flow (REFR) and CO₂-fixation for Tortula ruralis.*
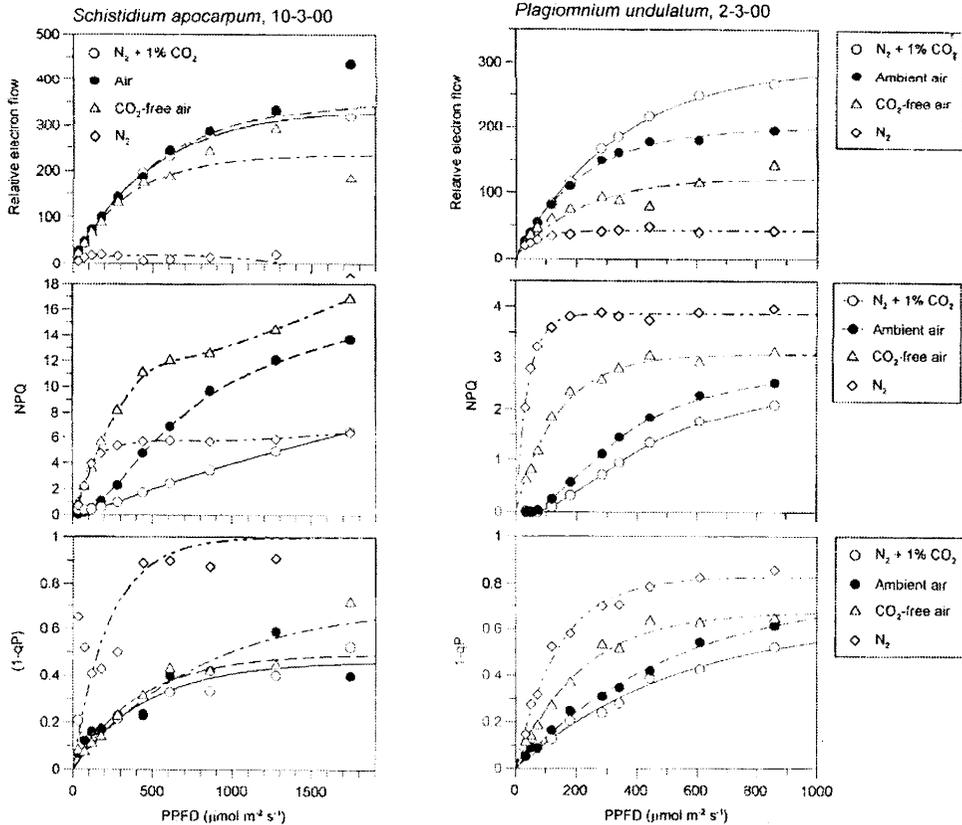
Figure 8: *PPFD response curves of relative electron flow (REFR), NPQ and 1–qP, from chlorophyll-fluorescence measurements for two contrasting species in various gas mixtures*

Acknowledgements

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