Stomatal aperture, photosynthesis and water fluxes in mesophyll cells as affected by the abscission of leaves. Simultaneous measurements of gas exchange, light scattering and chlorophyll fluorescence*

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Abstract. Carbon dioxide exchange, transpiration, chlorophyll fluorescence and light scattering of leaves of *Lycopersicon esculentum*, *Helianthus annuus* and *Arbutus unedo* were measured simultaneously before and after abscission of leaves. Scattering of a weak green measuring beam was used to monitor water fluxes across the thylakoid membranes of the mesophyll. When leaves were cut under water, stomata initially closed partially and then occasionally exhibited distinct regulatory oscillations. As stomata closed, light scattering decreased indicating water influx into the mesophyll. Stomatal oscillations were accompanied, with small but noticeable phase shifts, by oscillations of water fluxes at the thylakoid level. These fluxes could be distinguished from the water fluxes accompanying light-dependent ion pumping across the thylakoids by the concomitant chlorophyll fluorescence signals. The latter record energy-dependent ion fluxes in addition to redox changes of the electron-transport chain. As stomata closed partially after cutting a leaf under water, photosynthesis decreased. In *Arbutus unedo* and *Helianthus annuus* leaves, transient stomatal closure was insufficient to account for transient inhibition of photosynthesis which appeared to be brought about by transfer of an inhibitory solute through the petiole into the mesophyll. This solute also stimulated respiration in the dark. When leaves were cut in air, stomata opened transiently (Iwanoff effect) before wilting enforced closure. Photosynthesis followed the stomatal responses, increasing during opening and decreasing during closure.

Key words: Chlorophyll fluorescence – Light scattering – Photosynthesis – Transpiration.

* Dedicated to Professor H. Ullrich on the occasion of his 85th birthday

Introduction

Transpirational water loss of leaves is controlled by the stomata which close when water efflux cannot be balanced by water influx. The stomatal apparatus is embedded in the epidermis and stomatal aperture depends on several factors including water status and pressure relations in its surrounding tissue. During transpiration, the water columns in the water-conducting tissue elements are under tension. A water-potential gradient between mesophyll tissue and xylem elements is established which ensures water flux against flow resistances.

When the petiole of a transpiring leaf is excised under water, tension in the xylem vessels is suddenly relieved and water transport to the transpiring tissue increases due to an increased water-potential gradient. Consequently, the leaf water status improves and this, at first sight, should result in increased stomatal opening. Contrary to expectation, however, a partial closure of stomates is observed initially.

When a transpiring leaf is cut in air, tension in the xylem vessels is only transiently relieved until an increase of capillary forces prevents easy water recharge from the limited supply available in the water-conducting system. Subsequently, water loss by transpiration no longer can be replaced by water uptake. This should result in stomatal closure. However, stomata open in many cases under such circumstances and close subsequently only when water loss from the leaves has become so large that it results in wilting.

Transitory increase in transpiration due to temporary stomatal opening as a result of interrupted water supply after abscission of a leaf has long been observed (Darwin 1897; Darwin and Pertz 1911; Iwanoff 1928) and has been termed the 'Iwanoff effect' (see Ziegler 1983; Willmer 1983;
Stocker 1956). Iwanoff explained the momentary increase of transpiration after abscission by assuming that for the leaf tissue there is an improved availability of water which induces opening of stomata. This interpretation has been revised by Raschke (1970a, b) who demonstrated the phenomenon to result from an interplay of stomata with the neighbouring epidermal tissue: when leaves are cut in water, the improved water supply in the water-conducting elements results in water uptake by the epidermis. Swelling of epidermal cells then compresses the guard cells, closes the stomatal pores and thus decreases transpiration at least transiently. In contrast, cutting a leaf in air soon results in water deficiency and consequently shrinkage of epidermal cells permitting a transient expansion of the guard cells which in turn increases transpirational water loss. This was correctly explained by Darwin in 1897 when he stated with respect to the effect of cutting: “... therefore the opening of the stomata must be due to loss of turgor in the other elements of the leaf, especially of the ordinary epidermis cells.”

Stomatal aperture controls not only plant water loss but at the same time influx of carbon dioxide. We were interested in measuring simultaneously transpiration, photosynthetic CO₂ exchange, chlorophyll fluorescence and light scattering of leaves during changes in their water status produced during leaf abscission either in water or in air. The purpose of measuring fluorescence and light scattering was to obtain information on the energy state of the photosynthetic apparatus and on water fluxes in mesophyll cells while transpiration was altered.

Chlorophyll fluorescence is controlled by the redox state of the electron-transport chain (Duyens and Swers 1963), the phosphorylation state of the light-harvesting complex LHC (Bennett et al. 1980; Horton and Black 1982) and the magnitude of the transthylakoid proton gradient (Briantais et al. 1980; see also Sivak and Walker 1983) which drives phosphorylation of ADP. Fluorescence is low when the thylakoids are energized and the electron-transport chain is oxidized, and high when electron carriers are reduced and the proton gradient is small. Under high light intensities, the fluorescence yield of photosynthesizing leaves is usually low even though the electron-transport chain may be considerably reduced, because energy-dependent fluorescence quenching by large proton gradients and the associated Mg²⁺ distribution dominate the fluorescence yield (Schreiber et al. 1986).

Scattering of a low-intensity green measuring light beam by the thylakoid membranes of chloroplasts in leaves is highly sensitive to water fluxes across the thylakoids, as the water status of the thylakoid system determines the thylakoid shape which in turn determines light-scattering characteristics. This sensitivity has been used before to measure formation of the transthylakoid proton gradient in illuminated leaves (Heber 1969; Heber et al. 1978; Köster and Heber 1982). Electron transport drives proton uptake into the thylakoids. Charge compensation is maintained by a counterflow, mainly of cations (Dilley and Vernon 1965; Hind et al. 1974), and this increases the water potential inside the thylakoids as the main part of the protons taken up is consumed in protonation reactions and is therefore osmotically inactive. Water export then results in changes of the thylakoid shape which can be measured by an increase in light scattering. Similar changes should result when thylakoids lose water during excessive transpiration. Conversely, light scattering should decrease during water influx into the thylakoids.

We wished to know whether it is possible to use light scattering to gain information on the kinetics of changes in the water status of mesophyll cells during changes in transpiration and photosynthesis. We were also interested in the relationship between photosynthesis and transpiration when stomatal aperture is suddenly changed. In order to obtain rapid changes in transpiration and in water supply to the mesophyll, we cut leaves from the plant under water and in air.

Materials and methods

Potted plants of different leaf types (the mesophytic Lycopersicon esculentum and Helianthus annuus, and the Mediterranean sclerophyllous Arbustus unedo) were grown in the greenhouse or outdoors. Single leaves of intact plants were fitted into a temperature-conditioned sandwich-type cuvette and gassed with either air containing, in different experiments, between 390 and 470 ppm CO₂, CO₂-free air or nitrogen at a flow rate of 20 l·h⁻¹. Leaf temperature was recorded by an attached thermocouple, air temperature by thermistors. Water-vapor partial pressure of the incoming air stream was adjusted to desired levels by condensation of excess water at temperatures between 5 and 12°C. Transpiration of the enclosed leaf was determined through the differential signal in humidity of the gas streams entering and leaving the cuvette, measured by two capacitance humidity sensors (Coreci, Lyon, France). Exchange of CO₂ was recorded by an infrared differential gas analyzer (Binos, Leybold Heraeus, Hanau, FRG), recording the differential in CO₂ concentration across the cuvette. The leaves were illuminated with red actinic light (energyfluence rate usually 320 W·m⁻²; filters: RG 610: Schott, Mainz, FRG; K 65: Balzers, Liechtenstein; and a heat-absorption filter: Toshiba, Japan) and a very weak measuring light beam of 535 nm wavelength (about 0.08 W·m⁻²). Chlorophyll fluorescence peaking at 740 nm was measured by an UDT-photodiode protected