Stomatal frequency of *Quercus myrsinaefolia* grown under different irradiances

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Abstract

Stomatal and epidermal cell frequencies and leaf area were measured in leaves of *Quercus myrsinaefolia* grown in the field under different relative photon flux density (PFD), which was the ratio of integrated PFD at the leaf surface to that at an open site. Leaf area showed a linear relationship with the relative PFD. Stomatal and epidermal cell frequencies increased with increasing relative PFD. Numbers of stomata and epidermal cells per leaf, and stomatal index (ratio of stomatal number to epidermal cell number) increased with increasing relative PFD.

Additional key words: cell frequency; epidermis; irradiance; leaf area; photon flux density; stomatal index.

Introduction

Stomata are components of the leaf epidermis that facilitate the diffusion of gases between the leaf inside and the ambient air. Stomata have the most important control system of CO_2 influx for photosynthesis and water vapour efflux for transpiration, and optimize these two contradictory processes (*e.g.*, Farquhar and Sharkey 1982).

The objective of the present study was to determine if stomatal development is affected by PFD during growth and development. The significance of the effects on stomatal density is discussed with concurrent changes in epidermal cell density.

Materials and methods

Leaves of *Q. myrsinaefolia* Blume developed under different PFD at the National Institute for Environmental Studies were selected in the present experiment. Measurements of stomatal and epidermal cell frequencies were made on fully mature leaves, the 5th to the 10th leaf counted from the shoot apex. The study was done in winter (January to February) when leaf areal expansion had terminated.

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Diurnal changes in PFD (photon flux density) at the leaf surface and the open space were measured using quantum flux sensors (*LI-Cor*, model *LI-190SB*) connected to a data-logger (*LI-Cor*, Model *LI-1000*). The relative PFD at various PFD conditions was determined by dividing by PFD at the open space after integrating PFD from 8:00 to 16:00 h. The leaf area was measured using an area meter (*LI-COR*, model *LI-3000*).

Impressions were made on the epidermis using thin films of cellulose acetate, and the stomata and epidermal cell frequencies were determined under a light microscope. Stomatal index (SI) was estimated according to Meidner and Mansfield (1968):

$$SI = SD/(SD + ED)$$

where SD and ED are stomatal and epidermal cell densities, respectively.

Results

A linear relationship between leaf area and relative PFD was found in leaves of *Q. myrsinaefolia* (Fig. 1). Leaf area of leaves developed under 6 % relative PFD was approximately four times larger than that of leaves developed under 94 % relative PFD.

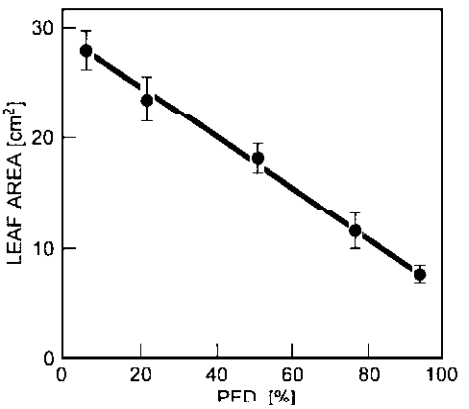

Fig. 1. Relationship between relative photon flux density (PFD) and leaf area of *Q. myrsinaefolia*. Vertical bars indicate + standard error ($n = 7$).

The stomatal frequency increased with increasing relative PFD (Fig. 2). The stomatal frequency was 1225 mm^{-2} in leaves developed under high irradiance (94 % relative PFD), and 567 mm^{-2} in leaves developed under low irradiance (6 % relative PFD). Also epidermal cell frequency was significantly higher in leaves developed under high irradiance (4370 mm^{-2}) compared to leaves developed under low irradiance (3638 mm^{-2}).

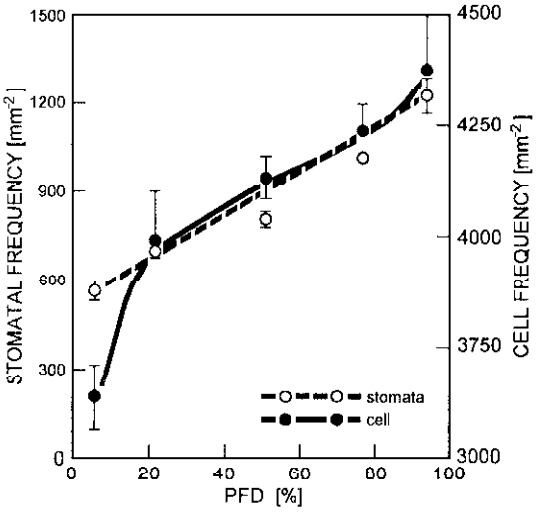

Fig. 2. Stomatal (o) and epidermal cell (●) frequencies of leaves of *Q. myrsinaefolia* developed under various relative photon flux densities (PFD). Vertical bars indicate ± standard error (n=7).

The maximum value of total stomatal number (density × leaf area) was observed in leaves developed at a relative PFD of 20 %, and was 1.64×10^6 stomata per leaf (Fig. 3). Though the number of stomata per leaf decreased with increasing relative PFD from 50 to 94 %, no significant difference in the stomatal number was detected in leaves developed below 50 % relative PFD. The total number of epidermal cells

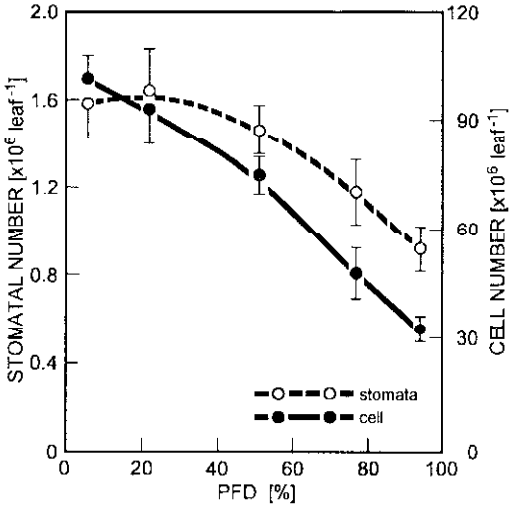

Fig. 3. Total numbers of stomata (o) and epidermal cells (●) per leaf of *Q. myrsinaefolia* developed under various relative photon flux densities (PFD). Total numbers of stomata and cells were obtained by multiplying the leaf area by stomatal and cell frequencies, respectively. Vertical bars indicate ± standard error (n=7).

per leaf decreased linearly with increasing PFD. Leaves developed under 6 % relative PFD had more than three times as many epidermal cells per leaf as leaves developed in 94 % relative PFD.

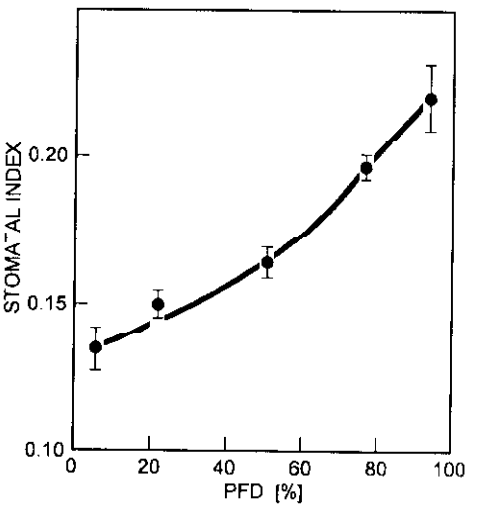

Fig. 4. Ratio of stomatal number to epidermal cell number (stomatal index) of *Q. myrsinaefolia* developed under various relative photon flux densities (PFD). Vertical bars indicate + standard error ($n=7$).

The stomatal index increased with increasing relative PFD (Fig. 4): in leaves developed under 94 % relative PFD it was 1.6 times that of leaves developed under 6 % relative PFD.

Discussion

Q. myrsinaefolia is hypostomatous, having stomata only on the abaxial surface. The stomatal density increases during the expansion of the leaf after the initiation of stomata. With increasing leaf area, the stomatal density decreases gradually, and stabilizes when the leaf expansion terminates. In contrast to the stomatal density, the total number of stomata per leaf reaches its maximum in the first days after leaf unfolding, and attains a relatively constant level (Gay and Hurd 1975, Furukawa 1992), indicating that stomata are initiated at an early stage of leaf development (Tichá 1982).

The stomatal density and stomatal index of various plant species are sensitive to changes in environmental factors, such as CO_2 concentration (Woodward 1987, Woodward and Bazzaz 1988, Ceulemans *et al.* 1995), humidity (Rawson *et al.* 1980), and irradiance (Gay and Hurd 1975, Wild and Wolf 1980, Liang *et al.* 1995), although there are some conflicting reports indicating that there are no consistent effects of these environmental factors on stomatal density.

The effect of PFD on stomatal density is consistent in many plant species, and stomatal density increases with increasing PFD. However, Mott and Michaelson (1991) reported that the adaxial stomatal density of *Ambrosia cordifolia* (*Compositae*) decreased, while the abaxial density increased under high PFD. According to Mott *et al.* (1982), the stomatal distribution of this species changes from hypostomatous in shaded conditions to amphistomatous in sunny conditions. Thus this species has an unusual stomatal adaptation to PFD.

The stomatal index, but not stomatal density or total stomatal number, is considered to be influenced by humidity rather than radiation (Tichá 1982). However, in my experiments the SI of *Q. myrsinaefolia* was higher in leaves developed under high irradiance than under low irradiance. Thus environmental factors, in particular irradiance, drought and CO_2, might have some influence on the initiation of stomata, though the mechanism of the regulation of stomatal differentiation by these environmental factors is not clear.

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