A Possible Evaporation Site in the Guard Cell Wall and the Influence of Leaf Structure on the Humidity Response by Stomata of Woody Plants

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Summary. Gas exchange measurements made with single leaves from Wych elm (Ulmus glabra Huds.), Lombardy poplar (Populus nigra ‘Italica’ L.) and common oak (Quercus robur L.) seedlings grown at high irradiance showed significant interspecific differences in the stomatal response to variation in atmospheric humidity. Elm and poplar seedlings showed low conductances at high vapour pressure differences (VPD), while the stomatal conductance of oak was little influenced by an increase in VPD between the leaf and the surrounding air. Mild water stress (leaf water potentials of approximately 0.4 MPa) reduced the sensitivity of stomata of elm to humidity and caused almost complete stomatal closure in poplar. Oak seedlings grown at low irradiance showed enhanced stomatal sensitivity to changes in VPD and comparatively high water use efficiencies.

Significant reductions in stomatal conductance and transpiration of elm leaves at high VPD suggest that some water loss must occur directly from the external surfaces of the guard cell complex. At high VPD, this loss may maintain the guard cells in a flaccid condition and therefore the stomatal pore will remain closed, even though the plant may be relatively turgid. Differential staining of guard cell walls in light microscope sections of elm and oak leaves suggested an area of the wall that may be permeable to water and transmission electron micrographs revealed a corresponding cuticle-free area on the inner wall of the guard cells of elm leaves. S.E.M. and T.E.M. pictures and light micrographs form the basis of a hypothesis to explain the modifying influence of mild water stress and leaf structure on the response of elm and oak stomata to humidity.

Introduction

The stomata of many plants close in response to an increase in the vapour pressure difference between the air inside and the air surrounding the leaf (VPD) (see Lösch and Tenhunen 1981). The exact nature of the relationship between stomatal conductance and VPD seems to depend upon the water status of the plant (Lange et al. 1971). This is not surprising, since the movements of the guard cells depend upon variation in the relative turgor of guard cells and subsidiary cells. Several workers have reported that increasing plant water stress can increase the sensitivity of stomata to decreasing humidity (Ludlow 1980; Osonubi and Davies 1981) but others report that decreasing water potential may reduce the stomatal sensitivity to humidity changes (Schulze and Küppers 1979; Black and Squire 1979).

A decrease in stomatal conductance in dry air is brought about by an increase in the rate of water loss from the leaf and a consequent reduction in guard cell turgor. This situation may arise following a general loss of leaf turgor (see Davies et al. 1981), but to postulate a direct linkage between the guard cells and the water status of the air one must demonstrate stomatal closure despite a constant leaf water potential and possibly even an increase in the bulk turgor of the leaf (Schulze et al. 1972). Two prerequisites for this type of response are a substantial rate of water loss directly from the epidermis of the leaf (Meidner 1975) and a significant hydraulic resistance between the epidermis and the xylem conduits in the mesophyll (Sheriff 1977). Only if these two criteria are satisfied can the low turgor of the guard cells be sustained against a relatively high turgor in the mesophyll.

Farquhar (1976) and Landsberg and Butler (1980) have discussed the influence of decreasing stomatal conductance with increasing vapour pressure deficit on the rate of water loss by the leaf: A significant reduction in conductance at high VPD can result in transpiration decreasing as the air dries. This can only occur, however, if the guard cells have a means of sensing the water status of the air from the outside of the leaf (Jarvis 1980). If the guard cells lose water in dry air through their external walls, even though the pores are closed, they may remain flaccid and therefore remain closed, even if the bulk mesophyll is relatively turgid. Seybold (1961/1962) recorded a high level of cuticular transpiration from guard cells and subsidiary cells and introduced the term peristomatal transpiration to differentiate between this water loss and water loss from the mesophyll cells of the leaf. Subsequently Maercker (1965a, b) and others (see Maier-Maercker 1979a) have provided evidence that the flux density of water loss from the guard cell complex directly to the ambient air may be appreciable and that this water loss can have substantial effects upon guard cell turgor and stomatal behaviour.

In the experiments described in this paper, we have investigated the responses of stomata of three woody plants to changes in humidity. To investigate the influence of plant water stress on these responses, stomatal behaviour was monitored in both well-watered and water-stressed plants.
Plants were grown under both high and low irradiance in an attempt to alter leaf structure and thus the nature of the hydraulic linkage between the epidermis and the mesophyll. Light and electron microscopy were used to investigate the possibility that guard cell structure has some influence on the capacity of the stomata to respond to changes in ambient humidity.

Materials and Methods

a) Gas Exchange. First year seedlings of Ulmus glabra Huds. and Quercus robur L. and rooted cuttings of Populus nigra ‘Italica’ L. were potted in 13 cm diameter pots containing John Innes No. 2 compost and grown on in a controlled environment chamber (16 h day, 21°C C day and 15°C C night, 50 ± 5% RH) for several months before experimental treatments were initiated. Plants were grown either at 100 µmol m⁻² s⁻¹ or 400 µmol m⁻² s⁻¹ (PAR) and designated either as low-irradiance or high-irradiance plants. Half of the seedlings at each irradiance level were not watered immediately before the experimental treatment, until a reduction in predawn plant water potential of 0.4 MPa was recorded with a pressure bomb. Under the growth conditions described this corresponded to approximately 0.06 mPa of soil moisture stress which was determined using a small tensiometer placed in each pot.

Gas exchange measurements were made on both well-watered and water-stressed plants using a cuvette system in which two leaf chambers were operated in parallel. Fully expanded, young leaves were sealed in water-jacketed, ‘Perspex’ leaf chambers with internal dimensions of 10 cm × 8 cm × 6 cm, and supported in a horizontal position by nylon threads. Leaf temperature was measured using 0.08 mm copper-constantan thermocouples touching the lower leaf surface and was maintained at a constant 21 ± 1°C for the whole of the experiment. All gas exchange measurements were made at 450 µmol m⁻² s⁻¹ (PAR) provided by a 400 W Thorn Kolorarc metal halide lamp filtered through a 0.5 cm thick glass screen. Air from outside the laboratory (CO₂ concentration of about 330 cm⁻³ m⁻³) was brought to the required temperature and humidity before it was pumped into the cuvettes. Humidity was controlled by mixing dry and humid air streams. These were obtained by passing air through either a silica gel column (to provide ‘dry’ air) or through warm water (to provide ‘wet’ air). The flow rate through the leaf chambers was 2.1 min⁻¹ and a 3.5 cm diameter fan in the chamber provided rapid air movement over the leaf.

Net carbon dioxide uptake by the enclosed leaves was measured using a differential infra red gas analyser (Analytical Development Co. Ltd., Hoddesdon, England). The water vapour content of the air at the chamber inlet and outlet was determined with a digital humidity analyser (Analyticon E.G. and G. Model 911). Leaf area was measured, after each experiment, with a Paton electronic planimeter (Paton Development Co. Ltd., Hoddesdon, England). The water vapour content of the air at the chamber inlet and outlet was determined with a digital humidity analyser (Analyticon E.G. and G. Model 911). Leaf area was measured, after each experiment, with a Paton electronic planimeter (Paton Development Co. Ltd., Hoddesdon, England). The water vapour content of the air at the chamber inlet and outlet was determined with a digital humidity analyser (Analyticon E.G. and G. Model 911). Leaf area was measured, after each experiment, with a Paton electronic planimeter (Paton Development Co. Ltd., Hoddesdon, England).

b) Light Microscopy. Small fragments were cut from full expanded, mature, young leaves of well-watered elm and oak seedlings. Oak seedlings grown at both high and low irradiances were sampled. Fragments were fixed in formalin-aceto-alcohol (FAA). Using the procedure described by Johansen (1940), tissue was dehydrated through a 2 methyl propan 2-ol series and embedded in paraffin wax. Cross sections of leaves (10 µm) were cut using a Spencer 20 ultra microtome and mounted on glass slides. Wax was cleared with xylene and the sections were stained using safranin and fast green (Johansen 1940). Permanent slides were made and later examined under the microscope. In addition to these prepared sections, fresh, frozen leaves were sectioned on a sledge microtome with a freezing stage. Sections were stained with Sudan IV and examined under the microscope.

c) Scanning Electron Microscopy. Fresh leaf material removed from well-watered and water-stressed elm and oak seedlings were quickly quench frozen in slushy nitrogen. Leaves of comparable maturity were selected. Fragments were mounted on aluminium stubs and inserted into the microscope (JSM 50A) where they were coated with carbon and then with gold. An accelerating voltage of 20 kV was used and the viewing angle was 25°.

d) Transmission Electron Microscopy. Using a grease-free razor blade, small (3 × 1 mm) pieces of leaf tissue, were removed from young, fully expanded Ulmus leaves under ice-cold 2.5% gluteraldehyde dissolved in a 0.025 M phosphate buffer at pH 7.0. The leaf pieces were then transferred to a vial containing 5 ml of the same fixative, buffer solution and fixed for 6 h at 0°C. The tissue was washed 3 times in a 0.025 M phosphate buffer and left overnight to post fix in 2% osmium tetroxide in phosphate buffer at 0°C. Leaf pieces were then rinsed in buffer and dehydrated into absolute alcohol through a graded ethanol series, at 0°C. Leaf tissue was left for one h at each alcohol concentration. Once in absolute alcohol, it was allowed to warm to room temperature. Absolute alcohol was changed 3 times.

Spurr’s “C” resin (Spurr 1969) was added to the vial containing the tissue in alcohol and the vial was rotated.