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大叶黄杨叶片表皮组织的聚光效应及其对叶片内部光分布的影响

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摘 要 利用自制的光纤微探测器研究了大叶黄杨(*Euonymus japonicus* T.)叶片内部的光分布。叶片表皮组织具有聚光效应,用金相砂纸磨去叶片的上表皮组织可以去除这种效应。用 660 nm 的红光照射叶片时(从上表皮方向),叶片内部光量迅速下降,在不到 100 μm 的路径上光量下降到初始值的 20%。叶片内部光分布微分曲线说明:叶片表皮组织的存在有利于叶片内部各组织之间光吸收的均匀化。分析叶片内部红光分布曲线(照射上表皮方向以及照射下表皮方向),海绵组织对红光(660 nm)吸收较少,这可能是海绵组织的一种生理生态意义上适应性的反映。

关键词 光纤微探测器 表皮组织的聚光效果 叶片内部的光分布 微分分析

THE LEN EFFECT OF THE EPIDERMIC CELL LAYER OF THE LEAF OF *EUONYMUS JAPONICUS* T. ON THE LIGHT GRADIENTS WITHIN LEAF

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Abstract One underlying assumption of the photosynthetic model based on physiological and biochemical mechanisms is that different tissue layers within a leaf do not show much difference in light absorbance. However, this assumption does not consider the different optical parameters and photosynthetic capacity of different tissue layers within a leaf, resulting in a non-precise photosynthesis model.

To measure the optical properties of different tissue layers, Vogelmann and Njorn (1984) made a microscopic fiber optic probe that was inserted into the leaf. The electronic circuit for the driving stepping motor of their probe is somewhat complex, and could be simplified.

A home-made fabric microprobe was used to determine light distribution within the leaf of *Euonymus japonicus* T. The Len effect of epidermic cell layer was affirmed by our sand paper treatment. Light declines rapidly and exponentially within the leaf, the red light (660 nm) fell to 20% of initial value after passage through 100 μm from the adaxial (upper) surface of the leaf. Based on differential analysis of the light curve, it seems that the epidermic cell layer could facilitate the averaging light absorbance between different tissue layers within a leaf. Spongy tissue absorbed only a small proportion of red light (660 nm), for although about 20% of the red light transmitted leaf, it was not absorbed by the spongy tissue layer.

Our improvement of the microprobe produces good results, and can avoid the complex electronic circuitry of the driving stepping motor.

Key words Fabric microprobe, Len effect of epidermic cell layer, Light distribution within leaf, Differential analysis

In the research of primary productive structure of forest, the photosynthetic model is one of the fundamental methods. To predict the whole photosynthetic capacity of whole leaf, the photosynthetic model based on the physiological and biochemical mechanism (Farquhar *et al.*, 1980) should be used. One prerequisite is applied for this kind of model, which presumes that the light absorbance of different tissue layers within leaf do not show

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much difference. This assumption does not consider the different optical parameters and photosynthetic capacity between different tissue layers within a leaf, which will result in non-precise photosynthesis model (Richter & Fukshansky, 1996).

Although it is available to measure the optical parameters of a whole leaf with the aid of an integrating sphere, this method does not provide information about the distribution of light within the sample. Vogelmann and Njorn (1984) have developed a new method to make light measurements inside leaf. A microscopic fiber optic probe is inserted into leaf, which makes it possible to measure the different tissue layers' optical properties. Combined with the mathematical approaches, this method can help to describe the photosynthetic performance from a chloroplast to a leaf (Richter & Fukshansky, 1998).

The light microprobes contain two kinds: the radiance microprobes with the tip diameter down to 10 μm sense the incoming radiation within the solid angle of about 10°-20° around their axis and can therefore detect the distribution of radiance even in thin samples; the flucence rate optical probes, also called scalar irradiance probes, with the tip diameter down to 40 μm sense the total radiant flux from all directions thus providing the flucence rate (Richter & Fukshansky, 1996).

In this paper, we designed a flucence rate optical probe to measure the light distribution of leaves of *Euonymus japonicus* T. within leaf and investigated the different absorbance between different tissue layers.

1 Material and method

1.1 Plant

Euonymus japonicus was grown in the nursery of Beijing Forestry University. The newly grown leaves in spring were taken as samples.

1.2 Experimental set-up for the fiber optic measurements

Similar instrument had been built up according to reference (Vogelmann & Njorn, 1984); and some improvements had been made (Fig. 1). The fiber optic probes (quartz fiber were bought from Beijing Glass Institute, tip was produced by acetylene + air flame) with the diameter around 40 μm were chosen as experimental microprobes. Low speed motor bought from Beijing Micromotor Factory was used to drive microprobe that was fixed on the movable screw of a micrometer. Signal was amplified by a selected photoelectric diode and supplemental circuit. The amplified signal was transferred to a computer and afterwards for further analysis. The sampling frequency was 2.5 $\mu m \cdot s^{-1}$. A red light laser diode (660 nm, half peak width ± 5 nm, bought from Semiconductor Institute of the Chinese Academy of Sciences) was chosen as light source for its exact parallel light. Light intensity was 200 $\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$. Preliminary test about the stability and creditability of this equipment was carried out according to Vogelmann and Njorn (1984).

1.3 Measurement of the light distribution within leaf

The leaf was clamped by a clamp fixed on one end of the beam of micrometer. Turning on the micro-motor, the micro probe driven by micro-motor slowly moved towards the abaxial (lower part) leaf surface and pierced into leaf. A magnifier was used to roughly learn when it pierces into leaf. The value of signal was collected by computer. Experimental time was about 5 min. The software Excel was used to make the light distribution curve based on these data.

To evaluate the light absorbance of different tissue layers, it was necessary to differentiate the light distribution curve. By this method, the absorbance curve across the cross section of a leaf could be gained.

1.4 Photograph of the cross section of leaf

For anatomical studies of the cross section sample, small pieces of material were preserved in FAA for 24 h or longer. A routine histological paraffine procedure was followed. Sections (about 8 μm thick) were stained with saffranine followed by fast green. The photograph of the cross section of leaf was taken under microscope (Leitz, Orthoplan). Based on the measurements of the length of different tissue layers, it was possible to calculate the relative absorbance of different tissue layers by differential analysis on the light distribution curve. Fig. 2 show the cross section of leaf of *E. japonicus*.

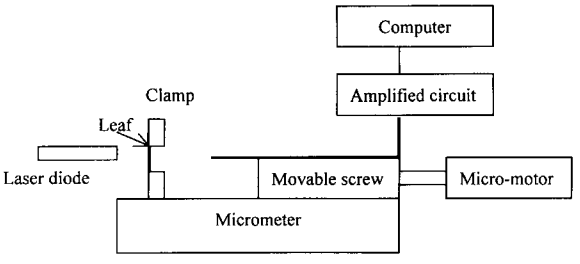

Fig. 1 The build-up of fabric microprobe according to Vogelmann and Njorn (1984) with some improvements

The micrometer was used as a supporter for fabric microprobe. Micro-motor was used to drive forward the movable screw fixed with fabric microprobe. Data was collected by computer. Sampling frequency can be adjusted manually

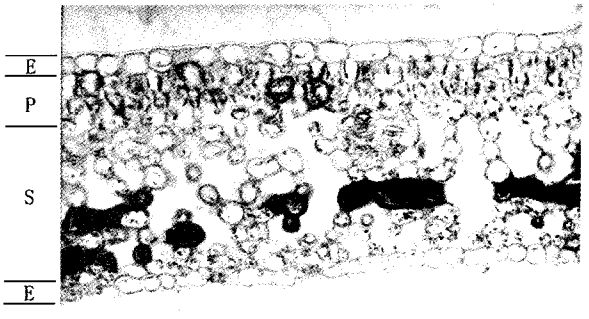

Fig. 2 The leaf cross section of *Euonymus japonicus*

Leaf thickness is about 200 ± 21 μm ($n = 3$). The thickness of epidermic cell layer (E) is about 18 ± 4 μm; palisade tissue layer (P) is about 70 ± 19 μm; spongy tissue layer (S) is about 120 ± 18 μm. Fig. 3, 4, 5, 6 are the same

2 Results

Based on the results of microprobe, light gradient in the leaf of *E. japonicus* show some characteristics. Firstly, a phenomenon of light focusing appeared, which can make the light stronger than the incident light. The effect of focusing was variable (in our experiment, it ranged from 1.3 to 5.8 times), depending on the state of leaves. According to our experience, the thicker the leaf, the stronger this effect. In Fig.3, the effect could bring about 1.5 times stronger light within a leaf; the highest light intensity must locate in the palisade tissue layer.

Vogelmann (1993) called this light focusing effect as "Len effect"; and believed that it was due to the epidermic cell layer. We used sand paper carefully to erase the epidermic cell layer, and diminished the "Len effect", which confirmed his conclusion.

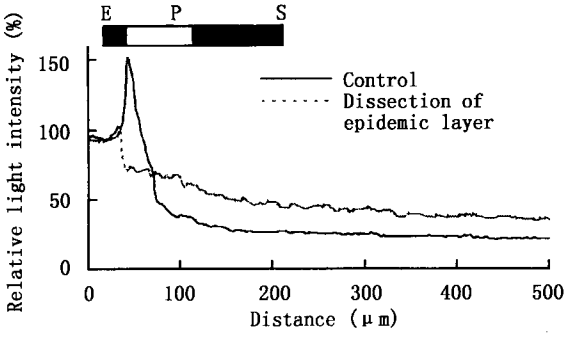

Fig.3 Light measurements through *E. japonicus* leaves

The adaxial leaf surface faced the collimated light beam and the probe was advanced from abaxial leaf surface towards the irradiated surface. Light was measured at 660 nm. At the distance 12.5 μm, probe was pierced into leaf (roughly determined by a magnifier). Sampling frequency is 2.5 μm·s^{-1}

Another characteristic of light gradient within leaf was that light declined rapidly and exponentially within the leaf, when the red light (660 nm) felled to 20% of initial value after passage through 100 μm from the adaxial (upper part) surface of leaf. This value is slightly larger than that of spinach leaf, from 50 to 90 μm (Cui *et al.*, 1991; Han & Vogelmann, 1999); it is maybe for different plant materials. It seems that about 80% light was absorbed by the palisade tissue layer.

The flucence rate optical probes could sense the total radiant flux from all directions thus providing the flucence rate, and needed not concern about the reflective, refractive and scattering light between different tissue layers within a leaf. It was possible to take differential analysis on the light distributional curve to gain the relative absorbance of different tissue layers.

To investigate how the Len effect influenced the relative absorbance of different tissue layers within a leaf, the sample without epidermic cell layer was used. Results showed in Fig.4 and Fig.5. It seemed that the epidermic cell layer could facilitate the averaging light absorbance between different tissue layers within a leaf. Leaf with epidermic cell layer showed slowly declination of light absorbance during the passage through 120 μm from the adaxial surface of leaf, while leaf without epidermic cell layer showed quickly declination. Within non-epidermic cell layer leaf, the light was strongly absorbed by 10 μm thick palisade tissue layer which apparently located on the upper part of leaf. But we could not clearly demonstrate in which tissue layer light was maximally absorbed, for the Len effect of epidermic cell layer concealed the real absorbance of upper part of palisade tissue layer.

It seemed the spongy tissue absorbed a little proportion of red light (660 nm), for although about 20% red light transmits leaf, it was not absorbed by spongy tissue layer. This could be supported by Fig.6, in which red light illuminate the abaxial surface of leaf and micro-probe moved towards the adaxial surface.

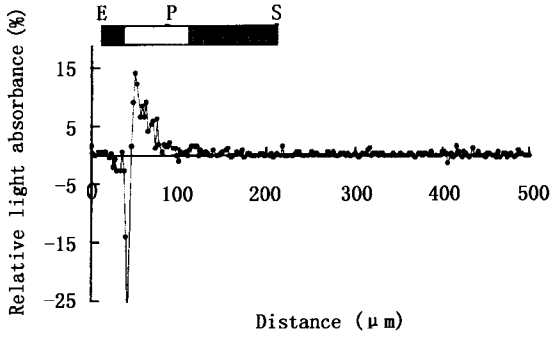

Fig.4 Differential analysis on the light curve within *E. japonicus* leave

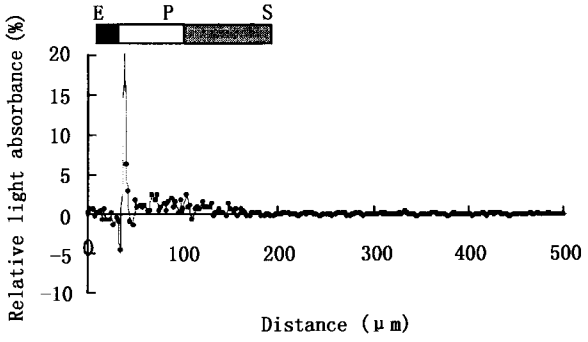

Fig.5 Differential analysis on the light curve within *E. japonicus* leave without epidermic cell layer

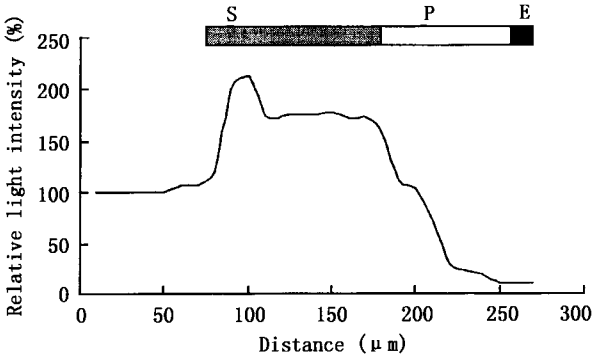

Fig.6 Light measurements through *E. japonicus* leaves

The adaxial leaf surface faced the collimated light beam and the probe was advanced from abaxial leaf surface towards the irradiated surface. Light was measured at 660 nm. At the distance 80 μm, probe was pierced into leaf (roughly determined by a magnifier). Sampling frequency is 10 μm·s^{-1}. Leaf thickness is about 200 μm. The light focus effect was caused by the epidermic cell layer of abaxial leaf surface

3 Discussion

3.1 The epidermal focusing

The Len effect of epidermic tissue layer observed by microprobe technique is an interesting problem discussed by many researchers in the field of light distribution within leaf. The light can approach to 1-6 times of the incident light (spinach leaf, Vogelmann, 1993). Only the parallel light can induce this effect, the scattering light does not induce it. This effect shows somewhat universality in different species (Vogelmann, 1993). Although it is not clear what role the Len effect of epidermic cell layer takes, some researchers believe that this effect lead the more homogeneous light absorbance between different tissue layers within leaf (Han & Vogelmann, 1999), which was supported by the differential analysis of our experiment.

However, there are still many questions about the physiological role of the Len effect of epidermic tissue layer. For example, photoinhibition was shown to affect primarily the quantum yield of the palisade chloroplasts when excessive illumination was applied from the adaxial leaf side (Schreiber *et al.*, 1996). So the Len effect maybe intensify the photoinhibition-induced damage of palisade tissue layer, especially during the noon period in field (considering the analogous parallel light of sun) (Sun *et al.*, 1996b). The significance of Len effect of epidermic tissue layer is still in dispute.

Richter and Fukshansky (1996) used paraffin oil to cover the adaxial surface of leaf, which could eliminate this effect because of the similar refractive ratio between paraffin oil and epidermic cell layer. If the epidermic cell layer was erased by sand paper, similar results could also be gained.

3.2 The photosynthetic performance across the section of leaf

Spongy tissue absorbed less red light than palisade tissue (Cui *et al.*, 1991) and absorb more green light than palisade tissue. This phenomenon may reflect an adaptively character of leaf anatomical structure.

It is generally acknowledged that the rate of photosynthesis has a Gaussian profile across the leaf by the measurements of the amount of carbon fixed by mesophyll layers of spinach (Sun *et al.*, 1996a; Nishio, 2000). The pattern of fixation across the leaf is due to the distribution of Rubisco (Nishio *et al.*, 1993), light absorbed by the reaction center and the Rubisco activation state across leaf section (Jerriann *et al.*, 1997).

However, in a recently report (Han & Vogelmann, 1999), oxygen evolution curve showed more flat than light distribution curve across the section of spinach leaf, which was illuminated by using a similar red light source of ours. The relatively flat profiles for O_2 evolution measured so far did not coincide very closely with estimates of profiles of absorbed light within spinach or other leaves. Oxygen evolution reflects the assimilative capacity and avoids the shortcoming of $^{14}CO_2$ isotope feeding measurement (Evans & Caemmerer *et al.*, 1996). This result may indicate that the energy transfer capacity of spongy tissue layer is higher than palisade tissue layer, which was supported by the results of fluorescence distribution within spinach leaf by using a microprobe and an instrument combined photoacoustic and fluorescence techniques (Schreiber *et al.*, 1996; Vogelmann & Han, 2000), as well as our results of photoacoustic measurement of the energy storage (Fan, unpublished data). Another possible explanation of this result is that a reductant and /or ATP shuttle may exist between the top and the bottom of a leaf (Outlaw *et al.*, 1976).

It is complex to describe the photosynthetic performance within leaf for the optical, biophysical and biochemical heterogeneity within leaf. For example, one puzzle is about the CO_2 accessibility across the leaf section. Although some researchers believe that CO_2 gradient within a leaf shows no significant difference (Nishio *et al.*, 1993), the calculation of the CO_2 resistance from membrane of chloroplasts to the act site of Rubisco is proven to be very difficult (Evans & Caemmerer, 1996).

Our improvement of the microprobe is successful for the good results, and can avoid the complex electronic circuit for driving stepping motor. Of course, it is necessary to further our research to clarify the role of Len effect phenomenon. The microprobe technique should combine with other techniques (for example, the photoacoustic measurement) (Malkin & Cannaani, 1994) to describe the photosynthetic performance of a whole leaf, which is the base of a more precise photosynthetic model.

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