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REVIEW

Stomatal patchiness

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Abstract

Different behaviour of small groups of stomata on a single leaf blade (stomatal patchiness) is reviewed. The occurrence of stomatal patchiness depends on plant species, age, leaf position, environmental conditions, *etc.* The possibility of errors in conventional evaluation of stomatal and non-stomatal (biochemical) limitations of photosynthesis resulting from patchy stomatal closure is analysed. The consequences of stomatal patchiness for leaf and plant photosynthesis and water economy are discussed. A brief survey of the techniques currently used for detection and quantification of stomatal patchiness is presented.

Introduction

Environmental stresses can decrease photosynthesis either by a direct effect on photosynthetic capacity of the mesophyll or by CO_2 limitation resulting from the stomatal closure. Several mathematical models have been developed (*e.g.* Jones 1973, 1985, Cornic *et al.* 1983, Prioul *et al.* 1984, Peisker and Václavík 1987, Assmann 1988, Atkinson *et al.* 1989) for calculating the magnitude of non-stomatal limitation relative to stomatal limitation of photosynthetic rate. The models have mostly been based on combined analyses of two functions: (*1*) demand function [the response of net photosynthetic rate (P_N) to leaf internal CO_2 concentration (c_i)] where the effect of stomata on photosynthetic CO_2 fixation is factored out; and (*2*) supply function [relating the air-leaf CO_2 concentration difference (c_a - c_i) to P_N] predominantly expressing the influence of stomata (see Fig. 1). More detailed evaluation of limiting factors in the biochemistry of CO_2 fixation *in vivo* derived

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from parameters of the $P_N(c_i)$ curve, is found in the model of Farquhar and Von Caemmerer (1982).

The parameters of demand and supply functions are frequently used for evaluation of stomatal and biochemical components in the inhibition of photosynthesis caused by many different factors such as drought, salinity, air humidity, irradiance, temperature, CO_2 concentration, pollution, *etc.* (Table 1). However, it now seems that

Table 1. Examples of determination of stomatal and non-stomatal limitations of photosynthesis.

Author and year of publication	Plant species	Factors affecting photosynthesis
Antolín and Sánchez-Díaz 1993	*Medicago sativa*	water stress
Assmann 1988	*Phragmipedium longifolium*	light quality
Atkinson *et al.* 1989	*Commelina communis*	calcium
Bunce 1988	*Lycopersicon esculentum*	air humidity
Cornic *et al.* 1987	*Phaseolus vulgaris*	water stress
Dingkuhn *et al.* 1989	*Oryza sativa*	water stress
Drew *et al.* 1990	*Cucumis sativus*	salinity
Dreyer *et al* 1991	*Quercus robur, Q. rubra, Q. petraea, Q. palustris*	waterlogging
Epron and Dreyer 1990	*Quercus ilex, Q. petraea Q. pubescens*	water stress
Ghashghaie and Saugier 1989	*Festuca arundinacea*	water stress, nitrogen
Grantz 1989	*Saccharum spp.*	low temperature
Grantz and Assmann 1991	*Saccharum spp., Glycine max*	blue light pulse
Grieu *et al.* 1988	*Pseudotsuga menziesii, P. macrocarpa, Cedrus atlantica*	air humidity
Guehl and Aussenac 1987	*Abies alba*	air humidity
Guehl *et al.* 1989	*Abies alba, A. normanniana, A. macrocarpa, A. cephalonica*	air humidity
Guehl and Garbaye 1990	*Pseudotsuga menziesii*	mycorrhizas
Kicheva *et al.* 1994	*Triticum aestivum*	water stress
Krieg and Hutmacher 1986	*Sorghum bicolor*	age, water stress, irradiance
Kropff 1987	*Vicia faba*	SO_2
Kubiske and Abrams 1992	*Quercus rubra*	water stress
Kuroda and Kumura 1990	*Oryza sativa*	air humidity, daily course
Lopez *et al.* 1987	*Cajanus cajan, Vigna unguiculata*	water stress, CO_2, irradiance
Martin and Ruiz-Torres 1992	*Triticum aestivum*	water stress
Ni and Pallardy 1992	*Quercus alba, Q. stellata, Acer saccharum, Juglans nigra*	water stress, CO_2
Nicolodi *et al.* 1988	*Medicago sativa*	water stress
Pezeshki *et al.* 1987	*Sagittaria lancifolia*	waterlogging
Reich *et al.* 1989	*Ulmus americana*	water stress, nitrogen
Ryan *et al.* 1987	*Triticum aestivum*	aphid infestation
Seiler and Cazell 1990	*Picea rubens*	water stress
Soldatini *et al.* 1989	*Helianthus annuus*	salinity, PEG
Tinoco-Ojanguren and Pearcy 1993	*Piper auritum, P. aequale*	irradiance
Van Rensburg and Krüger 1993	*Nicotiana tabacum*	water stress
Wallin *et al.* 1992	*Picea abies*	ozone

the conclusions based on analysis of $P_N(c_i)$ curves may in some cases be misleading due to an error in the calculation of c_i. The error arises from omitting variability in

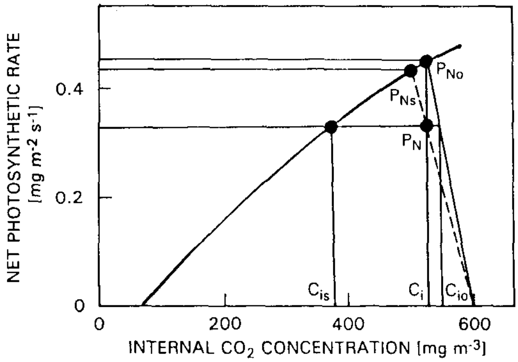

Fig. 1. Examples of calculations of the non-stomatal (S_n) and stomatal ($100 - S_n$) components in the decline of photosynthetic rate after treatment ⟨P_N; c_i⟩ according to Cornic *et al.* 1983 (*A*), and Peisker and Václavík 1987 (*B*). The curve is derived from the relationship between photosynthetic rate and internal CO_2 concentration in untreated leaves.

(*A*) $S_n = \dfrac{P_{Ns} - P_N}{P_{N0} - P_N} \times 100$ (*B*) $S_n = \dfrac{c_i - c_{is}}{c_{i0} - c_{is}} \times 100$

stomatal aperture and complexity in mesophyll anatomy. Conventional calculation of c_i includes an implicit assumption of uniform stomatal conductance (g_s) and uniform photosynthetic capacity of mesophyll cells over the investigated leaf surface. Consequently, invariable c_i should dominate the mesophyll air space in the measured part of the leaf. In reality, (*1*) densely packed palisade cells or bundle sheath extensions can represent more or less impermeable bariers for free lateral diffusion of CO_2. In extreme cases, bundle sheath extensions can extend up and down to both epidermes dividing the leaf mesophyll into separate hermetically sealed units called areoles (Sharkey 1985). This leaf type, more abundant in sun-adapted plant species, is refered to as heterobaric. (*2*) Stomatal distribution and/or mean stomatal conductance can vary significantly among adjacent microareas often corresponding to the surface of areoles. Such non-random, spatially arranged variation in g_s (and in aperture dimensions) is called "stomatal patchiness". As a consequence of (*1*) and (*2*), c_i beneath the epidermis of the individual areole will vary in proportion to g_s, even if the photosynthetic capacity of the mesophyll does not differ among areoles. Then the effective c_i can significantly vary from c_i calculated conventionally using the surface-averaged value of g_s for CO_2. Details of effective c_i calculation are given in Appendix 1. In contrast to stomatal patchiness as defined above, stoma-to-stoma variability also exists spread "randomly" over the leaf surface. Another type represents a mild gradient in g_s in one or two dimensions across the leaf lamina. The effect of both non-patchy types on c_i depends on lateral diffusion. Lateral diffusion exchange of CO_2 between two (*e.g.* 2 mm distant) points in the mesophyll is likely to be very small even in homobaric leaves (Farquhar 1989). Random variability over a microarea is not expected to cause "patchy" problems with c_i calculation, but a mild steady gradient can be a potential source of errors in c_i.

The above ideas were published first by Laisk *et al.* (1980, 1983) and stimulated the present interest in stomatal patchiness. As was pointed out, *e.g.*, by Terashima (1988), Farquhar (1989) and Chaves (1991) and is implied from Appendix 1, c_i can be overestimated if variability in g_s is omitted. Lower P_N at higher c_i implies an erroneous conclusion about biochemical limitation of photosynthesis. Nevertheless, a broad spectrum of information indicates that mesophyll photosynthesis can be affected by environmental factors (*e.g.* radiation quality, severe water stress) independently of the influence of these factors on leaf conductance (Lösch 1991).

The questions to be answered are: (*1*) What is the effect of stomatal patchiness on c_i in leaves of different anatomy (amphistomatous *vs.* hypostomatous; heterobaric *vs.* homobaric)? How often does stomatal patchiness occur under field or growth chamber conditions? (*2*) What are the consequences of stomatal patchiness in terms of the carbon or water economy of the leaf? With the aim of (at least partially) answering these questions, the main items of this review are the occurrence of stomatal patchiness, its consequences for evaluation of stomatal limitation of photosynthesis, and the causes and consequences of patchy stomatal response in plant performance. A brief survey of techniques for detection is added.

The random variability of stomatal aperture and mild systematic gradient of stomatal conductance across the leaf

Heterogeneity in the anatomy of a leaf blade was observed many years ago (for review see, *e.g.*, Tichá 1982, 1985, Weyers and Meidner 1990, Croxdale *et al.* 1992). Stomatal density usually declines from the leaf tip to its base, although opposite gradients have also been found. Differences in stomatal density on the edge and in the centre of the blade also exist, but are usually not significant. The differences in stomatal density may be analogous on abaxial and adaxial leaf surfaces though different trends have been described. The patterns in stomatal density on the leaf blade differ for leaves of different insertion levels and for leaves from individual crown layers of trees. The gradients of stomatal density on a leaf blade can be modified by various environmental factors during growth. For example elevated ambient CO_2 increased the number of stomata per unit of leaf area in rice (Rowland-Bamford *et al.* 1990). Changes in stomatal length on the leaf blade are usually opposite to changes in stomatal density and thus the actual area of the stomatal pores may be similar throughout the blade due to compensation of the smaller number of stomata by their larger size.

The heterogeneity in stomatal conductance over the leaf surface has been rarely studied until recently (for reviews see, *e.g.*, Solárová and Pospíšilová 1983, Čatský *et al.* 1985). The lowest g_s was observed at the leaf base and the highest at the tip of *Sorghum* (Kanemasu *et al.* 1973/74) and *Elaeis* (Zobel and Liu 1980) leaves. When stomata in the middle of the lamina of *Commelina* leaves were open, those near the leaf margins were rather closed and the gradient of stomatal aperture was as high as

1 µm mm^{-1} (Smith *et al.* 1989 - Fig. 2). A lateral and longitudinal gradient in stomatal sensitivity to air humidity or osmotic potential of the root medium was found in the leaf blade of *Commelina* (Maier-Maercker 1979, 1981 - Fig. 3). The response significantly increased from the centre towards the leaf margins. In contrary, width, area and length of stomatal apertures did not differ between the leaf tip, middle and base of *Chenopodium* leaves (Fig. 4 - Šantrůček *et al.* - unpublished).

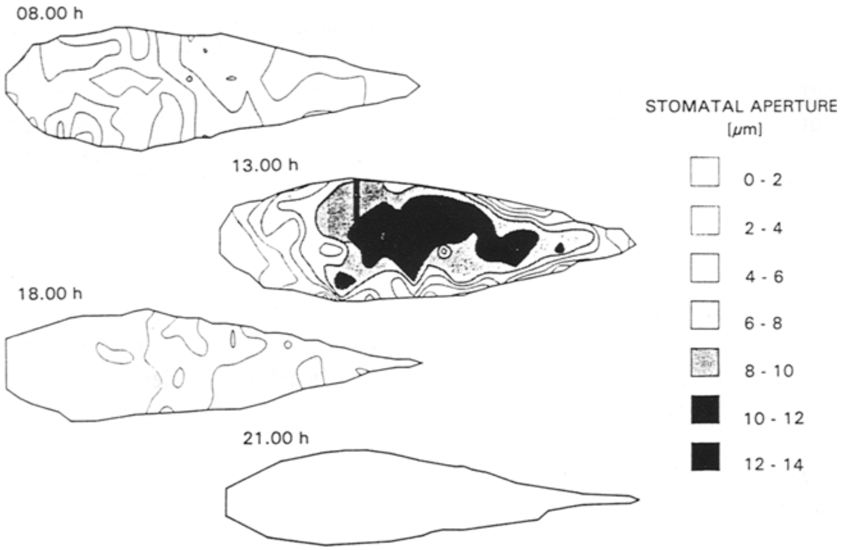

Fig. 2. Iso-aperture charts for stomata on the lower epidermis of *Commelina communis* sampled at times indicated. The key gives the shading used for the areas between the iso-aperture lines. (According to Smith *et al.* 1989.)

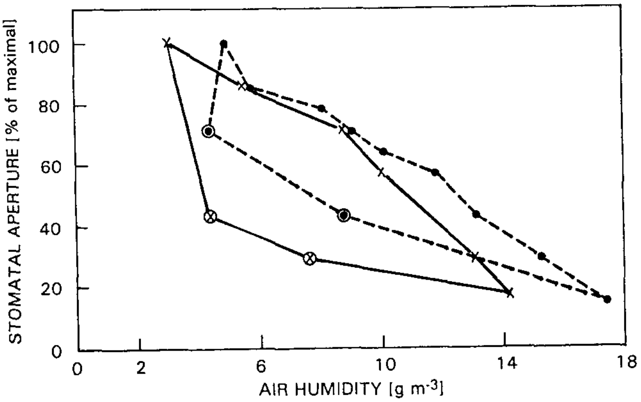

Fig. 3. Response of two stomata (*full and dashed line*) from different places in leaf blade of *Commelina communis* to descending and ascending air humidity. The *dashed line* represent a stoma in close vicinity to a vein. (Adapted from Maier-Maercker 1979.)

The aperture of stomata, distributed even in a small portion of the epidermis of *Oryza sativa*, showed much variation from each other (*e.g.* the range of pore width was from 0.5 to 1.2 µm), but these variations did not depend on the position in which the stomata were situated along the large or small veins, and near the edge or the midrib of the leaf (Ishihara *et al.* 1971). Random variability in stomatal apertures over the microarea of the homobaric tobacco leaf is demonstrated in Fig. 5.

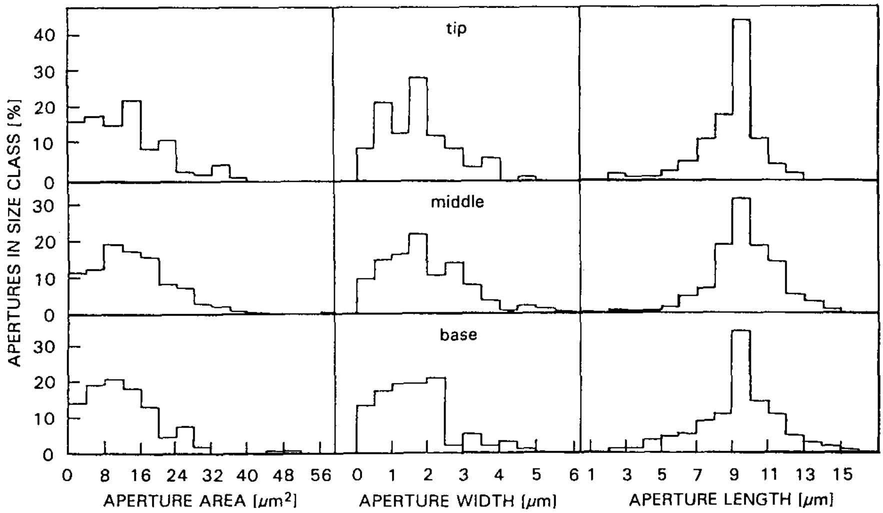

Fig. 4. Frequency histogram of area, width and length of stomatal apertures at the tip, middle and base of *Chenopodium album* leaf. (Šantrůček *et al.* - unpublished.)

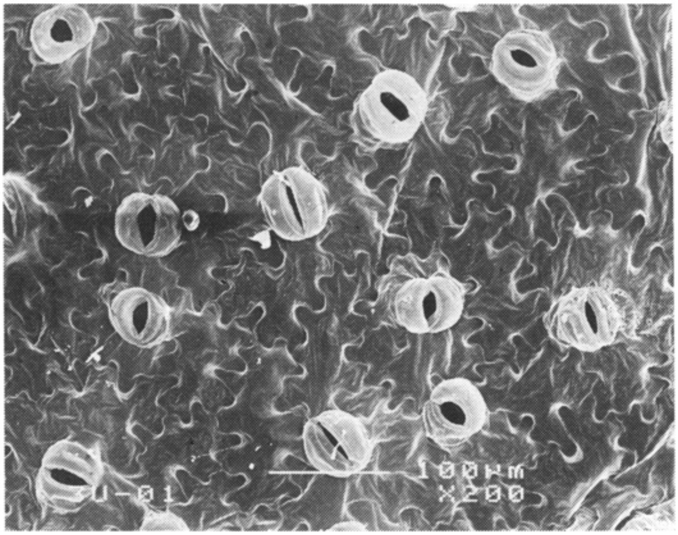

Fig. 5. Spatial variation in stomatal apertures over a microarea (about 0.3 × 0.5 mm) of a tobacco (*Nicotina tabacum*) leaf. (Šantrůček and Šimková- unpublished.)

Laisk *et al.*(1980) found symmetric statistical distribution of stomatal width in *Vicia faba* but asymmetric and variable shape of distribution during stomata opening and closure in *Hordeum vulgare*. The within-sample variance in stomatal aperture was reviewed by Spence (1987). He showed that the magnitude of variance depends on the plant species, but stomata of different leaves or plants of the same species respond with approximately equal variability to a given treatment. Therefore a stoma seems to react as an individual to environmental stimuli but a population of stomata keeps a species-specific response.

Occurrence of stomatal patchiness

Stomatal patchiness - non-uniform distribution of g_S and distinctive behaviour of small groups of stomata on the leaf blade - can be visualized as a non-random variability of stomatal apertures, arranged in clusters (Table 2). It has been observed on stomatal impressions (Farquhar *et al.* 1987, Terashima *et al.* 1988, Ward and Drake 1988, Smith *et al.* 1989) or *in situ* by an inverted microscope with video camera (Kappen *et al.* 1987), by scanning electron microscopy (SEM) with digital image analysis (Van Gardingen *et al.* 1989 - Fig. 6) and by a remote-control light microscope system (Omasa *et al.* 1985). It has been demonstrated by pressure infiltration techniques (Beyschlag and Pfanz 1990, Beyschlag *et al.* 1992, 1994) and

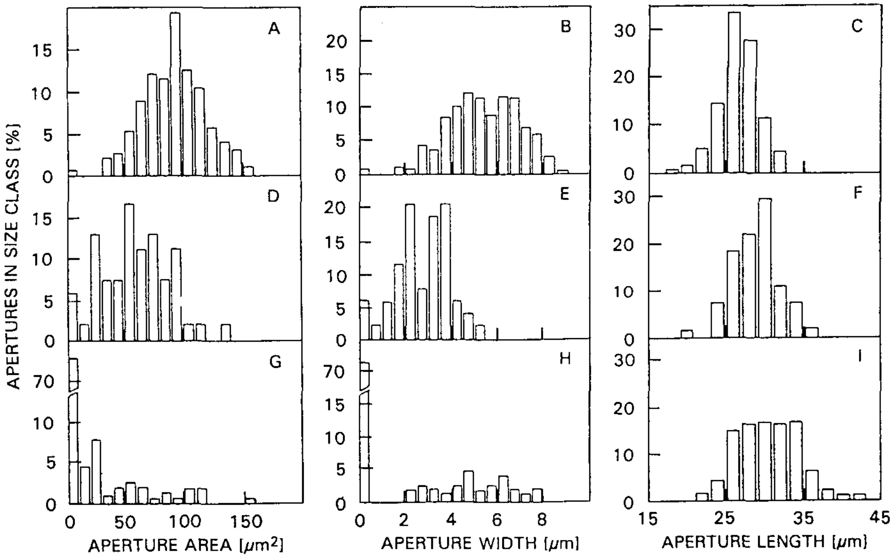

Fig. 6. Relative size class frequencies for aperture dimensions of *Avena fatua* stomata. The sizes of individual stomata were obtained by image analysis of electron micrographs. The relative size class frequences for area, width and length of aperture are shown for a range of leaf conductances (*A*, *B*, *C* - 712, *D*, *E*, *F* - 487, and *G*, *H*, *I* - 159 mmol m^{-2} s^{-1}). (According to Van Gardingen *et al.* 1989.)

Table 2. Survey of papers dealing with occurrence of stomatal patchiness. (ABA - abscisic acid; c_i - internal CO_2 concentration; g_s - stomatal conductance; P_N - net photosynthetic rate)

Author and year of publication	Plant species	Growing conditions	Measurement techniques	The most important results
Beyschlag and Pfanz 1990	*Arbutus unedo*	greenhouse, growth chamber	pressure infiltration	temporary occurence of stomatal patchiness during the phase of stomatal closure in the late morning and during stomatal reopening in the afternoon
Beyschlag *et al.* 1992	*Arbutus unedo* *Quercus ruber* *Q. coccifera*	greenhouse, growth chamber	pressure infiltration	temporary occurrence of stomatal patchiness during the phase of stomatal closure in the late morning and during the stomatal reopening in the afternoon
Beyschlag *et al.* 1994	*Abies alba* *Picea abies*	greenhouse	gas exchange, pressure infiltration	A patchy distribution of stomatal aperture would lower the risk of photoinhibition. By periodically exchanging the open and the closed areas the plant may be able to protect most of its leaf area from becoming severely photoinhibited
Brugnoli and Björkman 1992	*Gossypium hirsutum*	growth chamber	gas exchange, fluorescence	the relation between the ratio of external and internal CO_2 partial pressure and carbon isotope ratio indicates that the effect of patchy stomatal closure, if present, is not large
Bunce 1993	*Amaranthus hypochondriacus* *Glycine max* *Helianthus annuus* *Dactylis glomerata*	growth chamber, field	gas exchange	in some plant species the occurrence of stomatal patchiness is dependent on external CO_2 concentration
Cornic *et al.* 1989	*Phaseolus vulgaris* *Elatostema repens*	growth chamber	gas exchange, O_2-evolution at high CO_2	evidence of stomatal patchiness induced by water stress: significant drop in CO_2 uptake without response in c_i; lack of O_2 evolution and quantum yield response under high CO_2

Dai *et al.* 1992	*Ricinus communis* *Nicotiana tabacum* *Zea mays*	growth chamber	gas exchange	with decreasing air humidity c_i first decreased but then increased which is in agreement with the predicted behaviour when non-uniform stomatal closure is considered
Daley *et al.* 1989	*Xanthium strumarium*	greenhouse	fluorescence imaging, gas exchange	ABA induced drop in chlorophyll fluorescence over microareas coinciding with areoles when the leaf was in 2 % O_2; in the same time, proportional depression of P_N and g_s was observed
Downton *et al.* 1988a	*Vitis vinifera* *Nerium oleander* *Eucalyptus ficifolia*	greenhouse	gas exchange, fluorescence, autoradiograms	autoradiograms showed non-uniform gas exchange in water-stressed leaves, this can be accounted for the apparent non-stomatal inhibition of photosynthesis
Downton *et al.* 1988b	*Vitis vinifera* *Helianthus annuus*	greenhouse	gas exchange, PAM fluorimeter, autoradiograms	disagreements in c_i calculated from gas exchange measurements and from non-photochemical quenching, spatial variation of g_s visualized by autoradiograms
Downton *et al.* 1990	*Vitis vinifera*	greenhouse	autoradiograms, gas exchange	decline of the slope of $P_N(c_i)$ curves during severe salt stress was associated with patchiness in $^{14}CO_2$ uptake
Düring 1992	*Vitis vinifera*	greenhouse	pressure infiltration, gas exchange	lowering air humidity induced non-uniform stomatal closure in heterobaric grapevine leaves
Epron and Dreyer 1990	*Quercus petraea* *Q. ilex* *Q. pubescens*	greenhouse	gas exchange, chlorophyll fluorescence	mesophyll limitation of photosynthesis during drought determined in gas exchange measurements was not confirmed by fluorescence data; the explanation may be occurrence of stomatal patchiness
Epron and Dreyer 1993	*Quercus petraea*	greenhouse	gas exchange, chlorophyll fluorescence, autoradiograms	the use of calculated c_i to support the hypothesis of non-stomatal limitation to P_N may be misleading because of stomatal patchiness and/or changes in mesophyll conductance to CO_2 transfer

(continued)

Table 2 (*continued*)

Farquhar *et al.* 1987	*Helianthus annuus*	greenhouse	gas exchange, stomata impressions	when abscisic acid is injected into petioles of intact sunflower leaves, the stomata on the adaxial surface are completely closed, while those of the abaxial surface are closed only in certain patches
Flanagan and Jefferies 1989b	*Plantago maritima*	growth chamber	gas exchange, O_2-evolution at high CO_2	heterogeneity in stomatal closure caused by increasing salinity
Gunasekera and Berkowitz 1992	*Phaseolus vulgaris* *Triticum aestivum* *Spinacia oleracea*	growth chamber	gas exchange, autoradiograms	plant water deficit that caused leaf water potential depression to -1.1 MPa during a 4-d period resulted in heterogenous CO_2 assimilation patterns in *Phaseolus*; when the same stress was imposed more gradually (17 d) no patchy stomatal closure was evident; water stress in *Spinacia* and *Triticum* plants did not cause patchy stomatal closure
Kappen *et al.* 1987	*Vicia faba*	growth chamber	gas exchange, microscope connected to videocamera	in the light, parallel response of stomata to changes in air humidity although the change in individual pore width was different; no changes in the dark
Laisk *et al.* 1980	*Hordeum vulgare* *Vicia faba*	growth chamber	optical microscope	statistical distribution of stomatal width in *Vicia* was symmetrical, however distribution function in *Hordeum* was variable during stomatal opening and closure
Lloyd 1991	*Macadamia integrifolia*	nursery	gas exchange, mathematical model	relationship between P_N and c_i was strongly dependent upon the means by which variation in c_i was achieved; these differences arose as a consequence of non-uniform stomatal closure
Loreto and Sharkey 1990	*Olea europea*	greenhouse	gas exchange, autoradiograms	in mature leaves under decreasing air humidity ^{14}C labelling occurred uniformly, in young leaves patches of reduced CO_2 fixation occurred

Matthews and Omasa 1992	*Helianthus annuus*	greenhouse	fluorescence	for well-watered leaves fluorescence was spatially uniform; when low water potential was rapidly imposed (by excising of shoot or leaf) stomatal patchiness was observed; in contrast when low water potential was imposed by slow drying, patches were not observed
Mott and Parkhurst 1991	*Vicia faba* *Phaseolus vulgaris* *Glycine max*	growth chamber	gas exchange in air or helox	stomatal responses to air humidity may involve two separate but interdependent processes; the first is a simple hydraulic effect, when guard cell turgor is reduced by increasing transpiration; the second process occurs at low air humidity when high transpiration rate produces local water deficits and induces heterogeneous stomatal closure
Mott *et al.* 1993	*Xanthium strumarium*	growth chamber	fluorescence imaging, gas exchange	lowering of air humidity induced different patterns of patchy stomatal closure on adaxial and abaxial leaf surfaces which suggested that patchy stomatal closure did not result from a general mesophyll signal
Ni and Pallardy 1992	*Quercus alba* *Q. stellata* *Acer saccharum* *Juglans nigra*	greenhouse	gas exchange, autoradiograms	exposure of the leaves to $^{14}CO_2$ indicated apparent asymmetric stomatal closure for mildly water-stressed seedlings, but not for leaves of well-watered, severely stressed or rehydrated plants
Raschke *et al.* 1990	*Xanthium strumarium* *Zea mays* *Arbutus unedo*	greenhouse	fluorescence imaging, gas exchange	besides spatial non-uniformity (patchiness) also temporal stomatal oscillations were observed
Robinson *et al.* 1988	*Prunus armeniaca* *Helianthus annuus* *Spinacia oleracea*	greenhouse	CO_2 exchange, oxygen evolution, fluorescence	photosynthetic capacity of leaves under CO_2 saturation was inhibited very little by ABA; the decrease in quantum yield, initial slope and plateau of the curve expressing dependence of P_N on c_i can be explained by areas of complete stomatal closure

(continued)

Table 2 (*continued*)

Scheuerman *et al.* 1991	*Helianthus annuus* *Phaseolus vulgaris* *Zea mays*	growth chamber	autoradiograms	water stress resulted in occurrence of stomatal patchiness in sunflower and maize but not in bean; the occurrence of patchiness corresponded to enhanced CO_2 recycling in photorespiration as a preferable mechanism for dissipation of excess light energy
Terashima *et al.* 1988	*Helianthus annuus* *Vicia faba*	greenhouse	CO_2 exchange, oxygen evolution, starch distribution, stomatal impressions	apparent non-stomatal inhibition of P_N by ABA is an artifact which can be attributed to the non-uniform distribution of transpiration and photosynthesis over the leaf; stomatal patchiness was more expressed in *H. annuus* than in *V. faba* leaves
Van Gardingen *et al.* 1989	*Avena fatua*	nature	diffusion porometer, scanning electron microscopy	high variability of stomatal aperture, higher variability in pore area and width than in pore length; this variability is dependent on g_s
Ward and Drake 1988	*Glycine max*	growth chamber	gas exchange, autoradiograms	synchronous and equal relative stimulation of P_N and g_s in ABA-treated leaves by addition of osmoticum showed that apparent inhibition of mesophyll photosynthesis by ABA was an artifact due to heterogeneous closure of stomata, however, stomatal patchiness was not proved by microscopic examinations
Wise *et al.* 1991	*Helianthus annuus*	field	gas exchange, autoradiograms	although leaf water potential decreased from -0.5 to -1.1 MPa between pre-dawn and mid-afternoon, autoradiograms showed no evidence for inhomogeneities in stomatal aperture at any time of the day; patchiness could be induced only by severing tap root allowing the shoot to dry rapidly
Wise *et al.* 1992	*Gossypium hirsutum*	field, growth chamber	autoradiograms	Acclimation to drought caused decrease in leaf water potential at which stomatal patchiness occurred; at extremely low water potential (-2.61 MPa) patchiness persisted even under high CO_2

autoradiography (Downton *et al.* 1988a,b, Loreto and Sharkey 1990, Wise *et al.* 1991, Gunasekera and Berkowitz 1992, Ni and Pallardy 1992). It has been indirectly shown by the patterns of starch accumulation (Terashima *et al.* 1988 - Fig. 7) and by comparison of gas exchange measurements with measurements of chlorophyll fluorescence (Downton *et al.* 1988a,b, Robinson *et al.* 1988, Epron and Dreyer 1990, Dai *et al.* 1992, Matthews and Omasa 1992), with O_2 evolution (Robinson *et al.* 1988), or by comparison of gas exchange in air and helox (Mott and Parkhurst 1991).

Fig. 7. Distribution of open stomata (*closed circles*) on the abaxial surface of *Helianthus annuus* leaves in the presence of 30 (*A*) or 10 (*B*) µM abscisic acid in the transpiration stream. Irradiance 800 µmol m^{-2} s^{-1}. Hatched parts correspond to the bundle sheath extensions compartments which show the starch formation. (According to Terashima *et al.* 1988.)

Patchy stomatal behaviour depends on the plant species, and for example Gunasekera and Berkowitz (1992) found under the same conditions stomatal patchiness in *Phaseolus vulgaris* but not in *Spinacia oleracea* and *Triticum aestivum.* Stomatal patchiness was not found in some dicotyledonous plants with homobaric leaves (*e.g. Cucumis sativus* - Drew *et al.* 1990), and especially in grasses (*e.g. Triticum aestivum* - Martin and Ruiz-Torres 1992, Gunasekera and Berkowitz 1992, André and Du Cloux 1993, *Oryza sativa and Panicum maximum* - Kawamitsu *et al.* 1993). However, due to the apparent constancy in substomatal CO_2 concentration and variation in chloroplast CO_2 during water stress, the occurrence of stomatal patchiness remains open also for wheat (Renou *et al.* 1990). In *Vicia faba* leaves, which lack bundle sheath extensions, the distribution of starch accumulation (non-homogeneity of which indicated stomatal patchiness) was more homogeneous than in *Helianthus annuus* (Terashima *et al.* 1988). Usually stomatal patchiness is less frequent in homobaric leaves (typically in shade-adapted species) than in heterobaric leaves (sun-adapted). In contrast, stomatal patchiness occurred on abaxial but not adaxial surfaces of *Helianthus annuus* leaves when ABA was injected into petioles (Farquhar *et al.* 1987). Fall and Monson (1992) deduced a differential ABA effect on induction of patchiness on adaxial and abaxial sides from isoprene emission from cottonwood leaves. Loreto and Sharkey (1990) observed patchy patterns in young leaves but not in mature leaves of *Olea europea.*

Stomatal patchiness did not occur continuously, but only temporarily, *e.g.*, in *Arbutus unedo, Quercus coccifera* and *Q. suber* during the phase of stomatal closure in the late morning and during reopening of the stomata in the afternoon (Beyschlag and Pfanz 1990, Beyschlag *et al.* 1992). Frequent periodic changes in stomatal patchiness were also reported. Delayed luminiscence image analysis revealed that the spatial distribution of photosynthetic activity was not fixed in time but oscillated (Rand and Ellenson 1986) due to wave-mode fluctuations of stomatal aperture. Oscillations of stomata in groups have also been reported by Raschke *et al.* (1990).

The occurrence of stomatal patchiness was also dependent on the type of environmental factor and on the way it acted. In *Phaseolus vulgaris* water stress that caused leaf water potential depression to -1.1 MPa during a 4-d period resulted in stomatal patchiness, however, when the same level of stress was imposed more gradually no patchy stomatal closure was evident (Gunasekera and Berkowitz 1992). Similar results were observed in *Helianthus annuus* affected by rapidly and slowly imposed water stress (Matthews and Omasa 1992). In the same plant species stomatal patchiness was not observed during temporary diurnal water stress when leaf water potential decreased from -0.5 to -1.1 MPa but it occurred when roots were severed (Wise *et al.* 1991). Terashima *et al.* (1988) showed that exogeneously applied ABA induced patchiness in *Helianthus annuus.* However, it remains to be solved whether endogeneous ABA can triger the "patchy" responses to water potential depressions mentioned above. In *Quercus alba, Q. stellata, Acer saccharum* and *Juglans nigra* stomatal patchiness was observed in leaves of mildly water-stressed seedlings, but not in leaves of well-watered, severely-stressed and rehydrated plants (Ni and Pallardy 1992). The leaf water potential at which patchiness occurs probably decreases during dry acclimation, and in field grown fully acclimated plants only uprooting or similar extreme treatment can induce patchiness (Wise *et al.* 1992). However, in the same experiments stomatal patchiness persisted even under 3000 μmol (CO_2) mol^{-1} at extremely low leaf water potential (-2.61 MPa). Thus patchiness in biochemistry of photosynthetic CO_2 fixation can be a potential source of apparent stomatal patchiness under severe drought stress. Taking into account the above finding, stomatal patchiness seems to be a transient response to progressive development of severe water stress in plants which experience the first-ever water stress (*i.e.* in plants non-acclimated to drought). We concluded from experiments with differentially acclimated homobaric leaves of tobacco that heterogeneous stomatal response occurred transiently during the acclimation process. Apparent 'patchy' response was accompanied by establishment of a coupling between photosynthetic CO_2 fixation and stomata (Šantrůček - unpublished).

The occurrence of stomatal patchiness in *Glycine* and *Helianthus* during stomatal closure induced by low air humidity was dependent on external CO_2 concentration (Bunce 1993). We experienced from our experiments with *Chenopodium* plants grown at 75 Pa CO_2 that the irregularities of the CO_2-response curve of photosynthesis (indicating occurrence of patchiness) were more frequent than in plants grown at 35 Pa CO_2 (Šantrůček and Sage - unpublished results). Stomata in high CO_2 grown plants can be more sensitive to lowering of CO_2 during the experiment and massive loss of water from widely open stomatal pores can induce the patchy response. High leaf-air vapour pressure deficit (above 3.2 kPa) induced patchiness even in well watered plants of castor bean (Dai *et al.* 1992) and *Xanthium* *strumarium* (Mott *et al.* 1993). Thus, it seems that not only the depression of leaf water potential is a patchiness inducing stimulus but also the direct loss of water from individual guard cells can play this role.

Consequences for evaluation of stomatal and non-stomatal limitations of photosynthesis

Many authors have showed that occurrence of stomatal patchiness may influence the determination of internal CO_2 concentration from gas exchange measurements and so the evaluation of stomatal and non-stomatal limitations to photosynthesis. The most simple explanation is given in the model of Terashima *et al.* (1988) in which he supposed a biphasic distribution of conductance. Let half of the leaf area be occupied with patches having conductance g_{s1}, and the other half with patches of conductance g_{s2}. It is assumed that there is no lateral diffusion of gas through the mesophyll between patches. It follows from the non-linear saturation type of photosynthesis *versus* stomatal conductance curve (Fig. 8) that the mean rate of photosynthesis in the 'patchy' case is lower than at uniform g_s over the whole leaf. As P_N is lower when g_s varies among the patches, intercellular CO_2 concentration as calculated from Eq. (*1*) in Appendix 1 is higher than it would be if g_s was uniform. So the curve expressing the dependence of P_N on c_i is doubly affected by patchiness: for a given g_s, P_N is lower than and intercellular CO_2 concentration is higher than they would be if g_s were uniform (Terashima *et al.* 1988). This does not mean that some corrections for depression of P_N should be used. An adequate calculation of c_i, as suggested, *e.g.*, in Appendix 1, is sufficient to eliminate the deviation of the 'patchy' point. The source of the 'patchy' errors in calculating c_i was convexity of the $P_N(g_s)$ curve in the above example. However the linear relation of P_N *versus* g_s could

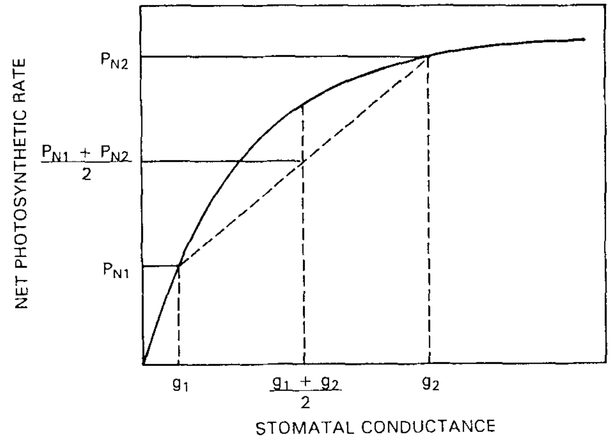

Fig. 8. Simple model of the relationship between net photosynthetic rates and stomatal conductances. Stomatal conductance of the patches of the halves of leaf blade are g_1 and g_2. The average P_N $[(P_{N1} + P_{N2})/2]$ is less than it would be if all patches had the conductance $(g_1 + g_2)/2$. (According to Terashima *et al.* 1988.)

also produce apparent depression of the $P_N(c_i)$ curve: if, *e.g.*, stomata on one half of the leaf area close and those on the other half remain unchanged, both P_N and transpiration rates drop to about one half of their original value. The proportional decrease in P_N and g_s will keep c_i apparently constant (see Eq.(*1*), Appendix 1).

When lateral diffusion of gas in the mesophyll is sufficiently large, c_i tends to equilibrate, and a mesophyll located under the patches of closed stomata may photosynthesize to some extent. Therefore some authors (Drew *et al.* 1990, Martin and Ruiz-Torres 1992, Kawamitsu *et al.* 1993) assume that the influence of any heterogeneity in stomatal closure across the leaf will have only a small effect on estimation of c_i especially in grasses because they lack small compartments seggregated by bundle sheath extensions and have huge numbers of stomata per unit leaf area (Kawamitsu *et al.* 1993). Also the relation between the ratio of external and internal CO_2 partial pressure and carbon isotope ratio of leaf sugars in *Gossypium* indicate that the effect of patchy stomatal closure under salinity stress is not large (Brugnoli and Björkman 1992). Similarly, lack of changes in the $P_N(c_i)$ curve was predicted by Cheeseman (1991) under a normal distribution of g_s. Only bimodal types of distribution were expected to cause significant deviation of c_i. Due to partitioning of the total leaf diffusive conductance into stomatal and boundary layer conductance, the effect of patchiness on c_i calculated conventionally increases with the rising thickness of boundary layer (Van Kraalingen 1990).

The conventional method of calculating the mesophyll resistance involving the subtraction of average stomatal resistance from total leaf resistance may also be incorrect (Laisk 1983). Lloyd *et al.* (1992) analyzed the effect of patchiness, expressed as variance of g_s, on the estimation of internal CO_2 conductance (conductance to CO_2 transfer from substomatal cavity to carboxylating sites). The internal conductance calculated from carbon isotope discrimination and gas exchange data decreases with increasing variability in g_s; the effect was dependent on carboxylation efficiency and on mean g_s (Lloyd *et al.* 1992).

The occurrence of stomatal patchiness has led to the development of some new approaches in modelling stomatal responses to environment (*e.g.* Downton *et al.* 1988a, b, Lloyd 1991, Soldatini and Guidi 1992). For example Downton *et al.* (1988a) used for corrected internal CO_2 concentration ($c_{i,\mathrm{corrected}}$) the following equation:

$$c_{i,\mathrm{corrected}} = [(1 - R)\,\Gamma + c_{i,\mathrm{IRGA}}\,R]$$

where $c_{i,\mathrm{IRGA}}$ is derived from gas exchange measured by a infra-red gas analyser and R is the ratio of P_N of stressed and control plant at the same $c_{i,\mathrm{IRGA}}$. Thus R represents the fraction of leaf area where stomata remain open and (1 - R) is the fraction of leaf area where stomata are closed. c_i is assumed to be at the CO_2 compensation concentration (Γ) under the closed stomata.

There are two ways of avoiding the illusion of a non-stomatal (biochemical) inhibition of photosynthetic CO_2 uptake. First, the direct (non-stomatal) effect has to be proved by other techniques (*e.g.* by measurement of O_2 evolution under high CO_2 concentration, by measurements of gas exchange in isolated chloroplasts or by determination of parameters of chlorophyll fluorescence). Second, stomatal patchiness has to be checked during the experiment, the 'patchy' leaf excluded or the effective c_i should be quantified.

The causes and possible consequences of stomatal patchiness for the leaf and plant performance

The cause of the variability can be analysed from the point of view of individual stomata (why is the environmental response of two adjacent stomata as different as it is?) or from the point of stomatal populations (why is the distribution of frequencies of stomatal aperture bell-shaped, bimodal and/or asymmetrical?).

The cause of the variance among several closely spaced individual stomata has not been unambiguously answered up to the present. Differences in morphology (size) and in the related functional parameters (mechanical advantage) of the guard cells and/or subsidiary cells of adjacent stomata may play a role; differences in functional contact of mesophyll, epidermal and guard cells, in viability of epidermal cells or in the microenvironment of the guard cells have been hypothesized (see Spence 1987 and Mansfield and Atkinson 1990 for reviews).

The patchy pattern of transpiration rate and related parameters (surface temperature) are the consequences of the non-uniformity. Stomatal response to an experimental treatment was hypothesized to consist of two distinguishable components: (*1*) a proportional one leading to unidirectional closing or opening of each stoma, and (*2*) a binary one leading to the patchy, bimodal distribution of stomata opening (Daley *et al.* 1989, Van Gardingen *et al.* 1989, Raschke 1990). We analysed the leaf surface temperature images of *in vitro* and *ex vitro* grown tobacco plants before and after feeding with abscisic acid (ABA). The relative changes of temperature (*i.e.* change in temperature of a pixel normalized to maximum change registered on the investigated leaf area) in Fig. 9 A, B show the portion of both the proportional and the binary components in stomatal response. We propose that the proportional component in stomatal behaviour becomes dominant at the expense of the binary component during acclimation to dry atmosphere (during acclimation from *in vitro* to *ex vitro* conditions). In contrast, the quantitative effect of ABA on stomata was more pronounced in acclimated (*ex vitro* grown) plants and minimal in non-acclimated (*in vitro* grown) plants (compare dT_{max} in Fig. 9A and 9B). Interestingly, when the proportional component was subtracted as was done in Fig. 10 for *ex vitro* grown plants, the range in which temperature fluctuated (dT_{max}-dT_{min}) was about the same for both *in vitro* and *ex vitro* grown plants. However SEM observations and other indirect measurements indicated that stomata of *ex vitro* grown plants had a bell-shaped distribution with large variance, while stomata of *in vitro* grown plants remain either fully open or completely closed. Hence the type of variance among stomatal apertures seems to reflect the variability in environmental conditions experienced by the plant during its life history (Šantrůček - unpublished).

After the patchy pattern of stomatal opening has been induced, local deficiencies in CO_2 appear. The full closure of a group of stomata causes a shutting-off of the

CO_2 flow into the underlying mesophyll, and internal CO_2 content falls until the CO_2 compensation concentration is reached. The corresponding patchy topography of

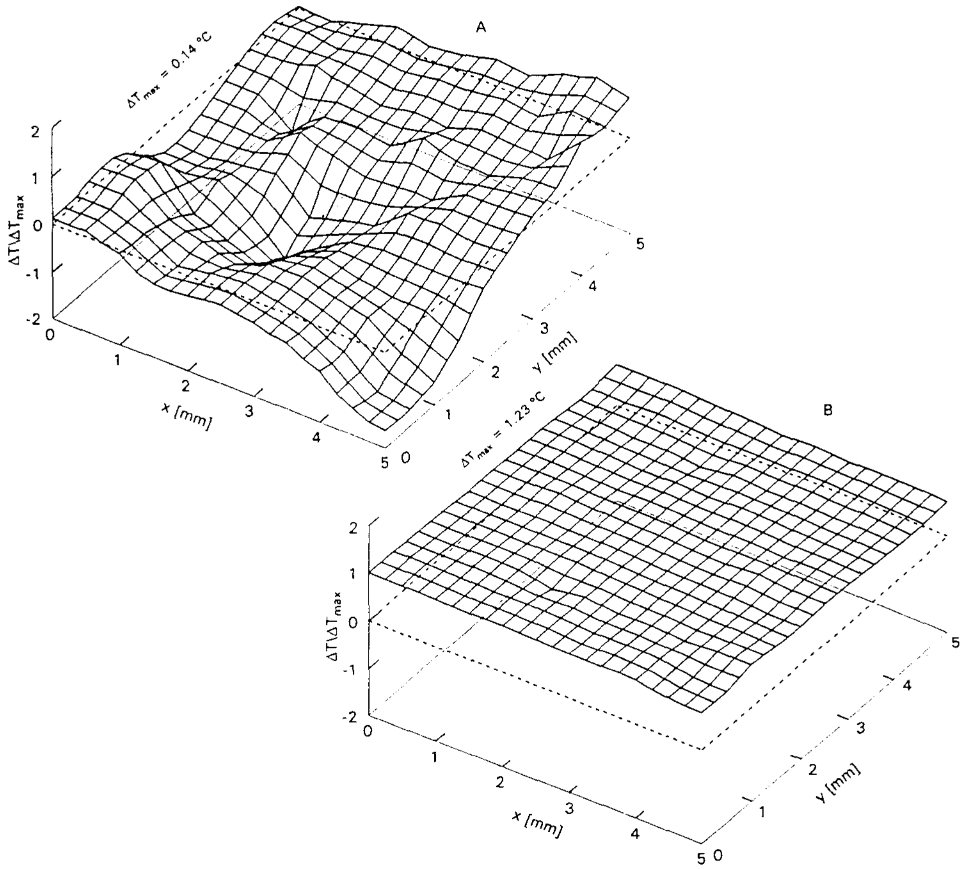

Fig. 9. A map of relative changes of leaf surface temperature induced by feeding a tobacco leaf with abscisic acid. The tobacco plants were grown in closed glass vessels under stable temperature, high air humidity, low irradiance and day-night fluctuating CO_2 (*A*) or in a greenhouse under varying humidity, irradiance and temperature (*B*). The part of $\Delta T/\Delta T_{max}$ which is common for all points in the net and which could be subtracted represented the proportional component. The subtraction was possible only for *ex vitro* grown plant; no proportional response was found for *in vitro* grown tobacco plants (Šantrůček - unpublished).

parameters of all the processes coupled with CO_2 fixation can be expected: electron transport rate through photosystem 2 (PS2) will decline due to the declining consumption of NADP and, therefore, nonphotochemical fluorescence quenching will rise in the parts of mesophyll supplied by CO_2 through the closing stomata (Daley *et al.* 1989, Terashima 1992); P_N and starch accumulation will follow the variable stomata opening (*e.g.* Terashima *et al.* 1988). Non-uniform c_i will affect light response of P_N measured over total leaf area in both the initial slope (apparent quantum yield efficiency) and in the maximum (light saturated) rate of P_N. In contrary, apparent convexity of light response curves seems to be affected only marginally (Olsson and Leverenz 1994). CO_2 depletion in the regions having low g_s will stimulate the photorespiratory pathway (Downton 1990). Of course, the above scene is valid only for heterobaric leaves and can be modified by changes in the activity of carboxylating enzymes, in photosynthate allocation, and by variability in boundary layer resistance (Van Kraalingen 1990). Non-uniform P_N does not always reflect non-uniform c_i, since non-uniform biochemistry and/or photochemistry may also occur (for review see Terashima 1992).

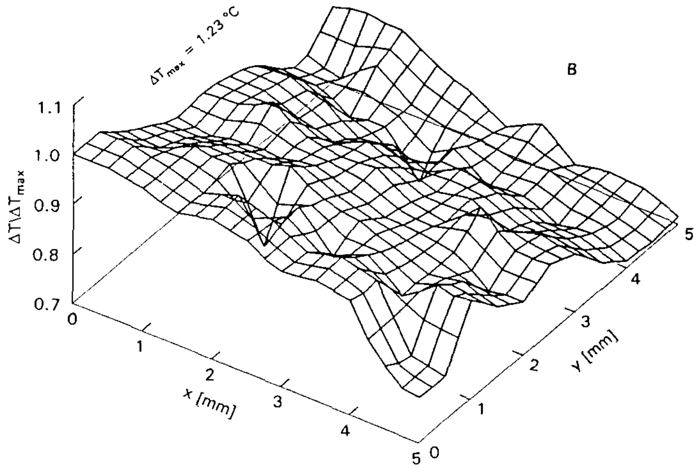

Fig. 10. A map of relative changes of leaf surface temperature induced by feeding leaves of greenhouse grown tobacco plants with abscisic acid when the proportional component was subtracted. (Šantrůček - unpublished).

Originally, the non-uniform opening (oscillations in time, or bimodal distribution in space) of stomatal populations over the individual leaf surface was theoretically predicted to be more advantageous for C_4 plants with high mesophyll conductance to CO_2 transport than for C_3 plants. Improvement of water use efficiency was the model-predicted benefit of the non-uniformity (Cowan and Troughton 1971). However, if the non-uniformity of stomatal response should be beneficial for the plant only in terms of the Cowan's and Troughton's concept, two other predictions have to be made: (*1*) only heterobaric leaves should exhibit patchiness, and (*2*) the capacity of the photochemical and carbon dioxide reducing processes should be unaffected by patchiness. In contrast to (*1*), patchiness was observed also in homobaric leaves (*e.g. Phaseolus* - Mott and Parkhurst 1991).

A patchy distribution of stomatal aperture would lower the risk of photoinhibition. By periodically exchanging the open and the closed areas the plant may be able to protect most of its leaf area from becoming severely photoinhibited (Beyschlag *et al.* 1994).

Another role of stomatal patchiness can be drawn from the work of Scheuermann

Table 3. Survey of types of distribution of patches.

Author and year of publication	Characteristics of the distribution	Measured value (method used)	Range of stomatal conductance at which the distribution was observed [mol m^{-2} s^{-1}]	Plant species	Growth conditions (experimental treatment)
Terashima *et al.* 1988	frequency of open stomata relative to all: 0.007 - 0.0014	stomatal aperture (silicon rubber impressions)		*Helianthus annuus*	greenhouse (ABA feeding)
Van Gardingen *et al.* 1989	bell-shaped, bimodal	aperture width (low temperature SEM)	0.487, 0.159	*Avena fatua*	field
Daley *et al.* 1989	skewed (relative frequences in four classes: 0.8, 0.1, 0.07, 0.03)	non-photochemical quenching (image of Chl fluorescence)	0.125	*Xanthium strumarium*	greenhouse (ABA feeding)
Laisk 1983	skewed	stomatal aperture (optical microscopy)	0.06-0.12	*Hordeum vulgare*	growth chamber (opening by light)
Šantrůček 1991	skewed, bimodal	stomatal resistance (modelled *vs.* experimental transpiration)	0.286	*Nicotiana tabacum*	*in vitro* (air flow through leaf)

et al. (1991), who investigated species-specific differences in mechanisms dissipating excess radiant energy, together with interspecific differences in stomatal patchiness. They found patchy behaviour predominantly in species which adopt CO_2 recycling (photorespiration) as a mechanism for dissipation of energy (*e.g.* in sunflower). Bean, which shows nonphotochemical quenching for radiant energy dissipation, did not have 'patchy' leaves within the applied range of drought stress. Thus, patchiness could allow the leaf to dissipate excess energy without loss of water and, at the same time, to synthesize saccharides for respiration.

Bell-shaped or bimodal distribution of patches?

It is of methodical as well as of heuristical interest whether g_s in the population of patches have bell-shaped, bimodal, skewed or other types of frequency curve (Table 3).

As shown above, the type of distribution of stomatal opening is of primary importance for quantification of the effect of patchiness on c_i assessment. However, with the methods currently used, it is often impossible to evaluate the type of distribution.

Methods

An ideal method should provide a map of stomatal opening and/or g_s over the leaf surface by a quick, nondestructive and noninvasive way. Quantification of the resistance patterns should be followed by computation of effective c_i. The currently used methods accomplish the above requirements only partially (Table 4).

Direct microscopic observations are usually time consuming and/or destructive. Direct investigations of the whole leaf surface by infiltration give only qualitative patterns.

Indirect methods based on the surface variability of transpiration rate or temperature (hygrophotography, temperature imaging) do not provide quantitative results; the same holds for indirect techniques based on the variability in CO_2 diffusive flux and in related photosynthetic processes (microautoradiography, histochemistry of starch). More useful is the method based on the determination of a map of nonphotochemical quenching of chlorophyll fluorescence published by Daley *et al.* (1989). This noninvasive and nondestructive technique may be an ideal method as it monitors closely linked consequences of non-uniform stomatal opening: stomatal closure is expected to induce CO_2 deficiency in the underlying mesophyll which reduces the rate of electron transport through PS2 and, under constant incident radiation, depresses quantum yield efficiency of the electron transport. Then, the monitored nonphotochemical fluorescence quenching is increased. Unfortunately, fairly expensive electronic equipment is needed and a quantification of stomatal conductance or stomatal aperture patterns is not possible.

Table 4. Survey of recent methods used for investigation of the within-sample variability in dimensions of stomatal apertures or for construction of the leaf maps showing iso-aperture areas (stomatal patchiness).

Author and year of publication	Method	Advantage	Drawbacks
Direct methods			
Kappen *et al.* 1987	optical microscope and videocamera	non-destructive, quantitative	low resolution
Terashima *et al.* 1988	leaf surface impressions	expeditious	unsatisfactory for small stomatal opening, suits widely open stomata
Van Gardingen 1989	low-temperature scanning electron microscopy	resolution well, below 1 μm	destructive, non-expeditious, hard to detect leaf pattern
Beyschlag and Pfanz 1990, Beyschlag *et al.* 1990, 1992, 1994	liquid infiltration into the leaf under pressure	expeditious	destructive, only for heterobaric leaves, semiquantitative
Indirect methods (experimentally based)			
Omasa and Croxdale 1992, Šantrůček (unpublished)	leaf surface temperature imaging	related to stomatal opening	expensive, limited resolution
Long *et al.* 1989	gas exchange measurement of small leaf areas	nondestructive	low accuracy, low resolution
Downton *et al.* 1988a,b, 1990, Sharkey and Seemann 1989, Ortiz-Lopez and Ort 1990, Wise *et al.* 1990	microautoradiography of the leaf surface		destructive, semiquantitative
Terashima 1988	histochemistry of the starch content		long dark pretreatment, semiquantitative

Downton *et al.* 1988a,b, Daley *et al.* 1989, Long *et al.* 1989, Raschke *et al.* 1990, Mott *et al.* 1993	chlorophyll fluorescence parameters scanned over leaf surface	nondestructive, noninvasive	laboratory, expensive, semiquantitative
Flanagan and Jefferies 1989a,b, Lloyd *et al.* 1992	calculation of c_i from ^{13}C discrimination and comparison with c_i computed conventionally from gas exchange measurements		qualitative, expensive
Dai *et al.* 1992	c_i estimated from quantum yield of PS2 and compared with c_i computed from gas exchange measurements		
Indirect methods (based on comparison of model and experimental data)			
Flanagan and Jefferies 1989a,b	stimulation of P_N by low O_2 concentration as determined from experiment and from model	nondestructive	only for quantitative assessment of effective c_i
Bongi 1990	comparison of the actual value of P_N at a particular c_i, adjusted by manipulating ambient CO_2 and calculated potential P_N from a model		
Šantrůček 1991	comparison of the modelled and experimentally estimated values of depression in transpiration rate after viscous flow of air through stomatal pore has been established		only for amphistomatous leaves, invasive

'Negative' methods suitable for checking whether patchiness plays a role in CO_2 uptake or not are based on measurements of O_2 production by leaf discs under very high ambient CO_2 concentration (*e.g.* 5 %). Such high concentration is able to saturate P_N even if the stomata are fully closed. The lack of response of O_2 evolution to such experimental treatment confirms the suspicion that patchiness takes part in the depression of CO_2 uptake. Depression of O_2 evolution indicates the possible role of a factor in biochemistry and/or photochemistry of photosynthesis.

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Appendix 1

Assuming that stomatal apertures over the investigated leaf surface are uniform (*i.e.* all have the same dimensions and stomatal density is invariable over the measured part of the leaf lamina), c_i is routinely calculated by the simplified relation

$$c_i = c_a - P_N/g \qquad (1)$$

where g is total leaf conductance for CO_2 diffusion from the ambient atmosphere through all the stomatal apertures into the leaf interior. Due to the variability in stomatal apertures, the rate of carbon fixation in mesophyll cells beneath an individual stoma or group of stomata will also vary (supposing that P_N responds only to c_i and that the mesophyll cells are not supplied by CO_2 laterally or from the opposite leaf side). In an extreme case when both g and P_N on a part of the measured leaf blade (*e.g.* on one half) will drop to zero, the total P_N and g will change in proportion and P_N/g remains unchanged. Then, the estimated (apparent) c_i stays constant but, in fact, it is valid only for the "open" part of the measured leaf. c_i in the other part, which is turned off for gas exchange, is equal to the CO_2 compensation concentration (Γ). The situation when P_N drops to, *e.g.*, one half and c_i remains constant is interpreted as a strong biochemical limitation of photosynthesis. The illusion of the non-stomatal limitation manifests itself by the flattened $P_N(c_i)$ curve (see Fig.1). To solve the problem, the distributions of P_N and distribution of the rate constants of CO_2 supply and consumption over the investigated leaf surface has to be estimated. In the first approximation, P_N can be expressed as

$$P_N = k\,(c_i - \Gamma) \tag{2}$$

where k represents apparent carboxylation efficiency (an increase in P_N per unit of added CO_2). Substitution for P_N into (*1*) and rearangement yields:

$$c_i = (c_a \times g + \Gamma \times k) / (g + k) \tag{3}$$

Treating c_a, Γ and k as constants, c_i depends non-linearly on g. The same is true for relation of c_i and k if g instead of k is kept constant (Fig. 11). When a variable (*e.g.* c_i) is influenced non-linearly by some factor (*e.g.* g or k), distribution of the factor levels has to be known to calculate the correct mean (effective) value of the variable in question. The effect of variability in k on calculation of effective c_i relevant to the leaf (surface-averaged) photosynthesis was analyzed by Farquhar (1989). As two different processes (supply and consumption) govern c_i, two effective values of c_i ($c_{i\ \mathrm{eff}}^{g}$ and $c_{i\ \mathrm{eff}}^{PN}$) can be found. The former is relevant to CO_2 supply and the latter to photosynthetic CO_2 fixation. Let c_{ij}, g_j and k_j are parameters of particular patch j.

Both effective values of c_i represent the g- or k-weighted average of c_{ij} over n patches:

$$c_{i\,\mathrm{eff}}^{g} = \sum_{1}^{n} (c_{ij} \times g_j) / \sum_{1}^{n} g_j \tag{4}$$

$$c_{i\,\mathrm{eff}}^{PN} = \sum_{1}^{n} (c_{ij} \times k_j) / \sum_{1}^{n} k_j \tag{5}$$

The difference between $c_{i\ \mathrm{eff}}^{PN}$, calculated using eqs. (*3*) and (*5*), and c_i using Eq. (*1*) represents the error caused by the false assumption of uniformity in stomatal apertures. It can be shown from Eq. (*3*) that the error will increase with depressing of g due to the increasing non-linearity in $c_i(k)$ relation (Fig. 11). Thus the impact of non-uniformity in stomatal opening on errors in c_i estimations will increase at lower mean stomatal conductance, *e.g.* by imposing stress.

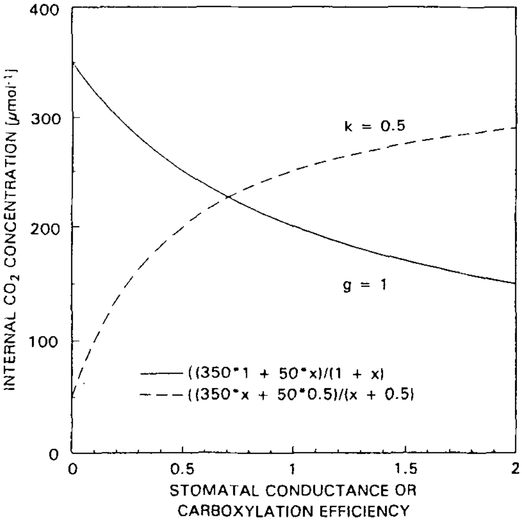

Fig. 11. Dependence of intercellular CO_2 concentration (c_i) on leaf CO_2 conductance (g) when the carboxylation efficiency k is kept constant (k = 0.5) or on carboxylation efficiency when g is assumed to be a constant (g = 1). The relations are modelled according to Eq. (*3*) using the constants for c_a and for Γ as indicated in the legend.