# ASPECTS OF LEAF ANATOMY OF TROPICAL KUDZU RELATED TO WATER AND ENERGY BALANCE[1]

ADAUCTO BELLARMINO DE PEREIRA NETTO[2], ANTONIO CARLOS GABRIELE[3] and HILTON SILVEIRA PINTO[4]

ABSTRACT - Tropical kudzu (*Pueraria phaseoloides* Benth, Leguminosae-Faboideae) has been established in southeastern Brazil in a region characterized by the transition between subtropical and tropical biomes. The seasonal changes in temperature and water availability found in this region are very distinct from those found in the region where tropical kudzu is native. The objective of this paper was to describe characteristics of leaflet anatomy related to water and energy balance in tropical kudzu. The lower epidermis of tropical kudzu showed a higher frequency of stomata (213 stomata.mm$^{-2}$) than the upper epidermis (101 stomata.mm$^{-2}$). Trichomes were present in both lower and upper epidermis. The average number of trichomes per square millimeter was 9 for the upper epidermis and 13 for the lower epidermis. The average trichome length was 300 μm for the upper epidermis and 460 μm for the lower epidermis. Cuticle thickness was not considerably different between lower and upper epidermis. The leaflet blade consisted basically of two layers (upper and lower) of unicellular epidermis and two layers of both palisade and spongy parenchyma. One layer of paraveinal mesophyll was found between palisade and spongy parenchyma.

Index terms: cuticle thickness, ecophysiology, leaflet anatomy, stomata, trichomes.

## ASPECTOS DA ANATOMIA FOLIAR DE PUERÁRIA ASSOCIADOS AO BALANÇO DE ÁGUA E DE ENERGIA

RESUMO - No Brasil, a puerária (*Pueraria phaseoloides* Benth, Leguminosae-Faboideae) é tradicionalmente empregada como cultura de cobertura em seringais na região amazônica, onde as condições climáticas se assemelham às condições climáticas tropicais encontradas no sudeste asiático, centro de dispersão da espécie. Puerária foi também introduzida na região sudeste do Brasil, região esta caracterizada pela transição entre os climas tropical e subtropical. Este trabalho teve como objetivo descrever algumas das características da anatomia foliar de puerária relacionadas à economia de água e energia. A epiderme inferior apresentou maior frequência de estômatos (213 estômatos.mm$^{-2}$) quando comparada à epiderme superior (101 estômatos.mm$^{-2}$). O número médio de tricomas por milímetro quadrado foi 9 para a epiderme superior e 13 para a epiderme inferior. O comprimento médio dos tricomas foi de 300 μm para a epiderme superior e 460 μm para a epiderme inferior. A espessura da cutícula não diferiu significativamente entre as epidermes inferior e superior. A lâmina foliar consistiu basicamente de duas camadas de ambos, parênquima paliçádico e parênquima lacunoso. Uma camada de mesófilo paranerval foi encontrada entre os parênquimas paliçádico e lacunoso.

Termos para indexação: espessura da cutícula, anatomia de folíolos, tricomas, ecofisiologia, estômatos.

## INTRODUCTION

Tropical kudzu (*Pueraria phaseoloides* Benth) is a robust perennial legume native to East and Southeast Asia, where it has been used for years as a cover crop (sometimes grazed) in rubber and oil palm plantations (Kretschmer Junior & Pitman, 1995). Tropical kudzu is a fast growing, nitrogen fixing vine able

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[2] Biólogo, M.Sc., Dep. de Botânica, S. Ciências Biológicas, Univ. Fed. do Paraná (UFPR), CEP 81531-970 Curitiba, PR.

[3] Biólogo, Dr., Dep. de Morfol. e Sistemática Vegetais, Univ. Estadual de Campinas (UNICAMP), CEP 13083-970 Campinas, SP.

[4] Eng. Agr., Dr., Dep. de Fisiol. Vegetal, UNICAMP.

to withstand water logging and light frosts (Kretschmer Junior & Pitman, 1995).

As a strategy to reduce damage caused by *Microcyclus ulei* infection, new rubber tree plantations have been established in southeastern Brazil, between tropical and subtropical areas, which is seasonally subjected to changes in temperature and water availability. Tropical kudzu have been established in small areas in southeastern Brazil and it presents potential to be used as a cover crop in the new rubber tree stands, even though, air temperature and water availability in southeastern Brazil are usually lower than what is found in East or Southeast Asia. Tropical kudzu has previously demonstrated broad intraspecific physiological plasticity while colonizing new environments and this physiological plasticity certainly contributed to the establishment of tropical kudzu in southeastern Brazil.

The objective of this paper was to describe characteristics of leaflet anatomy related to water and energy budget in tropical kudzu.

## MATERIAL AND METHODS

### Plant material

Seeds of tropical kudzu, *Pueraria phaseoloides* (Roxb.) Benth, Leguminosae-Faboideae, were imbibed overnight in pre-heated (75°C) distilled water. The seeds were then individually sown in five liter plastic pots containing haplortox soil. The pots were kept in a naturally-lit glasshouse in the southeastern Brazil (Campinas, SP, Brazil, lat. 22°49' S; long. 47°06' W). Pots were irrigated in a daily basis in order to keep soil water content close to the maximum retention capacity. The glasshouse had a coeficient of transmitance for light of 0.5. Average air temperature inside the glasshouse during the period of study ranged from about 25 to 35°C. The uppermost completely expanded terminal leaflet (28 days old) was used in this study.

### Frequency of stomata

The upper and lower epidermis of leaves of ten plants were washed in distilled water and rapidly peeled off, at a sharp angle to the epidermis of the leaflet. They were then immersed in 50% (v/v) ethanol, for 10 minutes, and immersed in 1% (v/v) sodium hypochlorite to complete tissue bleaching. Sections of the lower and upper epidermis measuring about 1 $cm^2$ were rinsed in distilled water and colored with toluidine blue. Each peel was placed on a slide and examined with a light microscope (Carl Zeiss, Germany). One hundred and fifty randomly chosen microscopic fields of each species were used to determine the number of stomata per unit of area. Each field included 0.18 $mm^2$ at 400X magnification.

### Frequency and length of trichomes

Leaflet segments (2 $cm^2$) were maintained under pressure between two glass slides. Thirty randomly chosen stereomicroscopic (Carl Zeiss, Germany) fields for ten plants were used for countings and measurements of trichomes in both epidermis. Each field included 20 $mm^2$ at 40X magnification. Trichome measurements were performed on three indiscriminately selected trichomes, in each stereomicroscopic field, with the help of a micrometric ocular.

### Cuticle thickness

Fresh leaflet cross-sections were treated with ethanol and sodium hypochlorite, as described above , and colored with SUDAN IV. The cuticle thickness of the upper and lower epidermis of thirty leaflets sections of six plants were measured through a light microscope (Carl Zeiss, Germany) supplied with a filar micrometric eye-piece.

### Leaflet structure

Leaf strips were fixed in FAA (formalin:acetic acid:ethanol), dehydrated in ethanol, immersed in a xylene series and embedded in wax. Thin lamina cross-sections were cut from the strips with a microtome and stained with toluidine blue. Photomicrographs of thirty slides of five plants were taken using a Carl Zeiss-2 (Germany) light microscope.

## RESULTS AND DISCUSSION

### Frequency of stomata

At the average, the lower epidermis showed a number of stomata per square milimeter (frequency of stomata) equal to 213 ± 98 ($\bar{x} \pm sd$), while the upper epidermis showed a frequency of stomata equal to 101 ± 36 ($\bar{x} \pm sd$).

Stomatal frequencies found in this study were in the stomatal frequency range commonly found for other crops such as tobacco (Turner & Begg, 1973) and soybean (Manam et al., 1977). Low stomata frequency on the upper epidermis can be beneficial by increasing water use efficiency, through the reduction of water loss, when high light intensity strikes the upper epidermis and by increasing the internal $CO_2$ concentration of the leaf (Kramer, 1969; Monteiro et al., 1985). The occurrence of stomata on the upper and lower leaf epidermis has been considered as an adaptative mechanism to maximize leaf conductance to $CO_2$ when light and water are not limiting factors (Mott et al., 1982). So, amphistomatic leaflets, as found in this study, might implicate in elevated capacity to accumulate photoassimilates, through the potentially higher photosynthetic rates.

**Frequency and length of trichomes**

Similar to stomata, trichomes were present on the lower and the upper epidermis (Table 1). Frequency of trichomes of the lower epidermis was 44% higher than the frequency of trichomes found for the upper epidermis. Lower epidermis trichomes were barely longer when compared to those of the upper epidermis.

The presence of trichomes is usually associated with species adaptation to high light intensity and low water availability (Ehleringer, 1980; Monteiro et al., 1985). Leaf trichomes have been considered as an important ecophysiological factor contributing to an increase in the leaf boundary layer resistance (Ehleringer, 1985). The expansion of the boundary layer in pubescent leaves allows reduction of excessive water losses, especially under high wind speed, when compared to non-pubescent leaves (Schuepp, 1993).

The increase in the boundary layer can also aid in the maintenance of an optimum leaflet temperature since the presence of trichomes can significantly change the energy budget of leaves. Pubescent and non-pubescent leaves can be respectively 11°C and 5°C warmer than air, when the air temperature is 5°C and 6°C and 3°C warmer than air when the air temperature is 20°C (Meinzer & Goldstein, 1985).

The effects of increased aerodynamics resistance on the photosynthetic rates would be expected to be relatively less significant, compared with the corresponding effect on transpiration or heat transfer, because of the larger mesophyll resistance to $CO_2$ exchange. Not surprisingly, water-use-efficiency (amount of $CO_2$ fixed in relation to the amount of water lost) has been found to be positively related to frequency of trichomes (Schuepp, 1993).

The presence of trichomes might also function as a mechanism to optimize control of the incoming solar radiation,as well as the heat and water budget through an increase in leaf reflectance (Ehleringer, 1985).

The increase in leaf reflectance due to the presence of trichomes might have a protective effect through the avoidance of thermal and photoinhibitory damage of the photosynthetic apparatus in leaflets subjected to excessive light intensity, which would be especially useful during cold weather, and excessive heat load, when leaflets would be exposed to direct solar radiation during warm whether.

The climbing mechanics presented by vines are related to the physiological adaptation to high light intensity environments (Carter & Teramura, 1988). Consequently, the presence of trichomes might be useful for the reflectance of part of the excessive light intensity (at midday) and for the reduction of the heat load, particularly when light intensity is very high and heat loss through convection is low (low wind speed).

**TABLE 1. Frequency and length of trichomes on the upper and lower epidermis of tropical kudzu.**

| Epidermis | Frequency (trichomes.mm$^{-2}$)[1] | Length (mm)[1] |
|---|---|---|
| Upper | 9 ± 2 | 300 ± 11 |
| Lower | 13 ± 4 | 460 ± 10 |

[1]Numbers refer to mean ± standard deviation.

**Cuticle thickness**

For the upper epidermis, cuticle thickness of tropical kudzu measured 1.1 ± 0.3 μm ($\bar{x}$ ± sd), while for

the lower epidermis, cuticle thickness measured $1.0 \pm 0.3$ µm ($\bar{x} \pm sd$).

Cuticle thickness of about 1 µm is comparable to cuticle thickness of other mesophytic species (Ashton & Berlyn, 1992) and is thin if compared to cuticle thickness of plants found in dry environments such as *Didymopanax macrocarpum* (cuticle thickness of 5.7 µm) (Franco, 1983). Working with plants adapted to dry environments, Ferri (1961) found that plants provided with thick cuticle showed higher cuticular transpiration rates when compared to plants with thin cuticle. Ashton & Berlyn (1992) found no relationship between cuticle thickness and drought tolerance among three species of *Quercus*. In addition, other studies have demonstrated that chemical composition and the pattern of deposition of hydrophobic compounds in the cuticle might be the main factors reducing peridermal transpiration instead of cuticle thickness (Price, 1982).

**Leaflet structure**

Tropical kudzu presented a tissue distribution typical of mesophytic species. The upper and lower epidermis were unicellular. Palisade parenchyma consisted of two cell layers while spongy parenchyma consisted of one cell layer (Fig. 1). Between the palisade and the spongy parenchyma a layer of paraveinal mesophyll was found. The stomata, present in the upper and lower epidermis, were located at the same level in the epidermis layer, showing small substomatal chambers (Fig. 2).

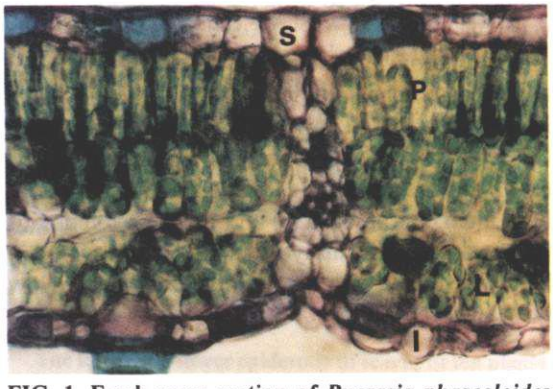


**FIG. 1. Fresh cross section of *Pueraria phaseoloides* leaflet at 50X magnification (S= upper epidermis; I= lower epidermis; P= palisade parenchyma and L= spongy parenchyma).**

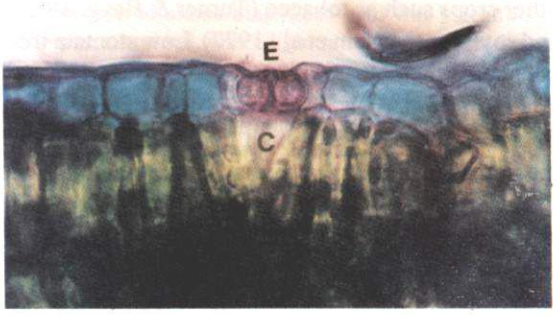


**FIG. 2. Fresh cross section of *Pueraria phaseoloides* leaflet at 80X magnification (E= stoma and C= substomatal chamber).**

Neither secretory nor structures that characterize adaptations to dry environments were found. The analyses of the kinds of tissues and the distribution of these tissues found in the leaflet blades in this study demonstrates that tropical kudzu leaflets do not present internal characteristics especially useful for the adaptation of this crop to colder and drier environments.

## CONCLUSIONS

1. Higher frequency of both stomata and trichomes in the lower epidermis indicates a trend to maximize $CO_2$ incoming and minimize water loss through the lower epidermis.

2. The presence of stomata and trichomes in the upper epidermis indicates a plant's ability to counteract excessive heat load avoiding excessive water loss.

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