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Change of whole-tree transpiration of mature *Hevea brasiliensis* under soil and atmospheric droughts: analyze in intermittent and seasonal droughts under the framework of the hydraulic limitation hypothesis.

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LIST OF ABBREVIATIONS

θ	volumetric soil water content
ψ_{crit}	critical minimum leaf water potential (MPa)
ψ_{crit_giv}	given critical minimum leaf water potential (MPa)
ψ_{Leaf}	leaf water potential (MPa)
$\psi_{predawn}$	predawn leaf water potential (MPa)
$\psi_{minimum}$	minimum leaf water potential (MPa)
ψ_{midday}	midday leaf water potential (MPa)
ψ_{Soil}	soil water potential (MPa)
ψ_{Xylem}	xylem water potential (MPa)
ΔS	soil water depletion (mm day^{-1})
Δt	A period of time (day)
$\Delta \mathrm{T_a}$	A transient or alternate signal
ΔT_{off}	temperature difference after the period of cooling
ΔT_{on}	temperature difference reached at the end of heating period
ΔT_{ua}	measured alternate signal at a given J_s
A_{sw}	the total cross sectional area of xylem
B	bark thickness
Ci	ratio of J_s to J_{s_out}
Cit.	*Citrus Maxima*
cm^3/100 cm^3 of soil	cubic centimeter per 100 cubic centimeter of soil
CTD	constant thermal dissipation method
D	leaf to air vapour pressure deficit (kPa)
E	tree water uptake (mm day^{-1})
E_{crit}	critical transpiration (mm day^{-1})
E_{Tree}	tree transpiration (mm day^{-1})
ET_0	reference evapotranspiration (mm day^{-1})
F_{tree}	total flow (L dm^{-2} h^{-1})
g_L	whole-tree hydraulic conductance (L dm^{-2} h^{-1} MPa^{-1})
g_s	stomata conductance

LIST OF ABBREVIATIONS (Cont.)

Hev.	*Hevea brasiliensis*
J_s	sap flux density (L dm^{-2} h^{-1})
J_{s_crit}	critical maximum sap flux density (L dm^{-2} h^{-1})
J_{s_est}	estimated maximum sap flux density (L dm^{-2} h^{-1})
J_{s_midday}	midday sap flux density (L dm^{-2} h^{-1})
J_{s_out}	sap flux density measured in the outmost ring (L dm^{-2} h^{-1})
K	flow index in CTD method
K_a	flow index in TTD method
K_{Tree}	conductance of whole sap pathway (mm day^{-1} MPa^{-1})
L dm^{-2} h^{-1}	liter per decimeter per hour
L dm^{-2} h^{-1} MPa^{-1}	liter per decimeter per hour per mega Pascal
Man.	*Mangifera indica*
mm day^{-1}	millimeter per day
mm h^{-1}	millimeter per hour
MPa	mega Pascal
REW	relative extractable soil water
RMSE	root mean square error
$RMSE_{rel}$	relative root mean square error
R_t	total radius
R_{Tree}	hydraulic resistance
TTD	transient thermal dissipation method
VPD	vapour pressure deficit (kPa)
z	depth of the root zone

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Chapter I

Introduction

INTRODUCTION

Rubber tree (*Hevea brasiliensis*) is grown for the production of natural latex. Commercial plantations are displayed over several continents of the world: Asia, Africa and South-America, but the main region of growing is south-east Asia.

Hevea brasiliensis is a brevi-deciduous tree, native from the tropical rainforest of the Amazon Basin. Its habitat is characterized by small variation in air temperature and precipitation throughout the year. Annual rainfall range between 1800 and 2500 mm (Pakianathan et al., 1989) and rainy days range between 100 and 150 (Watson, 1989) are considered as optimum for rubber tree growing. However, the rubber tree is now more and more cultivated in marginally suitable environmental zones or “non-traditional” areas, which are too cold or too dry. For instance, Thailand, the first world producer, had policies which have supported the extension of rubber tree plantation to the North and Northeast regions. In several areas of the Northeast, rubber tree plantations inevitably face atmospheric and soil droughts.

The south of northeastern Thailand is a new and large rubber growing area despite relatively low annual rainfall amount (1,200 mm) and five months of dry season. Hence, soil and atmospheric droughts occur mainly during the long seasonal dry period, from November to April, while defoliation occurs between January and February. However short intermittent droughts frequently occur during the rainy season, between June and July, when rains stop for several weeks. Such droughts should have dramatic consequence because the trees are in full canopy with newly mature leaves.

The importance of the water relations for growth and latex production is acknowledged (Pakianathan et al., 1989) and several authors have studied the impact of water constraints on tree water status, girth increment, canopy architecture and latex production (Chandrashekar et al., 1990; Chandrashekar, 1997; Chandrashekar et al., 1998; Devakumar et al., 1999; Gururaja Rao et al., 1990; Silpi et al., 2006). Detailed study on rubber tree water relations are few and focused on young trees (Ranasinghe and Milburn, 1995; Sangsing, 2004; Sangsing et al., 2004b). Little is known of the relationships between whole-tree transpiration and soil and atmospheric droughts, particularly for mature rubber trees in natural conditions.

Hydraulic models of the soil-plant-atmosphere continuum based on electric analogy and the cohesion-tension theory provide a simple integrated approach of the regulation of tree transpiration (Cochard et al., 1996; Sperry et al., 1998; Sperry et al., 2002; Tyree and Zimmermann, 2002). Evaporative demand is the driver of leaf transpiration. These models assume that the transpiration or xylem sap flow depend directly of the water potential gradient between the leaf-atmosphere interface and the soil-root interface and of the whole-tree hydraulic conductance, when the water storage is neglected.

Basically, atmospheric drought or high evaporative demand increases transpiration rates and lowers midday or minimum leaf water potential. On the other hand, soil drought decreases soil water potential and the conductivity at the soil-root interface and by consequence the whole-tree hydraulic conductance. To satisfy the same evaporative demand, the decline of the whole-tree hydraulic conductance induces a further decrease of the minimum leaf water potential. Then, when the decreasing xylem water potential reaches cavitation thresholds, embolism of xylem vessels provoke a further decrease of the whole-tree hydraulic conductance and a lethal cycle of runaway embolism can be engage (Cruiziat et al., 2002). A this point, several authors support the “hydraulic limitation hypothesis” which states that for a majority of species (i.e. isohydric species) regulation of transpiration through stomatal closure maintains xylem water potential above a certain threshold of cavitation to prevent this runaway embolism of xylem vessels (Cochard et al., 1996a; Jones, 1998; Sperry et al., 1998; Cruiziat et al., 2002).

Previous studies on young rubber trees found that rubber tree is relatively vulnerable to cavitation (Ranasinghe and Milburn, 1995; Sangsing et al., 2004b) and that stomatal control operates at the onset of xylem embolism in the petiole (Sangsing et al., 2004b). These results suggest that the hydraulic limitation hypothesis may well apply to rubber tree. Stomatal closure is the major short term response that regulates transpiration under both atmospheric and soil drought. However, reduction of transpiring leaf area and root growth in wetter soil layers are long term processes that may change dramatically the hydraulic conductance at the canopy-atmosphere and soil-root interface, respectively (Breda et al., 2006). These long term processes of leaf and root phenologies concern particularly the regulation of transpiration for deciduous trees facing seasonal drought such as rubber trees.

The general objective of this study was to test the framework of the "hydraulic limitation" hypothesis to describe the change of whole-tree transpiration of mature *Hevea brasiliensis* under both soil and atmospheric droughts. Continuous and accurate estimate of whole tree transpiration over a long period of time was a key measurement of this study. To achieve an accurate measurement of xylem sap flow rates, we applied a transient thermal dissipation method (TTD) developed by Do and Rocheteau (2002b) which has several advantages but which was never tested in rubber wood

Therefore, this work had four related objectives:

1. The first step was to test and validate for rubber tree wood, the measurement of xylem sap flow density by a simple transient thermal dissipation method.
2. The second objective was to assess particular thresholds of transpiration decline versus atmospheric drought, soil drought and tree water status in full canopy conditions.
3. The third objective was to test the ability a simple "hydraulic limitation" model, based on whole-tree hydraulic conductance and a critical minimum leaf water potential, to describe the main changes of transpiration under drought in full canopy conditions
4. The fourth objective was to study the change of transpiration in the seasonal dry period where long term responses to drought (leaf senescence and shedding, leaf flushing, root growth in the subsoil) are susceptible to interact and change the relationships.

These objectives were addressed in this thesis under the form of three scientific publications. Before the papers, a first part reviews the literature about general features of Rubber tree, drought definition and measurements, whole-tree transpiration and hydraulic parameters, hydraulic limitation hypothesis and modeling. A final part provides a general conclusion.

Chapter II

Literature Review and Experiments

LITERATURE REVIEW AND EXPERIMENTS

1. Botany, plantations, tree characteristics and yield

1.1 Botany

The genus Hevea is a member of the Euphorbiaceae family which comprises 10 species, of which the Para rubber tree, *Hevea brasiliensis* Muell Arg., is the only one commercially planted (Webster and Paardekooper, 1989). The rubber tree originates from the Amazon forest. This species is virtually the only source of natural rubber (Cilas et al., 2004).

1.2 Rubber plantations

Rubber plantation in the world concerns more than 10 million hectares nowadays, 92.3% in Asia and 7.7% in Africa and others. Thailand is the first world producer. Rubber plantation covered 2.4 million hectares (or 15 million rais) which produced 3 million tons in 2007 (RRIT, 2009). The tree is normally suited to the wet tropical climate. The optimal temperature and annual rainfall are 28^{o}C and 1,800-2,500 mm, respectively. It is why in Thailand the traditional area of rubber growing is the southern part of the country.

According to the ever-increasing demand for natural rubber and to the lack of available lands, rubber tree growing is extended in non-traditional area such as in northeastern Thailand. In this region, the large rubber growing areas can be separated between favourable locations in the north-east part along the Mekong River and water limited locations in the south-east part.

In the south-east part, average annual rainfall ranges between 1,000 mm and 1,200 mm. The rainy season generally lasts from April to October and the seasonal dry period comprises approximately 5 months. Therefore, the rubber trees planted in this area currently experience 4 months of both soil and atmospheric droughts. Moreover, intermittent drought or “mid-drought” of several weeks occurs regularly in the middle of the wet season, between June and July. Moreover, this area is assumed to have a low availability of water in the subsoil.

1.3 Tree characteristics, leaf phenology, growth and yield

The rubber tree is a quick-growing, erect tree with a straight trunk and a bark which is usually grey and fairly smooth. In the natural wild, it may grow up to over 40 m and live for over 100 years, but in plantations they rarely exceed 25 m height, and they are replanted after 25-35 years when yield falls (Webster and Paardekooper, 1989). The leaves are trifoliate, and the laminae hang downwards with a bronze color when emerge. The leaf expansion follows a sigmoid curve. During the first 5 days after leaf unfolding, the expansion increases slowly and then rapidly from 5-12 days; the leaf becomes fully expanded thirteen days after unfolding (Sangsing et al., 2004a). The mature laminae are shiny dark green on their upper surface and light green below. Samsuddin et al. (1978) reported that the period from emerged to mature of leaves lasts 35 days. The leaves exhibit a full rate of photosynthesis 50 to 60 days after emergence (Samsuddin and Impens, 1979).

Rubber trees older than 3 or 4 years are subject to 'wintering', which is the term used to describe the annual shedding of senescent leaves. The leaf shedding is partial or complete for a short period of few weeks (Webster and Paardekooper, 1989). Latex yields usually decreased slightly at the onset of leaf fall, and are more markedly reduced during re-foliation (Webster and Paardekooper, 1989; Sanjeeva Rao et al., 1998). Leaf fall is normally followed within 2 weeks by the terminal bud bursting and by the expansion of new leaves within further weeks (Webster and Paardekooper, 1989). In the south part of northeastern of Thailand, usually leaf yellowing starts at the end of December, massive leaf shedding occurs between the end of January and the onset of February, and bud emergence is noticed at the end of February. The phenological stage of fully mature leaves eventually last from May to November-December.

Rubber tree starts to be tapped according to the average trunk girth in each plot. In Thailand, the tree is considered mature when the girth attains 50 cm at 1.5 meters height above ground. This maturity is usually achieved around 5-6 years after planting in traditional conditions and around 8-10 years in sub-optimal conditions (Chardrashekar et al., 1998). More generally, growth of rubber tree varies with clones (Chandrashekar, 1997; Chandrashekar et al., 1998; Pathiratna et al., 2006), planting density (Pathiratna et al., 2006), climatic season (Silpi et al., 2006), air temperature

(Jiang 1988), drought occurrence (Devakumar et al., 1999), irrigation (Vijayakumar et al., 1998), tapping systems (Gunasekara et al., 2007; Silpi et al., 2006) and others. Typical radial growth pattern in water-limited areas of Thailand, starts at the onset of rainy season and lasts until the onset of dry season, girth growth completely ceases in the driest period (Silpi et al., 2006).

Latex is issued from a secondary metabolic pathway and exuded from the trunk after a deep tapping of the bark. The latex contains on average 60 to 70 % of water, so the tree water status and the availability of water in the soil are important limiting factors of rubber production (Pakianathan et al., 1989). Accordingly, several authors report that rubber yield decreases in the dry season (Chandrashekar et al., 1990; Gururaja Rao et al., 1990). Consistently, the highest flow rates of latex generally occur during wet months when growth rate are maximal (Pakianathan et al., 1989). Several meteorological parameters have been related to yield: temperature, sunshine duration, pan evaporation and vapor pressure deficit are negatively correlated, whereas only rainfall is positively correlated (Sanjeeva Rao et al., 1998).

2. Drought

2.1 Definition and primary effects

Drought is difficult to define precisely. It is a period of time where water limited conditions induces prolonged plant water deficits and reduces growth. Larcher (2001) quoted that drought causes stress in plants if not enough water is available at a suitable thermodynamic status. This situation can occur for a variety of reasons, such as soil dryness, high evaporation, osmotic binding of water in saline soils or in frozen soil. Breda et al. (2006) pointed out that drought stress occurs whenever soil water availability drops below a threshold inducing restriction to transpiration and growth. Frequently, but not invariably, soil dryness is coupled with strong evaporation caused by dryness of the air (Larcher, 2001). Reversely an atmospheric drought may occur despite available water in the soil.

The primary effects of the water deficit in the plant are to decrease cell water content, turgor, and the free energy status or potential of the remaining water (Kozlowski et al., 1991). Finally, ecophysiologists can evaluate plant water stress in several terms: turgor loss, growth reduction, stomatal closure, transpiration decrease and inhibition of processes such as photosynthesis and disturbance of the normal course of other processes such as nitrogen and carbohydrate metabolism.

2.2 Evaporative demand

2.2.1 Definition

The evaporative demand corresponds basically to the existence at the leaf level of a vapour pressure gradient between the leaf and the surrounding air ("leaf to air" VPD; Larcher, 2001). This is the driving force of the leaf transpiration which pulls water fluxes along the soil-tree-atmosphere continuum. Hence, whole-tree transpiration or sap flow rate are highly related to the evaporative demand when other factors are non limiting (Granier et al., 1996; Meinzer et al., 1997; Irvine et al., 1998; Meinzer et al., 1999; Meinzer, 2003; David et al., 2004; Bush et al., 2008; Huang et al., 2009).

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2.2.2 Reference evapotranspiration measurement

Several climatic parameters or formula are used to represent the evaporative demand according to the background, scale and focus of studies. Air VPD is more often used by ecophysiologists who studied individual plant responses (David et al., 1997; Meinzer et al., 1997; David et al., 2004; Bush et al., 2008; Huang et al., 2009; Woodruff et al., 2010).

Studies considering water balance per unit of soil and larger scale use more complete formula based on the energy balance such as previously the potential evapotranspiration (*PET*) according to Penman equation or now the reference evapotranspiration (ET_0) according to Penman-Monteith equation and FAO recommended coefficients.

In this thesis, ET_0 was used to represent the evaporative demand according to the details given by Allen et al. (1998):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34 u_2)} \qquad (1)$$

where ET_0 = reference evapotranspiration (mm day^{-1}),
R_n = net radiation at the crop surface (MJ m^{-2} day^{-1}),
G = soil heat flux density (MJ m^{-2} day^{-1}),
T = air temperature at 2 m height (°C),
u_2 = wind speed at 2 m height (m s^{-1}),
e_s = saturation vapour pressure (kPa),
e_a = actual vapour pressure (kPa),
$e_s - e_a$ = saturation vapour pressure deficit (kPa),
Δ = slope vapour pressure curve (kPa °C^{-1}),
γ = psychrometric constant (kPa °C^{-1}).

2.3 Soil water availability

2.3.1 Definition

Water infiltrates the soil following precipitation and gradually percolates to the ground water table. The maximum soil water content of natural soils that remains after gravitational percolation is called field capacity. The plant can withdraw water from the soil only as long as the water potential (ψ) of its fine root is more negative than that of the soil solution in its immediate surrounding (Larcher, 2001). When the soil dries out sufficiently, the soil water potential (ψ_{soil}) fall below root water potential (ψ_{root}) and plant cannot withdraw water what is called the wilting point. Therefore, the available water in the soil is usually ranged between field capacity (ψ_{soil} between -0.01 and -0.03 MPa) and permanent wilting point (ψ_{soil} = -1.50 MPa). Hence, the state of water in the soils can be described in terms of quantity or water content, and in terms of energy status or water potential which is the most important for the availability to the plant (Rundel and Jarrell, 1989). There is a unique relationship between soil water content and soil water potential which changes according to soil texture (Figure 1).

The soil water availability is often expressed from actual water content as a relative value compared to values at field capacity and permanent wilting point. In the thesis, we have used the "relative extractable water" (*REW*) as defined by Granier et al. (1999) and Breda et al. (2006). *REW* may be computed at any given time, from soil water content in the root zone as follows:

$$REW = EW/EW_0 \tag{2}$$

where, EW is the actual extractable soil water and EW_0 is the maximum extractable water. With more details:

$$REW = (W-W_m)/(W_F-W_m) \tag{3}$$

where, W is the actual water content, W_m is the minimum soil water content (lower limit of water availability or permanent wilting point) and W_F is the soil water content at field capacity.

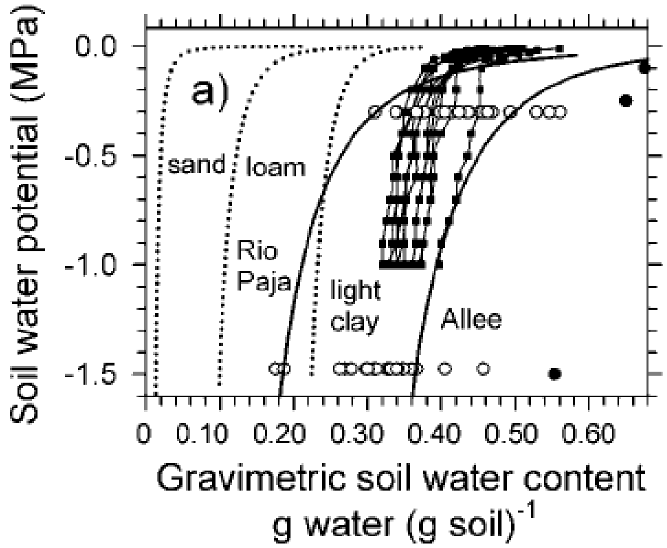

Figure 1 The relationship between soil water potential and gravimetric soil water content of several soil textures.
(Source: Kursar et al., 2005)

2.3.2 Soil moisture measurement

There are several techniques of soil moisture measurement such as the gravimetric method, the neutron probe count, the time-domain reflectometry and more recently the capacitive probe. In this thesis work, we used both the neutron probe and the capacitive probe.

The measurement principle of the neutron probe is based on the emission of fast neutrons which are slowed down when they strike a body of similar mass, such as a hydrogen nucleus. The number of slowed neutrons detected is proportional to the number of collision between neutron and hydrogen nuclei, which in turn reflects mainly the soil water content. Each individual probe should be calibrated for each general soil type and soil layer when the percentage of clay markedly changes. Usually, the soil surface layer requires a specific calibration. The calibration is generally done versus gravimetric method. This measurement is well proved and it usually gives the best accuracy but it is difficult to use in automatic monitoring (Rundel and Jarrell, 1989). Moreover its general use is more and more reduced due to the safety needed for the management of a radioactive source.

The capacitive probe is a new technique safe and well adapted to continuous monitoring. It measures the relative electric permittivity of soils which mainly depends of water content except in electrically conducting soils (Robinson et al., 1998). Usually the probe is vertical and includes several sensors corresponding to each depth of measurement. For absolute measurement, each sensor needs to be calibrated for each particular soil and textural layer. The sensor produces magnetic frequencies which have to be scaled between maximum value in the water and minimum value in the air. Then the scaled frequencies are converted in volumetric water content through calibration curves. The scaled frequencies can be calibrated versus gravimetric method or cross-calibrated versus another reliable measurement which may give a large range of soil water content values (e.g. neutron probe measurements (Girona et al., 2002).

3. Tree transpiration and hydraulic parameters

3.1 Whole tree transpiration

3.1.1 Estimate and sap flow measurement

Whole-tree estimates of water use are increasingly important in forest science and crop science (Wullschleger et al., 1998). Several techniques have been used such as weighing lysimeters, large-tree porometer, ventilated chambers, radio-isotopes, stable isotopes and xylem sap flow measurements (Rundel and Jarrell, 1989). Since twenty years, automatic measurements of xylem sap flow rate have became from far the most applied techniques to estimate whole-tree transpiration in the field (Wullschleger et al., 1998). They are now routinely used with concurring stand-level (energy balance, eddy correlations) or leaf-level measurements (porometry) to better understand the relationships between components and scales of the system. These techniques use the heat as a tracer with mainly three principles of measurement depending the methods: heat pulse, heat balance and heat dissipation.

The continuous thermal dissipation (CTD) method of Granier (1985, 1987) is largely applied due to its simplicity and low cost. This method uses two sensors, each containing a thermocouple inserted perpendicularly into the sapwood (Figure 2A and 2B). The downstream sensor is heated and the measured difference in temperature between the sensors narrows as sap flux density increases. Granier (1985) established the relationship between the temperature difference and sap flux density empirically by testing the system on cut stems in laboratory. To avoid the influence of natural thermal gradients between the two probes and to obtain more stable zero-flux references. Do and Rocheteau (2002a, 2002b) developed on the same probe basis a transient thermal dissipation method (TTD) based on cycles of heating and cooling.

Due to its analogy with the original CTD method, the response of the thermal index was assumed to be independent of the woody species and the first calibrations were performed on a synthetic porous media (sawdust). The TTD method has been used on several tree species including *Acacia tortilis* (Do and Rocheteau, 2002b; Do et al., 2008), *Adansonia* sp. (Chapotin et al., 2006a, 2006b), *Hevea brasiliensis* (Isarangkool Na Ayutthaya et al., 2007, 2008), and *Olea europea* (Abid-Karray et al., 2007).

However, to our knowledge no calibration-validation studies on these species have been performed. *Therefore, the first objective of the thesis was to test the validity of the calibration for rubber tree wood before to use it.*

3.1.2 Atmospheric drought effect on transpiration

The constraining effects of evaporative demand on transpiration are less documented than soil water shortage consequences. However, the midday stomatal regulation in well watered soil conditions is a well known phenomenon. Figure 3 from

Meinzer et al. (1997) illustrated a classical comparison of transpiration pattern between cloudy and sunny days.

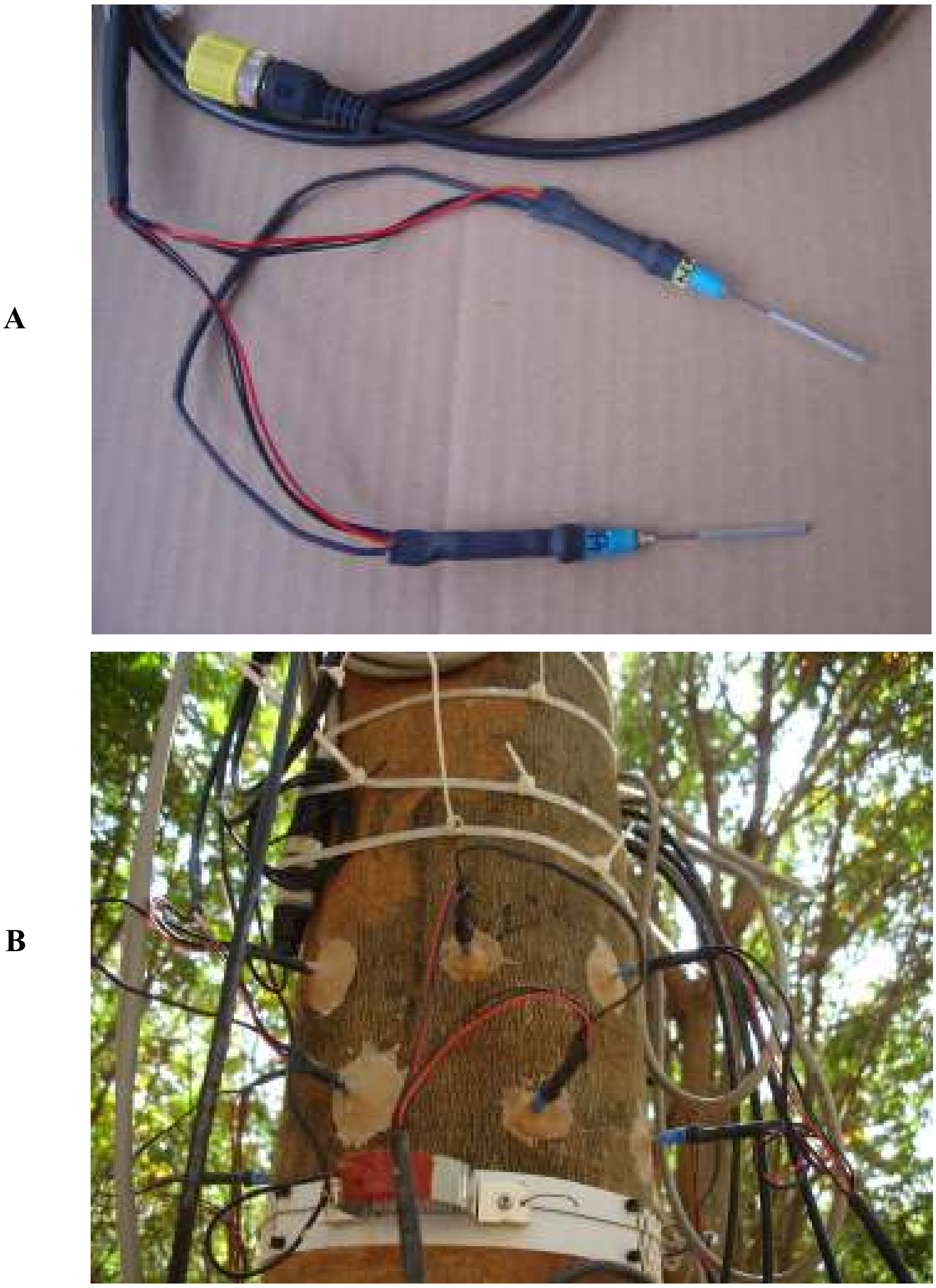

Figure 2 A) Granier-type probes of 2-mm diameter and 20-mm long sensors (UPgmBh, Cottbus, Germany). The yellow probe and blue probes are heating and reference probes, respectively.

B) Probes setting on the tree trunk, the heating probe is on the top and the reference probe is on the bottom.

For the cloudy day, i.e. with low evaporative demand, the transpiration of *Populus trichocarpa* x *P. deltoides* hybrid increased according to the *VPD* and radiation. For the clear day, i.e. with high evaporative demand, transpiration steeply increased in the morning and abruptly reached a plateau from 10 hr to 17 hr despite steadily changing radiation and *VPD*. Accordingly, the maximum value of canopy conductance was reached at 10:00 h and decreased steadily for the remaining hours of the day.

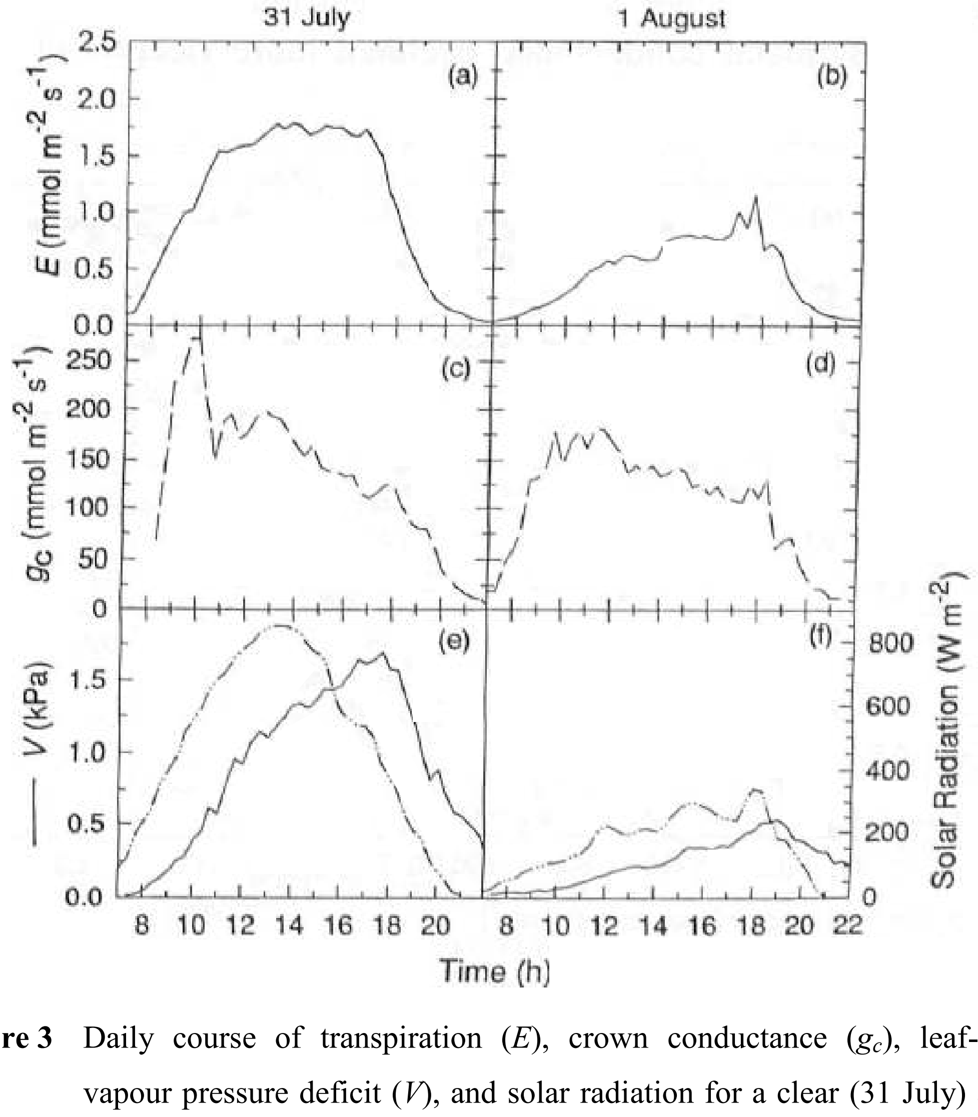

Figure 3 Daily course of transpiration (E), crown conductance (g_c), leaf-to-air vapour pressure deficit (V), and solar radiation for a clear (31 July) and a partly cloudy (1 August) day of *Populus trichocarpa* x *P. deltoides* hybrid. (Source: Meinzer et al., 1997)

Similarly, the response of stomatal conductance versus VPD has a well known pattern (Fernandez et al., 1997; Comstock and Mencuccini, 1998; Cochard et al., 2002; Meinzer, 2003; Woodruff et al., 2010). At low evaporative demand, stomatal conductance increases with increasing light and evaporative demand, it reaches its maximum value at intermediate evaporative demand, it steadily declines as evaporative demand continues to increase. The combination between increasing *VPD* and decreasing stomatal conductance induces a plateau of leaf transpiration. Similar conclusions were recently drawn for whole-tree transpiration of several species estimated with sap flow measurement.

In evergreen oak tree (*Quercus rotundifolia*), midday transpiration rates remained approximately constant for *VPD* higher than 1.5 kPa (David et al., 2004). The mean daily sap flux of *Populus grandidentata*, *Betula papyrifera*, *Acer rubrum* and *Quercus rubra* exhibits saturated values when VPD increased also above 1.5 kPa in either wet or dry soil (Bovard et al., 2005). Similarly, Bush et al. (2008) found that sap flux density (J_s) of *Gleditsia triacanthos*, *Quercus rubra*, and *Quercus gambelii* increased with increasing of *VPD*, and reached maximum J_s around 2 kPa of *VPD*. However, in *Platanus acerrifolia* which is a diffuse-porus wood species, transpiration increased almost linearly with *VPD* up to 5 kPa (Bush et al., 2008).

There are no available results on rubber trees in the literature despite the importance of this response for the extension in drier areas. More knowledge should allow to improve the choice of planting climatic areas, the clone selection and discussion of the interest of irrigation. *Therefore, one objective of the thesis was to study, for the main clone planted in Thailand and south-east Asia, the relationship between transpiration and evaporative demand and to check the existence of a particular threshold of regulation despite availability of water in the soil.*

3.1.3 Soil drought effect on transpiration

The negative effects of soil drying on transpiration at different scales of measurement have been extensively studied. Recent works applying sap flow measurements have confirmed in the field the dramatic decline of whole-tree transpiration versus soil drought in *Citrus limon* (L.) Brurm. fil (Ortuno et al., 2004; Ortuno et al., 2006), *Cyclobalanopsis glauca* (Huang et al., 2009), *Eucalyotus* *globulus* (David et al., 1997), *Olea europaea* L. (Tognetti et al., 2004), *Prunus armeniaca* (Alarcon et al., 2000; Ruiz-Sanchez, 2007), *Prunus persica* (Conejero et al., 2007), and *Quercus petraea* (Breda et al., 1993).

The plot of relative transpiration (E_{Tree}/PET or E_{Tree}/ET_0) or relative canopy conductance versus relative extractable soil water (*REW*), or any ratio of soil water availability, generally provides the same characteristic pattern.

The values are stable and maximum at high *REW* and they start to decreases below a threshold between 0.5 and 0.3 *REW* below 0.2 corresponds to severe soil droughts where relative transpiration could be reduced by 80 % (Granier et al., 1999; Sinclair et al., 2005; Breda et al., 2006). These results concerned mainly the temperate zone.

The change of transpiration has been studied under seasonal drought for mature rubber trees and compared between clones (Gururaja Rao et al., 1990). However, the authors analyzed change with time but did not provide relationship versus soil water availability and response thresholds. *Hence, a third objective of the thesis was to study the relationship between relative transpiration and soil drought.*

3.2 Water potential

3.2.1 Definition and measurement

The concept of water potential (ψ) is the key physiological parameter of plant water relations. It defines the thermodynamic or energy status of water within the plant (tissues and cells) and along the soil-plant-atmosphere continuum (Taiz and Zeiger, 1991; Kozlowski and Pallardy, 1997). The value of ψ is always negative or nil; however, in the cell the component of pressure potential (ψ_p) can be positive with turgor. The gradient of potential is the driving force of water flow and the water flows towards the more negative values across cell membranes, tissues and in the whole soil-plant-atmosphere continuum.

For instance, the atmosphere surrounding the leaf corresponds to water potential c.a. one hundred times lower than water potential in the leaf. Hence when stomata opens, the huge gradient of water potential dramatically draw the water from the leaf and the leaf water potential decreases.

The pressure chamber is the reference measurement of water potential or sap tension for plant samples (Scholander et al., 1965; Boyer, 1967). The sample of leaf, shoot or root is introduced within the chamber with a cut end protruding outside and exposed to the atmospheric pressure. The pressure is increased until xylem sap starts to appear. At this point, the positive pressure applied is assumed to equilibrate the sap tension existing in the intact stem.

3.2.2 Predawn and midday values

According to the Ohm's law analogy (Van den Honert, 1948) and the Cohesion-Tension theory (Tyree and Zimmermann, 2002), the water flow from soil to leaves can be efficiently described as simple hydraulic model where the flow is proportional to water potential gradients, the coefficient of proportionality being analog to a hydraulic resistance or its reverse a hydraulic conductance:

$$F \quad = \quad 1/R_{plant}(\psi_{Soil} - \psi_{Leaf}) \quad = \quad g_L(\psi_{Soil} - \psi_{Leaf}) \qquad (4)$$

where ψ_{Soil} and ψ_{Leaf} are soil water potential (MPa) and leaf water potential (MPa), respectively. F is the sap flux density (normalized by sap wood area; L dm^{-2} h^{-1}), R_{plant} is the plant hydraulic resistance (MPa L^{-1} dm^{2} h) and g_L is whole tree hydraulic conductance (L dm^{-2} h^{-1} MPa^{-1}) on the whole soil-to-leaf pathway.

Then a simple expression of ψ_{Leaf} may be deduced from equation (4):

$$\psi_{Leaf} \quad = \quad \psi_{Soil} - (F/g_L) \qquad (5)$$

It shows that the fluctuations in ψ_{Leaf} are determined by the variation in sap flux density, i.e. mainly by the transpiration, and by the hydraulic conductance if the soil water potential surrounding the roots remain constant.

Thus within the diurnal operational range two extreme values are characteristics: The maximum value measured at predawn ($\psi_{predawn}$) before the increase of transpiration which is assumed close to the soil water potential surrounding roots. And the minimum value measured at midday ($\psi_{minimum}$ or ψ_{midday}) which corresponds to the maximum of transpiration. The operational range of ψ_{Leaf} is species-specific (Tyree and Sperry, 1988; Cochard et al., 1996; Lu et al., 1996).

Logically drought generally induced a decrease of leaf water potential as observed for *Carica papaya* (Mahouachi et al., 2006), *Citrus* sp. (Ruiz-Sanchez et al., 1997; Ortuno et al., 2004; Ortuno et al., 2006; Garcia-Orellana et al., 2007), *Eucalyptus* spp. (Eamus et al., 2000; O'Grady et al., 2008), *Prunus salicina* Lindl. (Intrigliolo and Castel, 2006), *Prunus armeniaca* L. (Ruiz-Sanchez et al., 2007) and *Quercus petraea* (Breda et al., 1993).

Additionally, equation 5 shows why $\psi_{predawn}$ is often used as a reliable indicator of the average soil water potential surrounding the roots (Richter, 1997; Donovan et al., 2001). Hence, a threshold value of $\psi_{predawn}$ is often used as a surrogate of soil water availability to define the onset of water stress and transpiration regulation. However, the relative influence of soil or atmospheric droughts on $\psi_{minimum}$ varies depending on species.

Some plants exhibited reduction of both $\psi_{predawn}$ and $\psi_{minimum}$ whereas in other species $\psi_{minimum}$ remains stable such as for *Eucalyptus gomphocephala* (Franks et al. 2007), *Juniperrus osteosperma* (Linton et al. 1998), *Populus euramericana,* and *Zea mays* L. (Tardieu and Simonneau 1998). Hence, plants have been separated schematically between 2 groups: the isohydric and anisohydric species (Tardieu and Simonneau, 1998; Franks et al., 2007; Maseda and Fernandez, 2006; West et al., 2007). In isohydric plants, a tight control of transpiration through stomatal closure allows to stabilize $\psi_{minimum}$ above a discernable threshold. By contrast, anisohydric species have a less strict control by stomata, and express no discernible threshold of $\psi_{minimum}$ (West et al., 2007). These differences of stomatal regulation will have important consequences on carbon assimilation and growth under drought and on the speed of water depletion. Therefore, the pattern of $\psi_{minimum}$ under drought is an important indicator of the type of hydraulic regulation of the transpiration.

3.2.3 Drought effect on leaf water potential in rubber tree

The results from literature on mature rubber are not clear about the pattern of $\psi_{minimum}$ under drought. Comparing seasons, Chandrashekar et al. (1990) suggested an anisohydric behaviour under drought when $\psi_{minimum}$ decreased from -1.3 MPa in wet season to -1.8 MPa in dry season. But we are not sure that this comparison refers to similar sunny days, the difference could be related to difference of evaporative demand and magnitude of transpiration.

Additionally, lower values of $\psi_{predawn}$ have been noticed in seasonal drought but no threshold for water stress or transpiration decline was clearly assessed (Chandrashekar et al., 1990; Chandrashekar, 1997; Gururaja Rao et al., 1990). *Hence, another objective of the thesis was to study the operational range of leaf water potential under drought conditions and particularly to assess the pattern of* ψ_{midday} *under drought.*

3.3 Whole tree hydraulic conductance

3.3.1 Definition and measurement

As expressed in equation 4, the whole-tree hydraulic conductance (K_{plant}) is the coefficient of proportionality between the sap flow rate and the gradient of water potential. Hence, this relationship implies that K_{plant} (or its reverse the resistance) is the constant slope of a linear relationship between change of ψ_{Leaf} and sap flow rate. This assumes that the sap flux is conservative from soil to leaves, i.e. that the effect of water storage in the plant (capacitance) is quantitatively negligible which may be not true in diurnal kinetic depending species (O'Grady et al., 2008). The whole-tree hydraulic conductance can be also expressed per unit of leaf area instead of sapwood area which can be useful for comparison with stomatal conductance (Meinzer, 2003).

Basically, K_{plant} can be estimated from equation 4 by the linear regression method ("multipoint"), plotting diurnal change of sap flux density versus leaf water potential (Cochard et al., 1996, Lu et al., 1996). Equation 4 can be also simplify to estimate K_{plant} (also called g_L) from the two characteristic values of leaf water potential ("single point" method, Cochard et al., 1996):

$$g_L = J_{s\_midday} / (\Psi_{predawn} - \Psi_{midday}) \qquad (6)$$

where g_L is whole tree hydraulic conductance; J_{s_midday} is the daily maximum flux density; and $\Psi_{predawn}$ and Ψ_{midday} are predawn and midday leaf water potential, respectively.

Figure 4 from Lu et al. (1996) illustrates a successful comparison between the multipoint and the single point method to estimate g_L.

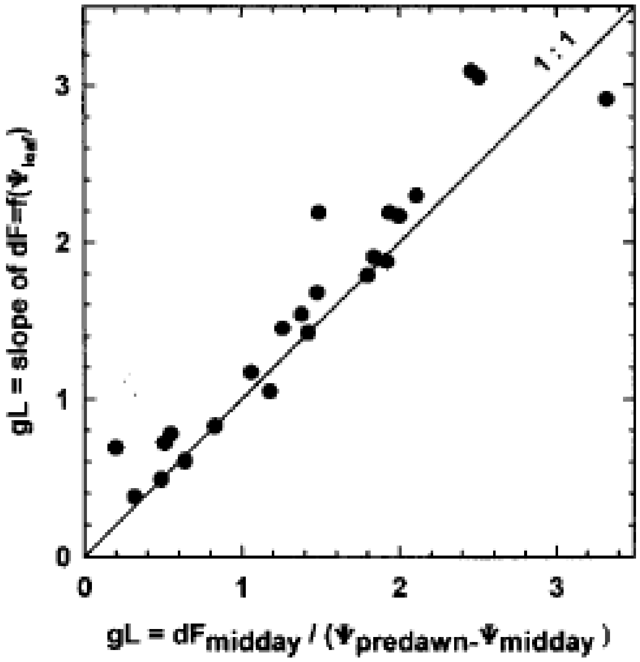

Figure 4 Whole tree specific hydraulic conductances (g_L) of *Picea abies* (L) Karst, g_L was calculated either as the slope of linear regression between the daily variations in leaf water potential (ψ_{leaf}) and sap flux density (dF; Y-axis) or as the ratio between the daily maximum flow density (dF_{midday}) and difference between the predawn and minimum leaf water potentials ($\Psi_{predawn}$ - Ψ_{midday}). The two techniques yielded similar results ($n = 24$, $r^2 = 0.91$, slope not different from one at $P = 0.05$).
(Source: Lu et al., 1996)

The components of g_L have been investigated to understand its processes of regulation. As simplified electric analog circuit, g_L, can be parted in four components (Sack and Holbrook, 2006): soil conductance (K_{soil}), root conductance (K_{root}), stem (K_{stem}), leaf conductance (K_{leaf}). g_L was found mainly dependent of K_{root} and K_{leaf}, which account together for more than 70% of the plant hydraulic resistance (Cruiziat et al., 2002; Sack et al., 2003; Sack and Holbrook, 2006; Domec et al., 2009; Passos et al., 2009).

3.3.2 Drought effect on whole tree hydraulic conductance

Drought-induced changes in g_L have been demonstrated in many species. For instance in *Bursera simaruba*, *Calycophyllum candidissimum*, *Enterolobrium cyclocarpum*, *Gliricidia sepium* and *Rhedera trinervis* (Brodribb et al., 2002), *Juglans regia* x *nigra* (Cochard et al., 2002), *Picea abies* (L) Karst (Lu et al., 1996), *Pinus sylvestris* L. (Irvine et al., 1998), *Prunus armeniaca* cv. Bulida (Alarcon et al., 2000), *Pinus palustris* Mill. (Addington et al., 2004), *Pinus taeda* L. (Domec et al., 2009) and *Quescus petraea* (Cochard et al., 1996a; Breda et al., 1993). It was related to the decrease of soil water availability. In this case, the decrease of g_L is mainly explained by the decrease of the hydraulic conductance at the soil-root interface. Moreover, a further decrease of g_L may be explained by xylem embolism due to the decrease of xylem water potential (Cruiziat et al., 2002).

Additionally, Domec et al. (2009) studied the relationship between whole tree hydraulic conductance (K_{tree}) and the conductance in roots and in leaves under both soil and atmospheric drought. They found that the change of K_{tree} and corresponding response of stomatal conductance (g_s) to VPD were mainly driven by K_{leaf} under high soil water availability and by K_{root} under low REW.

Moreover, recent studies have drawn attention on modifications of hydraulic conductance in leaves and roots by the effect of active processes. Diurnal changes in root hydraulic conductance have been ascribe to changes in plasmalemma or tonoplast aquaporins that act as water channels controlling water fluxes between cells (Martre et al., 2002). For instance, within the leaves, it has been shown that the main resistance in the liquid phase is extravascular (Tyree and Zimmermann, 2002). Hence, active processes may play a key role in the control under drought of the hydraulic conductances in the bottlenecks of leaves and roots.

For the rubber tree, the results of Sangsing (2004) in young potted trees support the good applicability of the whole-tree hydraulic conductance approach. A quick increase of R_{tree} was noticed under soil drought. *Hence, one objective of the* *thesis was to analyze for mature rubber trees under field drought how the decrease of transpiration is related to change of whole-tree conductance and thresholds of environmental conditions.*

3.4 Stomatal regulation

3.4.1 Interaction with whole tree hydraulic conductance

Stomatal conductance (g_s) is not a direct component of the whole-tree hydraulic conductance which concerns only the liquid phase. However, stomatal regulation plays the key role of coupling and short term adjustment between the gaseous phase and liquid phase water transfers in the soil-plant-atmosphere continuum while maximizing carbon assimilation (Whitehead, 1998; Franks, 2004).

To better understand the main interactions, Whitehead (1998) provided a simplified conservative equation neglecting water storage:

$$E = g_s D A_l = g_L(\psi_{Soil} - \psi_{Leaf}) A_{sw} \qquad (7)$$

where E is the canopy transpiration, g_s is stomatal conductance, D is leaf-to-air vapour pressure deficit, A_l is the leaf area, A_{sw} is the sapwood cross sectional area, others parameters are the same than in equation 4.

From this equation, it is understandable that an isohydric species growing in varying conditions of evaporative demand and soil water potential will have to dramatically adjust g_s, A_l, K_{plant} and A_{sw} to insure water potential homeostasis.

3.4.2 Drought effect on stomata

The decrease of stomatal or canopy conductance at high evaporative demand despite soil water availability is well known for temperate and tropical rain forest species (Meinzer et al., 1999; Granier et al., 2000a; Granier et al., 2000b; Meinzer, 2003). Such process may explain the observation of saturated whole-tree transpiration above a certain threshold of VPD or ET_0 (Breda et al., 1993; David et al., 2004; Bovard et al., 2005; Bush et al., 2008). However, the exact mechanism is not known and it is still a critical area of research.

Previous studies assumed a direct response of stomata to vapour pressure deficit is the feed forward response (Farqhuar, 1978). However, more recent studies suggest that this is a feedback response to leaf transpiration and whole plant water status (Franks et al., 1997; Meinzer et al., 1997; Monteith, 1995; Mott and Buckley, 1998; Domec et al., 2009)

The effect of soil drought is better understood. Basically, the induced decrease of ψ_{Leaf} may have a direct effect on g_s through its impact on leaf turgor (Cochard et al., 2002) or through interaction with stomata sensibility to ABA (Tardieu and Simonneau, 1998).

For young potted rubber trees, Sangsing (2004) found that a strong relationship between stomatal conductance and leaf water potential. For two clones (RRIM600 and RRIT251), the stomatal closure was maximum when leaf water potential reached to –2.2 MPa.

3.5 Influence of leaf phenology on drought responses

3.5.1 General features

As described in equation 7, to prevent excessive dehydration plant can express short term reversible responses like stomatal closure and long term responses like reducing transpiring leaf area, increasing root development in wetter soil layers, decrease of the active sapwood area (Breda et al., 2006).

Reducing leaf area is a major phenological adaptation of deciduous trees to seasonal drought in the dry tropics (Eamus and Prior, 2001). Moreover, the different stages of leaf phenology may correspond to particular changes of root phenology: root decay or root growth in different soil layers according to soil water availability. These processes should influence tree water relations and g_L, particularly the leaf and root parts which contribute to more than 70 % of the total hydraulic resistance (Becker et al., 1999; Nardini and Tyree, 2000; Brodribb et al., 2002; Domec et al., 2009).

Leaf senescence and shedding should decrease the total leaf hydraulic conductance. Following soil drying, root decay may decrease furthermore the soil to root conductance. Reversely, root growth in the wetter subsoil could increase soil to root conductance and K_{root}. At last, leaf flushing should increase the hydraulic conductance of leaves.

3.5.2 Phenology of rubber in seasonal dry period

In the water limited area of northeast Thailand, the period of leaf yellowing-leaf shedding-bud bursting-leaf flushing normally takes around five months, from December to April. Hence, such period of relatively low green leaf area should markedly reduce whole-tree transpiration. The dry season generally lasts from November to April.

In the same area, Gonkhamdee et al. (2009) had followed growth dynamics of fine roots of rubber trees down to 450 cm. They found that the onset of the dry season (November) corresponded to a period of active growth in the subsoil from 100 to 400 cm depth. After a rest period, root growth appeared again in the very deep soil between 300 and 400 cm around the time of massive leaf flushing in March. The onset of the rainy season (May) corresponded to an active growth in the top soil above 100 cm. The higher root length density was found above 50 cm. Root decay was observed above 100 cm at the end of the rainy season in September-October.

Guardiola-Claramonte et al. (2008) stress up for rubber tree the importance of including leaf phenology in soil water balance model to correctly predict the trend of water uptake in dry season. Moreover the same authors found a shift of root water uptake from topsoil in the onset of dry season to subsoil at the end of the dry season when leaf flushing occurs.

Hence, one particular objective of thesis was to compare the change of water regulation under drought between period with full canopy where dominate short term response like stomatal regulation and period of leaf shedding like in seasonal dry period where the leaf area dramatically changes.

3.6 Hydraulic limitation hypothesis

3.6.1 Embolism

Previous paragraphs explained that water in the xylem is under tension, and that this tension increases as transpiration rate increases or soil water potential decreases. If the tension in the water column becomes too great, embolism (gas bubbles) occurs within the xylem vessel, and cavitation (breaking of water column) occurs. Then the xylem conduit becomes permanently or temporarily dysfunctional and there is a loss of hydraulic conductivity of the xylem (Tyree and Sperry, 1989).

The vulnerability to cavitation is measured by the xylem pressure potential (ψ_{xp}) that induces cavitation; a more vulnerable vessel or tracheid will cavitate at a less negative ψ_{xp} (Tyree et al., 1993).

Additionally, Zimmermann (1983) introduced the hypothesis of "plant segmentation" which states that during periods of severe drought, embolism will first occur in the terminal part of trees (ie, leaves and small branches) where water potential are the lowest. This has been demonstrated in several temperate species (Tyree et al., 1993).

3.6.2 Critical minimum leaf water potential

Stomatal control of leaf transpiration and loss of hydraulic conductivity in twigs have been monitored in parallel in a range of species during the course of drought (Cruiziat et al., 2002). A tight coordination was evidenced between stomatal closure and induction of embolism: usually, embolism begins only when stomatal conductance drops below 10% of initial values. This supports the "hydraulic limitation" hypothesis that a tight control of water loss protects the xylem against drought-induced embolism (Jones and Sutherland, 1991; Tyree and Sperry, 1988). The range of water potential between full stomatal closure and onset of cavitation corresponds to a safety margin (Sperry and Pockman, 1993). In most species, this margin is narrow, meaning that tree transpiration operates close to the cavitation induction point.

The consequence of this tight regulation is the observation a critical minimum water potential (ψ_{crit}) above which the operational water potential is maintained. This value varies largely depending on species. For example, ψ_{crit} in shoot water potential of *Thuja occidentallis*, *Acer scacharum*, *Cassipourea elliptica* and *Rhizophora mangle* were -1.8, -2.0, -1.6 and -4.0 MPa, respectively (Tyree and Sperry, 1988). In *Quercus petraea* (Cochard et al., 1996a) and *Picea abis* (Lu et al., 1996), the Ψ_{crit} were -2.8 and -2.5 MPa, respectively.

The combination of equation 6 and hydraulic limitation hypothesis is the basis of RER model (Cochard et al., 1996a, Lu et al., 1996) which is similar to the ones developed by Tyree and Sperry (1988) and Sperry et al. (1989):

$$J_{s\_crit} = g_L(\Psi_{predawn} - \Psi_{crit}) \qquad (8)$$

where J_{s_crit} is the estimated critical maximum sap flux density and ψ_{crit} is the critical leaf water potential at the completely stomata closure period. This equation allows to estimating the critical value of minimum leaf water potential from recordings of leaf water potential and xylem sap flow rates.

3.6.3 Simple transpiration model in water-limited conditions

The 'hydraulic limitation' hypothesis provided a very simple but process-based model to analyze tree transpiration under water stress. According to equation 8 it is possible to compute a critical transpiration (E_{crit}) corresponding to ψ_{crit}:

$$E_{crit} = (\Psi_{soil} - \Psi_{crit}) \times g_L \times a \qquad (9)$$

where E_{crit} (mm day^{-1}) is critical maximum tree transpiration, g_L (L dm^{-2} h^{-1} MPa) is whole tree hydraulic conductance per sapwood area and a is coefficient to transform maximum sap flux density to total flow of tree transpiration per day and unit of soil area. ψ_{soil} and ψ_{crit} (MPa) are represented by predawn leaf water potential and critical minimum leaf water potential that estimated from equation 8, respectively.

According to the hydraulic limitation hypothesis, E_{crit} set a functional limitation to E_{Tree} that can be reached under drought conditions (low ψ_{soil} and low g_L values) but also when the evaporative demand is very high (ψ_{crit} and g_L max). The climatic conditions (e.g. ET_0) set also a maximum transpiration which cannot be exceeded by E_{crit}.

By combining both limiting effects of ET_0 and E_{crit} on tree transpiration (Cruiziat et al. 2002, Figure 5), it is possible to construct a simple model for E_{Tree}:

$$E_{Tree} = Min(E_{crit}; ET_0) \qquad (10)$$

In this model, it is assumed that soil drought affects E_{crit} by its effect on the g_L and $\psi_{predawn}$ or ψ_{soil}.

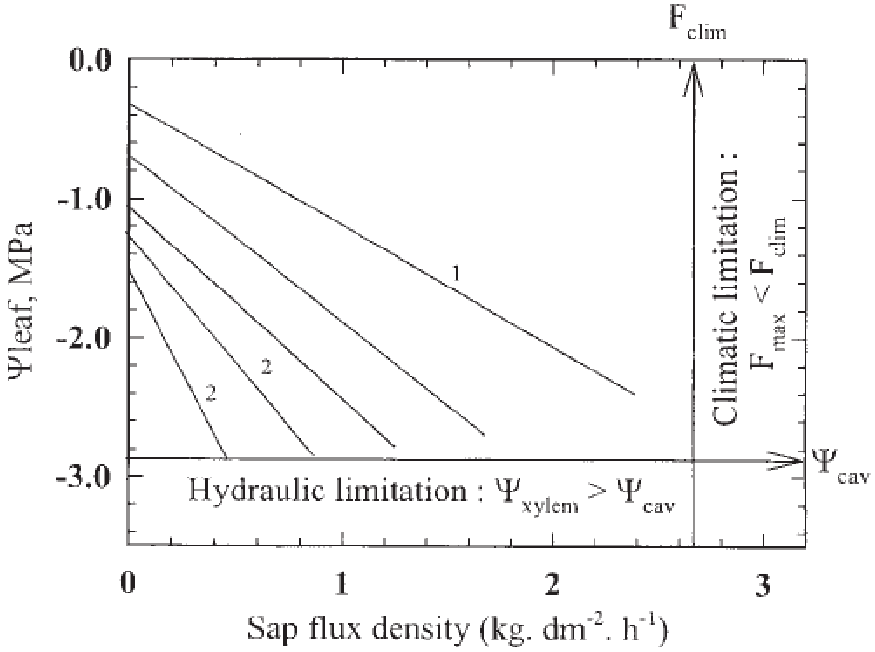

Figure 5 Factor controlling maximum water loss. Flux/Potential relationships help in understanding daily maximum transpiration rates in *Quercus*. For well watered trees (line 1), F_{max} is probably limited by climatic conditions such as light level, air vapor pressure deficit or CO_2 concentration. However, for water-stressed trees (lines 2), whole hydraulic resistances increased (steeper slopes) causing xylem water potential (Ψ_{xylem}) to reach values close to water potential at cavitation point (Ψ_{cav}).
(Source: Cruiziat et al., 2002)

3.6.4 Embolism on rubber tree

There are no published results on native embolism and vulnerability to cavitation on mature rubber trees. However, there are several insights that suggest an isohydric behaviour for mature rubber trees with relatively strict stomatal control of transpiration.

Studying young potted trees, Ranasinghe and Milburn (1995) and Sangsing et al. (2004b) both found high values of xylem tension corresponding to 50% of embolism in the petioles (between -1.5 and -2.0 MPa) which suggests that rubber tree is relatively vulnerable to cavitation. Moreover, Sangsing et al. (2004b) assessed that stomatal control operates at the onset of xylem embolism in the petiole. Finally, the minimum values of leaf water potential found under drought are within the same range (-1.8 to -2.2 MPa) for the rare results available on mature rubber trees (Chandrashekar et al., 1990; Gururaja Rao et al., 1990, Chandrashekar, 1997). *Therefore, the general objective of this thesis was to test the framework of the "hydraulic limitation" hypothesis to describe the change of whole-tree transpiration for mature Hevea brasiliensis under soil and atmospheric droughts.*

4. Experiments

4.1 General approach

To address the objectives detailed previously, we selected a mature (7 years old after planting and started tapping) and representative rubber tree stand in the drought prone area of Northeast Thailand. The key measurement was the continuous and long term monitoring of whole tree transpiration by applying xylem sap flow measurement. It was performed over a complete annual cycle including rainy season with intermittent short droughts and seasonal dry period with leaf shedding stage. Seasonal and diurnal variations of leaf water potential and whole-tree hydraulic conductance were measured with concurrent observation of canopy phenology and recordings of atmospheric and soil water conditions.

4.2 Materials

4.2.1 Location and characteristics of the field experiment

The plantation is located at Baan Sila site (N15^{o} 16′ 23″ E103^{o} 04′ 51.3″), Khu-Muang, Burirum province in northeast Thailand (Figure 6). The experiments were conducted in a monoclonal plot, clone RRIM600, planted at 2.5m x 7.0m spacing (the density 571 trees/ha) and tapped for 4 years or age 11 years old from planting (Figure 7A). The soil was a deep loamy sand. Mean contents of clay, loam, and organic matter varied from 9.9, 24.2 and 0.78% in the top soil (0-20) to 20.2, 23.6 and 0.34% at a depth of 1.5 m, respectively. In this non-traditional rubber tree plantation area, the environmental conditions are water limited for *H. brasiliensis*. The dry season lasts six months, from November to April, and average annual rainfall is 1,176 mm. In 2007, even drier climatic conditions occurred with an annual rainfall was less than 1,000 mm.

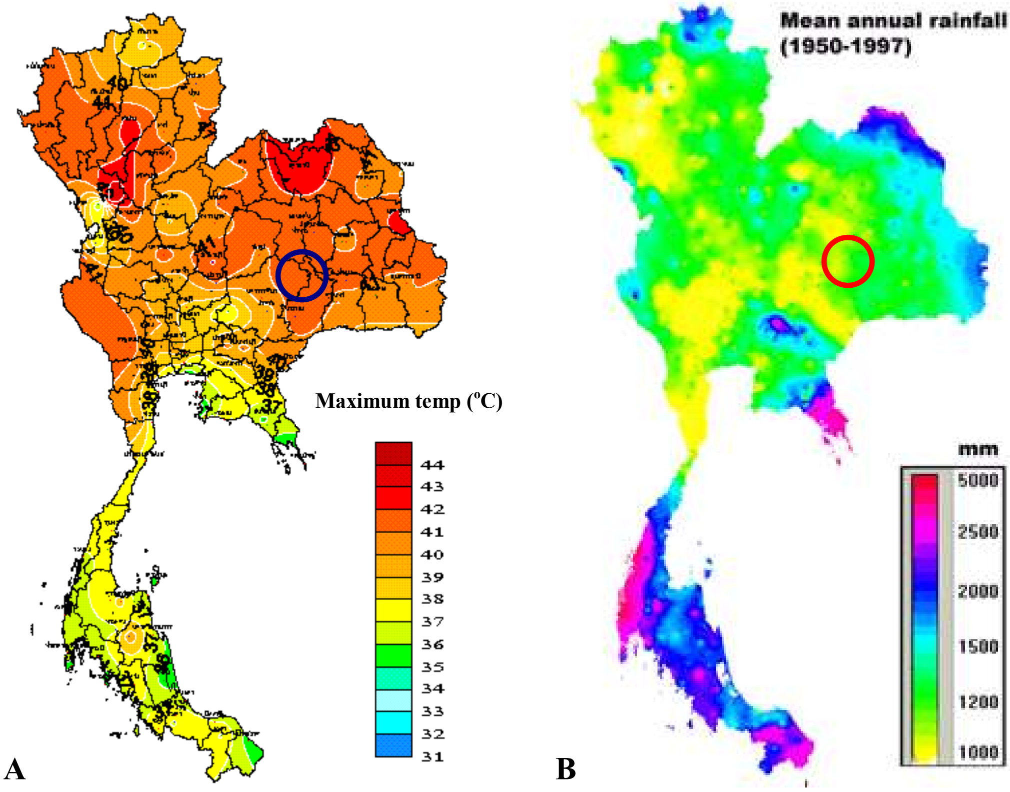

Figure 6 The maximum temperature (A) and annual rainfall (B) of Thailand and for Burirum province, which is in the southern part of northeast of Thailand, shown in the circles.

4.2.2 Detail in experimental trees

Figure 8 illustrates the layout of 11 experimental mature rubber trees which separated in 6 healthy trees (green with black cover) and 5 necrotic trees (red with black cover). The comparison between soil water depletion and tree transpiration in the multi-species xylem sap flow calibration part was done with all 11 experimental trees, while the investigation of effect of intermittent and seasonal droughts on tree transpiration was done on only 6 healthy trees.

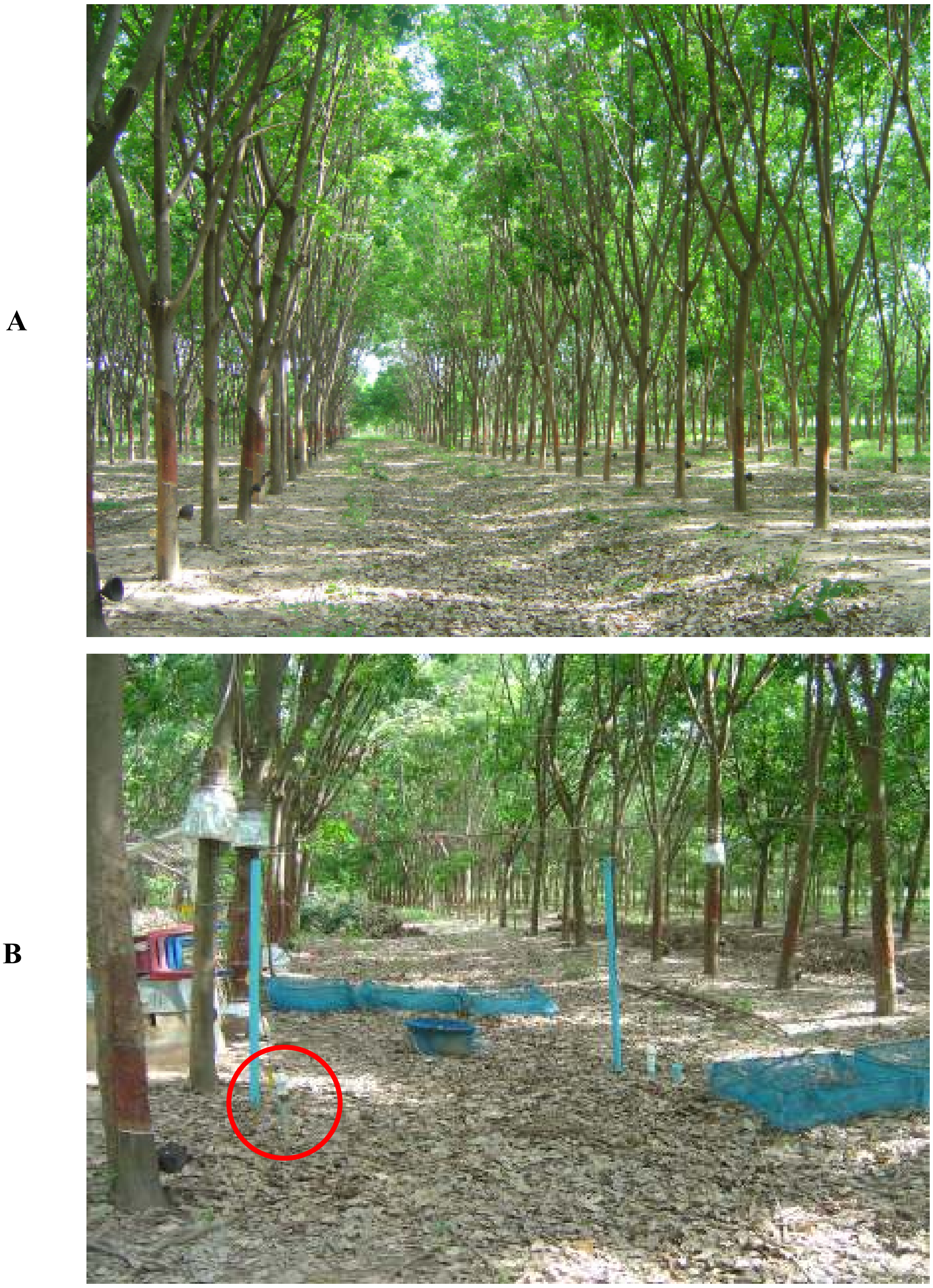

Figure 7 A) The plantation of rubber tree in this work that is located at Baan Sila (N15° 16′ 23″ E103° 04′ 51.3″), Khu-Muang, Burirum, northeast Thailand. The spacing is 7 x 2.5 m. and tapped for 4 years.

B) The instruments were installed in the experimental plantation such as neutron probe tubes, capacitive probe (red circle), and leaves collecting boxes.

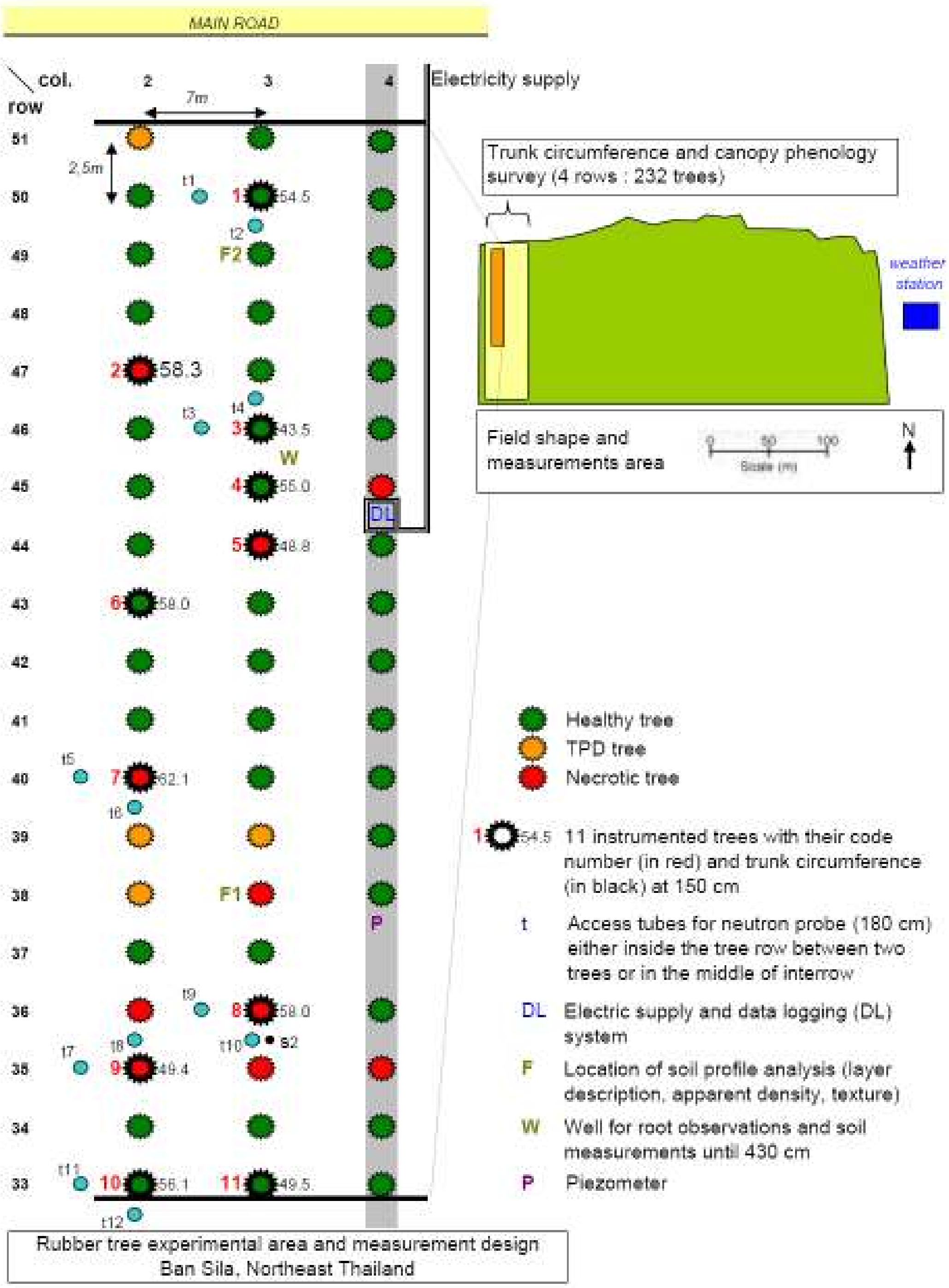

Figure 8 Schematic diagram showing the layout of 11 experimental mature rubber trees which separated in 6 healthy trees (green with black cover) and 5 necrotic trees (red with black cover). The sky blue circles indicate the position of installation of 12 neutron probe tubes.

4.3 Methods

4.3.1 Sap flow calibration on cut stem in the laboratory

The cut stems used for calibration experiments comprised three species of particular interest for our laboratory: *Hevea brasiliensis* (Rubber tree), *Mangifera indica* (Mango), *Citrus maxima* (Pummelo). The water flow rate (J_s) through the cut stem was controlled by a high pressure flow meter (HPFM, Dynamax Co., Houston, USA). The reference measurement of J_s was obtained by weighing water flowing out of cut segments (0.01 g accuracy balance, Adventurer^{TM}, Ohaus, Pine Brook, USA). Flow density ranged from 0.3 to 5.0 L dm^{-2} h^{-1}. Depending on the length of the cut segments and on the experiment, one or two sets of probes were inserted into the sapwood. The distance between needles of the same probe was 10 cm and the heated needle of probe 1 was separated from the reference needle of probe 2 by 10 cm too. Probe 1 was in upstream position. (Figure 9A) The same set of two probes was used for all tests and the probes were located at the same position. The probes were connected to a data logger (21X, Campbell Scientific, Leicester, U.K.). (Figure 9B)

4.3.2 Xylem sap flux measurements in the field

The measurements of xylem sap flow density were made using the transient thermal dissipation method (TTD) developed by Do and Rocheteau (2002) which is a modification of the continuous thermal dissipation method of Granier (1985). Probes were inserted into the trunks at a height of 1.8 m above the soil. After removal of the bark, the probes, 2-cm long probes were inserted into a hole of 2.5 cm deep within the sapwood, in such a way that the whole probe was inside the conductive sapwood. Three probes were inserted into each trunk to take circumferential variability into account. After the probe was inserted, the exposed parts of the needles were coated with silicone. The trunk area containing the probes was protected from direct solar radiation and rainfall by a deflector. Probes were connected to a data logger (CR10X, Campbell Scientific, Leicester, U.K.). (Figure 10)

A

B

Figure 9 A) Installation of cut stem with a high pressure flow meter (HPFM) and weighing water flowing out of cut segments by 0.01 g accuracy balance. Also, the two sets of Granier's type probe were inserted to the cut stem, which the distance between two set of probe was 10 cm.

B) A data logger 21X, Campbell Scientific with the relay electric boxes controlled turn on and turn off of heating in transient thermal dissipation method according Do and Rocheteau (2002b)

Figure 10 Installation of xylem sap flow probe in the experimental tree at a height of 1.8 m above the soil and the data logger (CR10X, Campbell Scientific, Leicester, U.K.) with multiplexer setting.

4.3.3 Leaf water potential measurements

Leaf water potential (ψ_{Leaf}) was measured on the six healthy experimental trees with a Scholander type pressure chamber (PMS 1000, PMS Instrument Company, Corwallis, Oregon, USA; Figure 11). Two trifoliate leaves with petiole were randomly selected from sunny locations on each experimental tree. ψ_{Leaf} measurements were performed in situ rapidly after cutting. Regular measurements of ψ_{Leaf} were carried out once or twice times per month, $\psi_{predawn}$, between 05:30 and 06:15 hours, and midday leaf water potential (ψ_{midday}), between 12:30 and 13:30

hours. Additionally, diurnal kinetics of ψ_{Leaf} measurements, i.e., every 1-2 hour from predawn to sunset, were performed on sunny days.

Figure 11 A Scholander type pressure chamber (PMS 1000, PMS Instrument Company, Corwallis, Oregon, USA).

4.3.4 Leaf area index

Leaf area index was calculated from total leave area, which collected by the leaves collecting boxes (Figure 7B), divide by spacing size of leaves collecting boxes (1 m^2). The leaves collection was done in leaves shedding period during November 2007 to February 2008. Leaf area was measured by leaf area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, Nebraska USA).

4.3.5 Climatic measurements

Local microclimate was automatically monitored in an open field, 50 m from any trees (Figure 12). A datalogger (Minimet automatic weather station, Skye Instruments Ltd, U.K.) recorded half hourly values of air temperature, relative humidity, incoming short wave radiation and rainfall. A reference potential evapotranspiration (ET_0) was calculated according to Allen et al. (1998).

Figure 12 Local microclimate (Minimet automatic weather station, Skye Instruments Ltd, U.K.) was automatically monitored in an open field, 50 m from any trees.

4.3.6 Soil water content measurements with a neutron probe

Volumetric soil water content (θ) was measured with a neutron probe (3322, Troxler, Research Triangle Park, North Carolina, USA; Figure 13A) calibrated for the experimental soil with separated calibrations between upper (0-0.2 m) and lower (below 0.2 m) layers (Figure 13B). The highly linear relation in both upper and lower layer express following these equations:

Upper layer

$$\theta = (0.0436NP) - 0.7957 \qquad ; R^2 = 0.94, n = 19 \qquad (11)$$

Lower layer

$$\theta = (0.04NP) - 4.0296 \qquad ; R^2 = 0.89, n = 126 \qquad (12)$$

where θ is volumetric soil water content and NP is neutron probe value.

Twelve tubes of 2.0 m in length were set up, six along the rows and six between the rows (Figure 8; sky blue circles). Measurements every 0.2 m, from 0.1m until 1.7 m depth, were performed every month or two weeks. According to soil water fluctuations, the soil profile was separated between two layers, a top soil (0-0.4 m) and a subsoil (0.4-1.8 m).

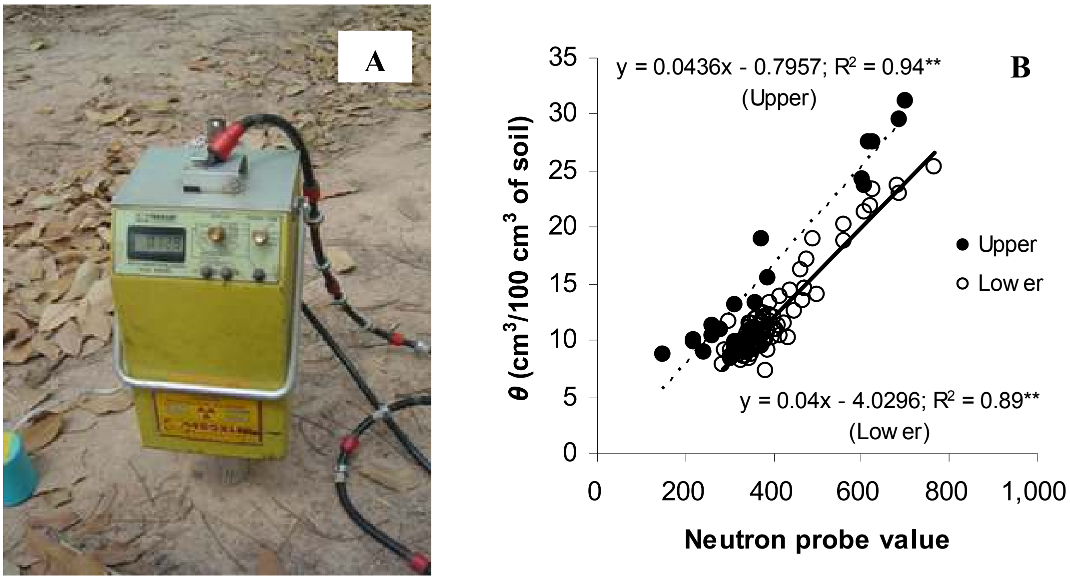

Figure 13 A) A neutron probe (3322, Troxler, Research Triangle Park, North Carolina, USA).
B) Relationship between volumetric soil water content (*θ*) and neutron probe value separated calibrations between upper (0-0.2 m; closed circles) and lower (below 0.2 m; opened circles) layers. The dotted line and continuous line indicate the tendency in upper layer and lower layer, respectively.

4.3.7 Continuous soil water content measurements

Continuous θ was measured with a capacitive probe (EnvironSCAN System, Sentek Sensor Technologies, South Australia, Australia; Figure 14) within a single tube close to a tube dedicated to neutron probe measurement (tube No. t4; Figure 7B and 8). Capacitive sensors were located at the same levels than neutron probe measurements. For each capacitive sensor, θ was estimated from a cross-calibration with the neutron probe measurements over the whole season range. To estimate continuous change of the average soil water profile, linear regressions were performed between θ of the average soil water profile (12 neutron probe access tubes) and θ of the profile continuously measured with the capacitive probe according Girona et al. (2002).

Figure 14 A capacitive probe (EnvironSCAN System, Sentek Sensor Technologies, South Australia, Australia).

Chapter III

Result I:
Transient thermal dissipation method of xylem sap flow measurement: multi-species calibration and field evaluation

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Transient thermal dissipation method of xylem sap flow measurement: multi-species calibration and field evaluation

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Summary The transient thermal dissipation (TTD) method developed by Do and Rocheteau (2002*b*) is a close evolution of the original constant thermal dissipation (CTD) method of Granier (1985). The TTD method has the advantage of limiting the influence of passive natural temperature gradients and of yielding more stable zero-flux references at night. By analogy with the CTD method, the transient method was first calibrated on synthetic porous material (sawdust) on the assumption that the relationship was independent of the woody species. Here, our concern was to test the latter hypothesis with a 10-min heating time in three tropical species: *Hevea brasiliensis* Müll. Arg., *Mangifera indica* L. and *Citrus maxima* Merr. A complementary objective was to compare the field estimates of daily transpiration for mature rubber trees with estimates based on a simplified soil water balance in the dry season. The calibration experiments were carried out in the laboratory on cut stems using an HPFM device and gravimetric control of water flow up to 5 L dm^{-2} h^{-1}. Nineteen response curves were assessed on fully conductive xylem, combining 11 cut stems and two probes. The field evaluation comprised five periods from November 2007 to February 2008. Estimates of daily transpiration from the measurement of sap flow were based on the 41 sensors set up on 11 trees. Soil water depletion was monitored by neutron probe and 12 access tubes to a depth of 1.8 m. The calibrations confirmed that the response of the transient thermal index to flow density was independent of the woody species that were tested. The best fit was a simple linear response (R^2 = 0.88, n = 276 and P < 0.0001). The previous calibration performed by Do and Rocheteau (2002*b*) on sawdust fell within the variability of the multi-species calibration; however, there were substantial differences with the average curve at extreme flow rates. Field comparison with soil water depletion in the dry season validated to a reasonable extent the absolute estimates of transpiration acquired with the 10-min TTD method. In conclusion, evidence for the independence of calibration from woody species and the simple linear response of the thermal index strengthen the interest of the TTD method with 10-min heating.

Keywords: Citrus maxima, cut stem experiment, Granier's sensors, *Hevea brasiliensis*, *Mangifera indica*, soil water balance, tree transpiration, validation.

Introduction

During the last 20 years, the wide use of automatic thermal techniques to measure sap flow has been crucial in improving our understanding of the hydrological cycle, community ecology, whole-plant physiology and tradeoffs between water use and carbon acquisition (Granier et al. 1996, Burgess et al. 1998, Wullschleger et al. 1998, Eamus and Prior 2001, Meinzer et al. 2003, Roberts et al. 2005, Breda et al. 2006, Sevanto et al. 2008).

One of the most commonly used techniques to measure sap flow in trees is the constant thermal dissipation (CTD) method developed by Granier (1985, 1987). This method uses two needle sensors inserted radially into the sapwood. Each sensor contains a thermocouple, and the sensors are connected and yield a differential measurement of temperature. The downstream sensor is constantly heated, and the measured difference in temperature decreases when the sap flux density rises because the flow increases heat dissipation through a convective effect. Granier (1985) developed a flow index (K), which is the ratio between the difference in temperature at zero flow and at measured flow. The non-linear calibration between K and flow density holds for several woody species (Granier 1985) and for synthetic porous media (Granier unpublished data). Because of its empirical basis, Smith and Allen (1996) recommended checking the calibration for each new woody species. Applying the CTD method and Granier-type probes, several authors observed values close or identical to the original calibration: Cabibel and Do (1991) on *Malus domestica* Borkh., Lu and Chacko (1998) on *Mangifera indica* L., Braun and Schmid (1999) on *Vitis vinifera* L., Clearwater et al. (1999) on *Eucalyptus deglupta* Blume, *Anacardium excelsum* (Bert. & Balb.) Skeels and *Bursera simaruba* (L.) Sarg., Lu et al. (2002) on *Musa* 'cavendish', Do and Rocheteau (2002*b*) on a sawdust column, McCulloh et al. (2007) on *Pseudobombax septenatum* (Jacq.) Dugand and *Calophyllum longifolium* Willd., based on the cut stem experiments in the laboratory, pot or lysimeter experiments. A few authors found differences which they assumed to be due to the effect of woody species (de Oliveira Reis et al. 2006 on *Carica papaya* L.), or due to the configuration of the probe (Roupsard et al. 2006). Hence, calibration of the CTD method is mainly considered to be independent of the species even if some sort of validation is still recommended (Lu et al. 2004).

To avoid the influence of natural thermal gradients between the two probes and to obtain more stable zero-flux references, Do and Rocheteau (2002*a*, 2002*b*) introduced a non-continuous heating system with a cycle of heating and cooling. The temperature signal used became time-related: it was the difference between the temperature reached at the end of the heating period and the temperature reached after the cooling period. the final temperature reached after the cooling period. By analogy with the original CTD method, the flow index (K_a) was considered as the ratio of the signal at zero flow to the signal at measured flow. Due to its analogy with the original CTD method, the response of K_a was assumed to be independent of the woody species and the first calibrations were performed on a synthetic porous media (sawdust). The authors found a similar K_a response to flow density with different cycles of heating and cooling: 45/15, 30/30, 15/15 and 10/10 min. The calibration was very different from the CTD method because of the transient conditions, i.e., after 10 min of heating, the temperature reached a slow kinetic phase, but for low and zero flow rates it was far from equilibrium and the equilibrium was not completely reached even after 30 min (Do and Rocheteau 2002*a*). This transient thermal dissipation (TTD) method has now been used on several tree species including *Acacia tortilis* (Forsk.) (Do and Rocheteau 2002*b*, Do et al. 2008), *Adansonia* sp. (Chapotin et al. 2006*a*, 2006*b*), *Hevea brasiliensis* Müll. Arg. (Isarangkool Na Ayutthaya et al. 2007, 2008) and *Olea europaea* L. (Abid-Karray et al. 2008). However, to our knowledge, no calibration study on these species has been published. Therefore, this work had two aims: the first was to test the hypothesis that calibration of the TTD method is independent of the woody species and porous media. The response to flow density of the K_a index calculated with 10 min of heating was evaluated in three tropical species: *H. brasiliensis*, *M. indica* and *Citrus maxima* Merr. Several sets of cut stems were processed with *H. brasiliensis* wood to assess the variability of response curves. We expected to find a calibration similar to the one obtained by Do and Rocheteau (2002*b*) on synthetic porous material (sawdust). The second related objective was to compare the estimates of daily transpiration between sap flow measurements and the soil water balance in the dry season in a mature stand of *H. brasiliensis*. We expected the values to be close if the hypothesis of tree transpiration estimates from the simplified soil water balance holds.

Materials and methods

TTD method of sap flow measurement

The TTD method (Do and Rocheteau 2002*a*, Do et al. 2008) is a close evolution of the original CTD method of Granier (1985, 1987). It is based on the change over time in the difference in temperature between two probes inserted radially into the xylem, one heated and other unheated, with a cyclic schedule of heating and cooling on the heated probe. Granier's probe of 2-mm diameter and 20-mm-long sensors were used (UP gmBh, Cottbus, Germany). When the heating power is adjusted to 0.200 W, it induces a maximum temperature difference of 8–12 °C after 10 min under zero flow conditions. Do and Rocheteau (2002*b*) found an empirical relationship between sap flux density (J_s; L dm^{-2} h^{-1}) and an index of the change in the temperature difference, denoted alternate flow index (K_a; dimensionless).

The calibration was performed on a synthetic porous media (sawdust in a plastic-glass cylinder). K_a was determined as below.

$$K_a = 1/(1 + 11.3 J_s^{-1.414}), \text{ or}$$
$$J_s = (11.3 K_a/(1 - K_a))^{0.707}.$$

A transient or alternate signal (ΔT_a) was defined as

$$\Delta T_a = \Delta T_{on} - T_{off},$$

where ΔT_{on} is the temperature difference reached at the end of the period of heating and ΔT_{off} is the temperature difference after the period of cooling.

The alternate flow index was calculated as

$$K_a = (\Delta T_{0a} - \Delta T_{ua})/\Delta T_{ua},$$

where ΔT_{0a} is the maximum alternate temperature difference obtained under zero flow conditions and ΔT_{ua} is the measured alternate signal at a given J_s.

To measure J_s every half hour with a heating period of 10 min, a cycle of 10-min heating and 20-min cooling was applied and the temperature signals were recorded every

Table 1. Characteristics of experimental cut stems used for calibration of the TTD method on three species: *H. brasiliensis* (*Hev.*), *M. indica* (*Man.*) and *C. maxima* (*Cit.*). Parameter K_h is hydraulic conductance measured by HPFM and normalized by length and xylem area. ΔT_0 is the maximum temperature difference at zero flow. Acronyms P1 and P2 indicate probe 1 and probe 2 when two probes were inserted into the same cut stem.

Cut stem acronym	Length (cm)	Diameter (cm)	Dry density (g/cm^3)	Stem water content (cm^3/cm^3)	K_h [kg s^{-1} MPa^{-1} dm^{-2}]	Sapwood area (dm^{-2})		ΔT_0 (°C)	
						P1	P2	P1	P2
Hev. 1	55.20	3.93	0.49	0.54	0.47×10^{-5}	0.115	0.105	7.9	9.3
Hev. 2	53.75	4.38	0.52	0.51	5.78×10^{-5}	0.123		9.1	
Hev. 3	55.30	4.30	0.48	0.56	0.49×10^{-5}	0.108	0.095	9.4	10.3
Hev. 4	55.00	4.90	0.50	0.58	6.32×10^{-5}	0.143	0.139	7.9	7.9
Hev. 5	38.55	4.88	0.52	0.58	9.76×10^{-5}	0.151		7.9	
Hev. 6	37.75	5.08	0.48	0.55	11.01×10^{-5}	0.166		10.3	
Hev. 7	52.10	5.33	0.49	0.51	6.90×10^{-5}	0.153	0.151	10.5	9.6
Hev. 8	56.00	4.71	0.47	0.54	7.50×10^{-5}	0.145	0.141	9.3	10.0
Man. 1	56.85	3.88	0.42	0.69	5.19×10^{-5}	0.084	0.080	8.2	8.3
Man. 2	53.30	4.82	0.41	0.63	2.72×10^{-5}	0.115	0.109	8.8	9.6
Cit. 1	54.00	5.14	0.80	0.72	10.64×10^{-5}	0.168	0.144	9.6	10.2

10 min. According to Do and Rocheteau (2002*b*), ΔT_{off} is the temperature after 10 min of cooling. Here, the calculation of ΔT_{off} was slightly modified; the final temperature after 10 min of cooling was averaged with the initial temperature before heating, so after 20 min of cooling. Such modification yielded the same K_a but reduced the errors due to a quick change in natural thermal gradient, which may occur within 10 min in the early morning (Do unpublished).

Cut stem experiment in the laboratory

The characteristics of the cut stems used for calibration experiments are listed in Table 1. They comprised three species of particular interest for our laboratory: *H. brasiliensis* (rubber tree), *M. indica* (mango) and *C. maxima* (pummelo). The cut stems of *H. brasiliensis* received special treatment to avoid vessel blockage due to latex exudates. They were soaked for one full night in a water bath. For all species, after re-cutting each cut stem, a 2-cm-thick disc from both ends of stem was cut off and set up to enable capillary rise of a bromothymol blue solution. Only cut stems where wood staining was coarsely homogenous were retained for further analysis. The cut stems were connected to a high pressure flow meter (HPFM, Dynamax Co., Houston), which allowed the pressure, J_s and conductance to be controlled. The reference measurement of J_s was obtained by weighing water flowing out of cut segments (0.01 g accuracy balance, Adventurer™, Ohaus, Pine Brook). Flow density ranged from 0.3 to 5.0 L dm^{-2} h^{-1}. Depending on the length of the cut segments and on the experiment, one or two sets of probes were inserted into the sapwood. Aluminum tubes were inserted into the stem before insertion of the probes. The distance between needles of the same probe was 10 cm, and the heated needle of probe 1 was separated from the reference needle of probe 2 by 10 cm too. Probe 1 was in upstream position. The same set of two probes was used for all tests and the probes were located at the same position. These were connected to a data logger (21X, Campbell Scientific, Leicester, UK). Data, such as the weight of water from the balance, were recorded every 10 min. To ensure best contact between the probes (especially the heated one) and the sapwood, only set-ups with ΔT_{0a} below or equal to 10.5 °C were used (Table 1).

Sap flow measurements in the field

The field comparison of estimates of transpiration from the sap flow measurements and soil water balance was carried out in a mature stand of *H. brasiliensis* in northeast Thailand in a plantation of RRIM600 clones (15°16′23″ N and 103°04′51.3″ E) that were located close to Khu-Muang, Buriram province. The spacing was 2.5 × 7.0 m and the trees had been tapped for 4 years. The soil was a deep loamy sand. Mean contents of clay, loam and organic matter varied from 9.9, 24.2 and 0.78% in the top soil (0–20) to 20.2, 23.6 and 0.34% at a depth of 1.5 m, respectively. In this non-traditional rubber tree plantation area, the environmental conditions are water limited for *H. brasiliensis*. The dry season lasts for 6 months, from November to April, and the average annual rainfall is 1176 mm. In 2007, even drier climatic conditions occurred with an annual rainfall of 990 mm. Eleven trees were selected within the main classes of trunk girth. The trunk girth (measured at 1.5 m above the soil) ranged from 40 to 60 cm, yielding an average of 55 cm [standard deviation (SD) = 6.03] that corresponded to 17.5 cm in diameter.

The xylem area was estimated from the observations of cores and whole sections of freshly cut trees or branches from the stand for a wide range of girths. There was a strong relationship between bark thickness (B) and total radius (R_t), which allowed the deduction of xylem radius (R^2 = 0.93, B = 0.0822 R_t – 0.0287 and n = 30). Dying experiments with bromothymol blue showed that xylem was completely conductive, except at a pith of an almost

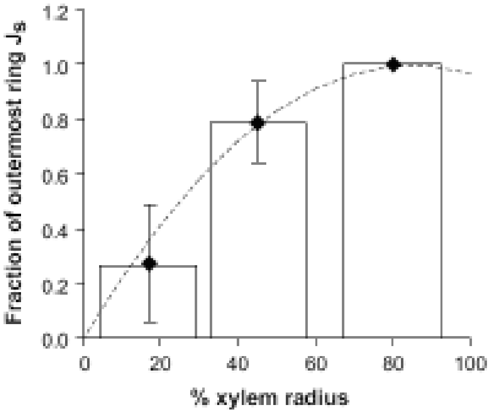

Figure 1. Radial pattern of relative sap flux density (J_s) in *H. brasiliensis*. The percentage of xylem radius starts at the center of the trunk. Bold points represent the average of four radial profiles (three depths) of daily maximum J_s recorded for 7 days in four representative trees taken within 11 instrumented trees. Vertical bars are SDs. Horizontal bars represent the average location of 2-cm sensors along the profile. Xylem radius averaged 7.25 cm, SD = 0.74. Maximum J_s in the outer ring averaged 2.36 L dm^{-2} h^{-1}, SD = 0.45.

constant radius of 0.3 cm. However, an area with slightly less staining was observed toward the pith. Probes were inserted into the trunks at a height of 1.8 m above the soil. At this height, the average xylem area was estimated at 2.04 dm^2 (SD = 0.47). The set-up took into account the circumferential and radial variability of J_s within the sapwood (Granier et al. 1996). Xylem area was schematically divided into four rings: the outer ring comprised between 100% and 60% of xylem radius, the intermediate ring between 60% and 30%, the inner low-conducting ring between 30% and 5% and the non-conductive pith. Standard equipment corresponded to three probes inserted into the outer ring of each trunk to a depth of 0.5–2.5 cm after removal of the bark. In four representative trees within the same sample of 11 trees, sensors were inserted at two complementary depths: 5 and 7 cm beneath cambium (xylem radius ranging from 6 to 8 cm and average = 7.33 cm). The exposed parts of the needles were coated with silicone. The trunk area containing the probes was protected from direct solar radiation and from rainfall with a waterproof deflector. The 41 probes were connected to a data logger (CR10X, Campbell Scientific, Leicester, UK). The zero-flux signal was determined every night, assuming that sap flow was negligible at the end of the night. Toward the dry season, nocturnal vapour pressure deficit of the air (VPD) (at the same time of $\Delta T_{\max}$ recording) differed from zero, and reached a maximum of 0.6 KPa. However, our assumption relied upon the facts that $\Delta T_{\max}$ of probes was quite stable over the 4-month period (variation coefficient = 1.8% on average), and overall there was no relationship between these small variations and the progressive increase of nocturnal VPD toward the dry season. The explanation of negligible night-time sap flows despite substantial VPD was likely due to several features: the dramatic decrease in the leaf area toward the dry season, the night-time stomatal behavior of the species and the low soil water availability. In a representative record of 7 days, the radial profile of J_s showed the classical shape of diffuse porous species (Figure 1). Midday J_s was maximum in the outer ring (2.36 L dm^{-2} h^{-1} and SD = 0.45). J_s decreased by 20% in the intermediate ring and by 70% in the low-conducting wood close to the center. The final calculation of total flow (F_{tree}) gave

$$F_{\mathrm{tree}} = A_{\mathrm{sw}} \times J_{\mathrm{s\_ot}} \times (0.64\mathrm{C1} + 0.27\mathrm{C2} + 0.0875\mathrm{C3}),$$

where A_{sw} is the total cross-sectional area of xylem at the level of the heating probe, $J_{\mathrm{s\_out}}$ is the J_s measured in the outermost ring and C_i is the ratio of J_s to $J_{\mathrm{s\_out}}$ in the inner rings. The latter formula showed that due to the relative areas, the measurements of J_s close to the center were of little importance. The ratio of J_s applied to the successive rings from the outside toward the center was 1, 0.79 and 0.27. The result was the application of a reduction coefficient of 0.874 to the J_s measured in the outer ring of conducting xylem. Hourly total flow over a period of 24 h was cumulated to calculate daily total flow. Additionally, daily total flow was an estimate of daily tree transpiration, ignoring the changes in tree water storage. Daily transpiration of an individual tree over a period of time (Δt) was computed as the sum of daily transpiration divided by the period of time. The final estimate of daily transpiration was the mean of the average daily transpiration of the 11 trees.

Simplified soil water balance

Tree transpiration can be estimated from the depletion of the soil water content (SWC) profile alone when the following conditions are assumed in the soil water balance: (i) zero water input (rainfall and irrigation), (ii) negligible soil evaporation, (iii) negligible lateral and deep water transfers and (iv) root water uptakes limited to the measured soil profile. Under these circumstances, the tree water uptake (E) equals the soil water depletion (ΔS) from the root zone for a period of time (Δt) according to the simple formula

$$E = \Delta S / \Delta t = [S(t) - S(t + \Delta t)] / \Delta t,$$

where E represents tree water uptake or transpiration in mm day^{-1}, ΔS is the soil water depletion expressed in mm, $S(t)$ is the soil water storage in mm at the initial date t and $S(t + \Delta t)$ is the soil water storage in mm after a period Δt expressed in days.

The soil water storage in the root zone $S(t)$ was derived from soil moisture measurement at different depths following the formula:

$$S(t) = \int_0^z \theta(z, t) \times dz,$$

where θ is the volumetric SWC and z is the depth of the root zone.

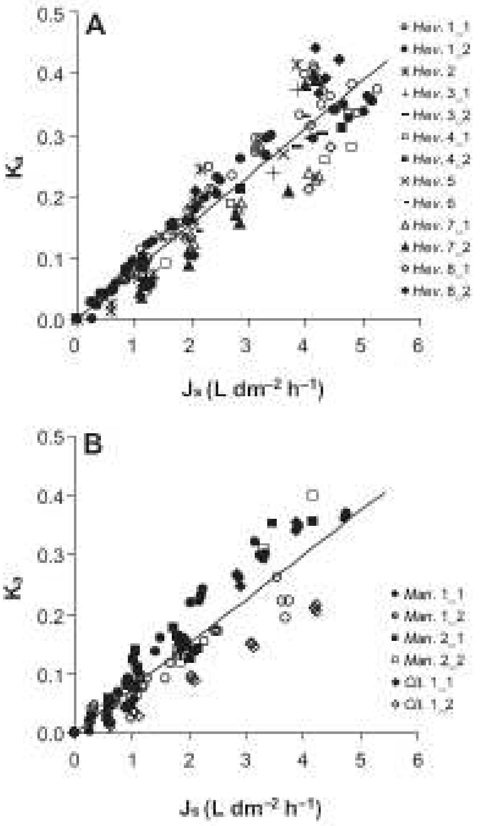

Figure 2. Values of the flow index (K_a) versus flux density based on the cut stem experiment with the 10-min TTD method: (A) *H. brasiliensis* (*Hev.*), numbers related to acronyms indicate the set of cut stems and probes (presented in Table 1) ($K_a = 0.0778\ J_s$; $R^2 = 0.89$ and $n = 155$); (B) *M. indica* (*Man.*) and *C. maxima* (*Cit.*) ($K_a = 0.0749\ J_s$; $R^2 = 0.84$ and $n = 121$). The lines indicate the respective linear regressions.

The soil water balance was assumed to fulfill these requirements during the dry season, long enough after the last rainfall and considering the soil profile to a depth of 1.8 m. Rubber tree roots are mainly concentrated within the first meter but a few roots are observed at a greater depth (Devakumar et al. 1999). Soil moisture was measured with a neutron probe (3322, Troxler, Research Triangle Park, NC) every 0.2 m to a depth of 1.8 m. Two gravimetric calibrations were applied, separating the top layer from the other layers. The SWC was not uniform in the horizontal direction due to run-off towards the inter-row in the rainy season. Therefore, we set up 12 tubes by couples; for each couple, 1 tube along the planting line between two trees, approximately 1.75 m from each, and 1 tube in the middle of the inter-row, approximately 3.5 m from each planting line. An average soil water profile was determined by couple of tube and statistics showed the variability related to the six repetitions. Tree water use was estimated from soil water depletion for five periods starting 15 days after the last rainfall (October 30) to February 21, at leaf fall peak. Average soil water depletion was computed as the mean of measurements made on six pairs of tubes. Soil moistures at 'field capacity' (−0.03 MPa) and 'permanent wilting point' (−1.5 MPa) were deduced from soil water retention curves modeled from soil properties (% clay, % silt, % OM, medium size of sand and bulk density) of the four main layers of the soil profile (0–0.2, 0.2–0.4, 0.4–1.0 and 1.0–1.8 m) using the van Genuchten–Mualem model adapted by Wosten et al. (1998).

Data analysis

Regression analysis and other statistics were performed using Sigmaplot Version 10.0 and SPSS Version 11.5. Linear slopes were compared using their confidence intervals at 95%. Curve fits and estimates were compared using the root mean

Table 2. Details of regression curves from cut stem experiments with the TTD method: (*Hev*_L), linear regression of *H. brasiliensis* data; (Oth_L), linear regression of data from other species, *M. indica* and *C. maxima*; (MS_L), multi-species linear regression including all the data; (MS_sig), multi-species sigmoid regression and (D&R_sig) sawdust sigmoid regression of Do and Rocheteau 2002*b*. The form of the sigmoid function is

$$K_a = \frac{a}{1 + \left(\frac{J_s}{X_0}\right)^b}$$

The parameters of the columns are *n*, number of data; *a*, slope of linear curve; X_0, constant value of sigmoid curves; *b*, power of sigmoid curve; SE, standard error of regression parameters (related to X_0 for MS_sig); CI_95, confidence interval at 95% of linear slopes; (R^2 and *P*), statistics related to each regression; Total RMSE, Total root mean square error ($n = 276$) and RMSE_{rel}, relative root mean square error at several ranges of flow rates.

	n	*a*	X_0	*b*	SE	CI_95	R^2	*P*	Total RMSE	% RMSE_{rel} range of J_s (L dm^{-2} h^{-1})			
										0–0.5	0.5–1.0	1.0–3.0	3.0–5.0
*Hev*_L	155	0.0778	–	–	0.0022	0.0045	0.89	< 0.0001					
Oth_L	121	0.0749	–	–	0.0030	0.0060	0.84	< 0.0001					
MS_L	276	0.0772	–	–	0.0018	0.0035	0.88	< 0.0001	0.52	41.4	30.6	26.3	20.5
MS_sig	276	1	6.8986	1.414	2.8519		0.87	< 0.0001	0.57	58.6	27.3	24.1	23.1
D&R_sig	–	1	5.5557	1.414									

square error (RMSE) according to the following formula:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (x_{1,i} - x_{2,i})^2}{n}},$$

where in the analysis of cut stem experiments, $x_{1,i}$ and $x_{2,i}$ are sap flow densities from weighed and calculated values at the same K_a, respectively, and n is the number of estimated values. In the field comparison, $x_{1,i}$ and $x_{2,i}$ are soil water depletion and tree transpiration for the same period of time, respectively and n is the number of tree transpiration values.

The comparison of curve fitting, which separated the ranges of flow rates in cut stem experiments, was made using relative root mean square error ($\mathrm{RMSE}_{\mathrm{rel}}$) according to the following formula:

$$\mathrm{RMSE}_{\mathrm{rel}} = \sqrt{\frac{\sum_{i=1}^{n} \left(\frac{x_{1,i} - x_{2,i}}{x_{1,i}}\right)^2}{n}},$$

where $x_{1,i}$ and $x_{2,i}$ are sap flux densities from weighed and estimated values at the same K_a, respectively and n is the number of values.

To determine if sap flow measurements and soil water balance resulted in similar estimates of daily transpiration, a paired t test was performed for each period of time.

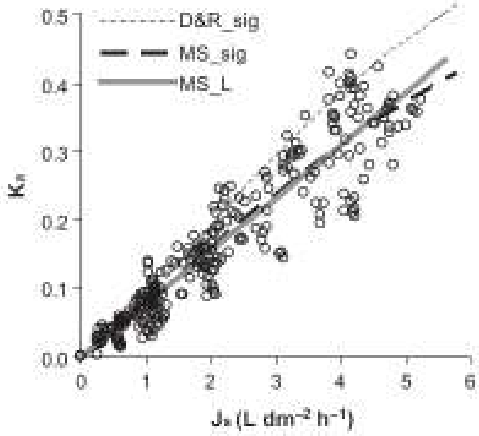

Figure 3. Multi-species calibration of the flow index (K_a) versus flux density with the 10-min TTD method including three species: *H. brasiliensis*, *M. indica* and *C. maxima*. The dotted line indicates the original sigmoid calibration performed by Do and Rochetean on sawdust (2002*b*, D&R_sig), the bold dotted line indicates the multi-species sigmoid calibration (MS_sig) and the continuous line indicates the multi-species linear calibration (MS_L: $K_a = 0.0772\ J_s$, $R^2 = 0.88$ and $n = 276$).

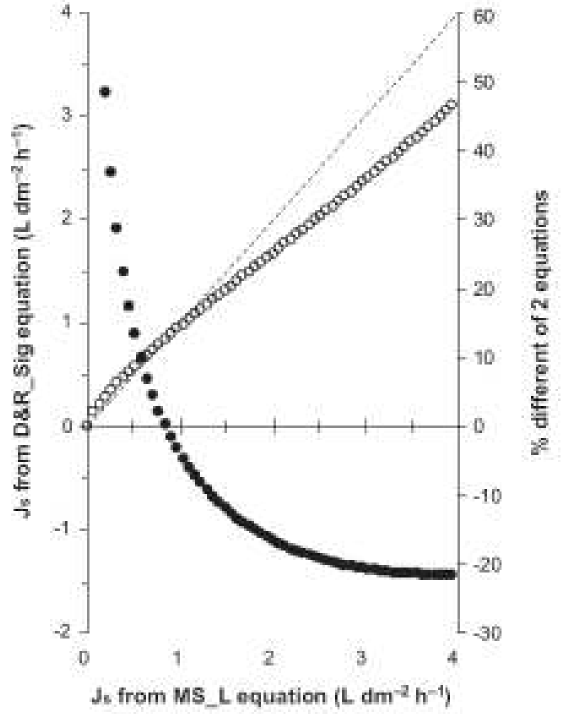

Figure 4. Sap flux density (J_s) estimated from the equation of Do and Rocheteau (2002*b*) (open circles) versus J_s estimated from the new multi-species linear equation (presented in Figure 3). The closed circles related to the second *Y*-axis indicate the differences between the two estimates relative to the estimates from the multi-species equation.

Results

Cut stem experiments

Firstly, for *H. brasiliensis* wood, the responses of the K_a index to J_s showed a strong linear relationship (Figure 2A; Table 2, $R^2 = 0.89$). A substantial discrepancy was observed over 13 response curves. Figure 2A shows that variability originated from the differences in response both between cut stems and between probes within the same cut stem. For example, between cut stems, the global response of *Hev.* 1, at the top end of the scatter, contrasts with that of *Hev.* 7 at the low end. And within the same cut stem, the response of *Hev.* 8_2 contrasts with that of *Hev.* 8_1. The data recorded using *M. indica* and *C. maxima* woods (six sets) displayed a linear relationship close to the one assessed using *H. brasiliensis* wood (Figure 2B; Table 2, $R^2 = 0.84$). The slope of *M. indica* and *C. maxima* data did not differ from that of *H. brasiliensis* (Table 2). Similarly to *H. brasiliensis* data, a substantial discrepancy was observed within the scatter of points coming from the differences in response both between cut stems and between probes within the same cut stems.

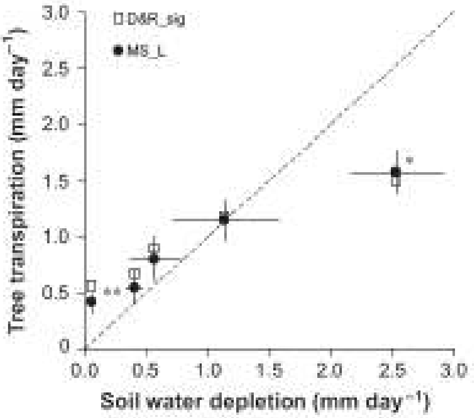

Figure 5. Tree transpiration estimated from sap flow measurements versus estimates from soil water depletion for five periods in the dry season. Estimates from sap flow measurement are the average of 11 mature trees (three probes per tree) of *H. brasiliensis*. Estimates from soil water depletion are the average of neutron probe measurements made on six pairs of tubes inserted to a depth of 1.8 m. The closed circles indicate tree transpiration estimates from the multi-species linear calibration (presented in Figure 3). Open squares are tree transpiration estimates from Do and Rocheteau's calibration (2002*b*). Vertical and horizontal bars indicate standard errors. * and ** indicate when estimates differ significantly from each other, which was the case during the first period at the onset of the dry season (November, $P < 0.05$) and during the last period (leaf fall peak, February, $P < 0.01$).

The whole data set was fitted to a linear function passing through 0 (Figure 3; Table 2):

$$K_g = 0.0772 J_s, \quad \text{or} \quad J_s = 12.95 K_g,$$

where r^2 equals 0.88 and n equals 276.

The fit to a sigmoid function similar to the form used by Do and Rocheteau (2002*b*) was very close to the previous linear function (Figure 3) and proved to be no better (Table 2). The RMSE (absolute and relative) were even slightly lower for the linear function. The calibration curve obtained with sawdust by Do and Rocheteau (2002*b*) is located at the upper end of the scatter of points (Figure 3). The comparison of the two sigmoid functions (MS_sig and D&R_sig in Table 2) showed that the uncertainty on parameter (X_0) in the multi-species calibration included the response curve assessed with sawdust by Do and Rocheteau (2002*b*). However, the estimates of J_s differed substantially between the average calibrations at very low flow and high flow rates. Compared to the multi-species calibration, the values from the Do and Rocheteau (2002*b*) calibration were overestimated at low J_s (< 0.8 L dm^{-2} h^{-1}); e.g., +30% at 0.5 L dm^{-2} h^{-1}, and were underestimated at high J_s, e.g., −20% at 4.0 L dm^{-2} h^{-1} (Figure 4).

Estimates of H. brasiliensis transpiration in the field

Tree transpiration estimated from sap flow measurements with the multi-species linear calibration decreased from 1.6 mm day^{-1} in November to 0.4 mm day^{-1} in February at leaf fall peak (Figure 5). Comparison with estimates from the depletion of soil water profile to a depth of 1.8 m showed a strong correlation ($R = 0.97$) and RMSE equal to 0.47 mm day^{-1}. However, there were large discrepancies at the borders, and for these two extreme points, the estimates differed significantly from each other ($P < 0.05$). In November, at the start of the dry season, the transpiration estimated from soil water depletion was two-fold higher (2.5 mm day^{-1}). Compared to the previous equation of Do and Rocheteau (2002*b*), the multi-species calibration has little effect on the transpiration estimates, it slightly lowered RMSE, from 0.55 to 0.47 mm day^{-1}, mainly through the differences at low J_s (Figure 5). The modest difference of transpiration estimates between the two calibrations was related to the measured range of medium to low J_s (< 2.0), and was foreseeable from the previous analysis of Figure 4.

Discussion

TTD calibration independent of woody species

By analogy with the CTD method of Granier (1985), the TTD method developed by Do and Rocheteau (2002*b*) was first calibrated on synthetic porous material (sawdust), assuming that the relationship was independent of the woody species and porous media. Our results demonstrated that the response of the K_a index to flux density, calculated with 10 min of heating, was similar for three tropical woody species: *H. brasiliensis* (with a large sample of cut stems and two probes), *M. indica* and *C. maxima*. All the species tested had diffusive-porous wood. So, if ring-porous wood had been used, would the responses have been different? The previous calibration performed by Do and Rocheteau (2002*b*) was done using sawdust. This is very different porous media than cut stems and likely induced heterogeneous J_s. However, it fell within the variability of the multi-species calibration. These results strongly support the hypothesis that the TTD empirical calibration is independent of the woody species, although it is recommended to check results by some sort of validation before interpreting any absolute values obtained from different species of wood and in different environmental conditions (Lu et al. 2004). Hence, the TTD method appears to share the property of universal calibration with the original CTD method. What is the explanation? Even concerning the CTD method, this point has not been much discussed, but this independence is likely due to Dr. Granier's design of the thermal index (K), which is also used in the TTD method. It is the ratio of signal response at zero flow to signal response at actual flow. Unlike most physical methods of measurement, each probe has no particular calibration coefficient. As it works, it appears that zero-flux response of the set-up system acts as a calibration coefficient for the whole probe-porous media system.

Limitations of cut stem experiment with TTD methods

Response curves showed an intra-species discrepancy for the different species. The discrepancy came from both differ-

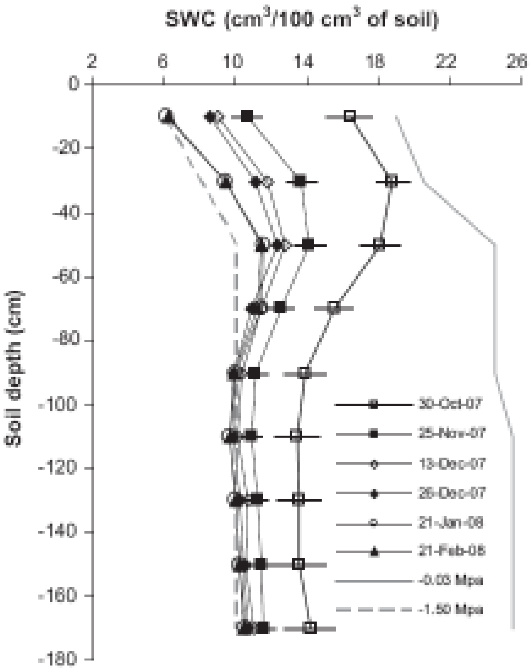

Figure 6. Changes in the volumetric SWC profile during the dry season from October 2007 to February 2008. Soil moisture at field capacity is indicated by the profile at −0.03 MPa, and soil moisture at wilting point is indicated by the profile at −1.5 MPa. Horizontal bars indicate standard errors (n = 6).

ences between the cut stem samples and the probe set-ups within the same segment of cut stem. Similarly to the CTD method, the discrepancy in the TTD method may come in particular from (i) probe contact with non-conductive sapwood, (ii) poor contact between the probe and the sapwood, (iii) heterogeneous flow density within the cut sample and poor representativeness of the probe position, (iv) heterogeneous flow density along the length of the probe and (v) the existence of transient natural thermal gradients. Firstly, contact between the probe and the conductive sapwood only has been a known requirement of TD methods since the start (Granier 1985, Braun and Schmid 1999, Clearwater et al. 1999). The response curves assessed for the multi-species calibration corresponded to fully conductive sapwood confirmed by staining. We did some tests on the cut stems of Longan (*Dimocarpus longan* Lour.) and observed a large non-stained area around the pith, implying that the 2 cm tip of the probe was in partial contact with non-conductive wood. The final results were a much lower response curve than the range presented in Figure 3 (data not shown). Secondly, correct contact between the probe and the sapwood is also a basic requirement of TD methods (Lu et al. 2004). This is particularly true when dealing with dead wood in cut stem experiments because there is no progressive tightening of wood as occurs in living trees. ΔT_0 is a good indicator of such contact. In this experiment, ΔT_0 below 10.5 °C insured a good contact and heat dissipation between probe and wood. However, there was still a substantial variability of ΔT_0, between 10.5 and 7.5 °C (Table 1), despite an accurate regulation of the power of each probe heating at 0.2 W. The data do not support a difference related to the probe itself because within the same stems, the mean and variation coefficient of ΔT_0 were similar, 9.0 °C (SD = 0.9) and 9.4 °C (SD = 0.9), for probes 1 and 2, respectively. Hence, the variability of ΔT_0, particularly within the same cut stem, reflects mainly the variations of heat dissipation by conduction due to variation in the quality of the contact between probe and wood. This could explain the substantial differences observed in probe responses within the same cut stem and between cut stems.

Thirdly, heterogeneous flows within cut samples are naturally expected. They could be due to the existence of tension wood counteracting the effect of wind or weight. The probe may have been inserted into a sector that was not representative of average flow. Moreover, Table 1 shows that conductance could vary considerably in similar cut segments of the same species, and such differences may also exist between sectors within the same sample. This could explain some differences in the response of a probe within the same cut sample. Fourthly, Clearwater et al. (1999) showed that with the CTD method, heterogeneous flow density along the probe may lead to underestimation of sap flow due to the non-linear response of J_s to the temperature signal. Sap flow along the length of the probe was likely heterogeneous in our cut stem experiments at a magnitude that varied with stem samples. This feature may have induced some discrepancy between responses. However, the more linear response of the TTD thermal index to flow density should decrease sensitivity to this effect. Finally, by contrast with the CTD method, the TTD method is insensitive to a stable natural thermal gradient (Do and Rocheteau 2002*b*). However, a change in the natural thermal gradient over the 10-min period of measurement may induce a large error in the measurement ($\Delta T_{on} - \Delta T_{off}$) as it was noticed in the field in the early morning when ΔT_{off} was not interpolated between values before and after heating. Even in the laboratory experiments, the use of an interpolated ΔT_{off} decreased the overall discrepancy (data not shown). There are certainly other unknown factors that contribute to the uncertainty and to the difficulty of laboratory calibration on cut stems. Nevertheless, the sources of variability are surely emphasized in the field with large trees. Even with small trees in containers, McCulloh et al. (2007) observed large individual errors, (up to 50%), when comparing the responses of the CTD method with gravimetric measurements. Consequently when working in the field, as in cut stem experiments, it appears important to estimate the mean response using a large number of set-ups and repetitions.

Comparison between current calibration and that of Do and Rocheteau (2002b)

The sawdust calibration of Do and Rocheteau (2002*b*) was included in the variability of response curves observed in cut stems of the three tropical species (Figure 3). However, the estimates using the average multi-species calibration differed substantially from the previous calibration at high flow and very low flow rates. The multi-species calibration was based on the 19 response curves mixing 15 wood samples and two probes. The calibration of Do and Rocheteau (2002*b*) on sawdust was based on two sawdust columns and one probe. The data basis of the multi-species calibration likely maximized variability and provided a more reliable average response curve. We assume that a larger sample of sawdust columns and probe set-ups would have yielded a mean response closer to our multi-species calibration.

Field evaluation of transpiration estimate in mature H. brasiliensis

The field evaluation compared the estimates of transpiration from sap flow measurements with the estimates from a simplified soil water balance. The comparison validated the magnitude of transpiration estimates from sap flow measurements with the multi-species calibration when considering the middle of the dry season. The estimate of 1.6 mm day^{-1} may appear quite low after only 1 month of dry season, when trees are still in full foliage and evaporative demand is relatively high. However, Isarangkool Na Ayutthaya et al. (2008) showed that at this time both J_s and whole-tree hydraulic conductance had already decreased about 40% compared to values measured at the end of the rainy season in October (2.5 mm day^{-1}). At the start and at the end of the soil drying period used for comparison, we found significant discrepancies between transpiration estimates from sap flow measurements and from the soil water balance. For the first period of comparison, starting 15 days after the last rainfall, the change in soil water profiles to a depth of 1.8 m did not support the hypothesis of negligible soil surface evaporation and water transfers outside the soil profile (Figure 6). There was still a large amount of water available in the soil surface layer, and at the lower end, the SWC had substantially decreased. Moreover, at the end of the dry season, the hypothesis of negligible root water uptake outside the measured profile was doubtful. At this time of leaf fall peak, mean transpiration estimated from the sap flow measurements was 0.41 mm day^{-1} while the 1.8 m soil water profile showed almost no change over a period of 30 days, yielding 0.1 mm of depletion. Figure 6 shows that the soil water profiles were not far from the reference profile at –1.5 MPa. In the same plantation, Gonkhamdee et al. (2008) reported root activity at a depth of 4 m in the dry season, so it is fairly possible that at this time the trees tap water from deeper soil layers. Additionally, in this study, the transpiration estimate of 0.4 mm day^{-1} corresponded to very low hourly J_s, below 0.5 L dm^{-2} h^{-1}. At this rate, the accuracy of absolute measurement with the TTD method is poor because of the calibration uncertainty (see $RMSE_{rel}$ in Table 2) and because of the relative influence of any inaccuracy in the measurement of ΔT_0 in the field. The relative inaccuracy of ΔT_0 and its assumed correspondence to zero flow are a common weakness of all thermal methods (Do and Rocheteau 2002*a*, Lu et al. 2004, Regalado and Ritter 2007). The CTD and more recently the TTD (Chapotin et al. 2006*a*, 2006*b*, Abid Karray et al. 2008, Do et al. 2008) methods have demonstrated their ability to accurately record the changes in hourly and seasonal transpiration. The accuracy of absolute estimates of transpiration is another step, which obviously requires a large number of probes and cross-validation with an independent method such as soil water balance or gas exchange measurements.

Conclusion

In conclusion, the evidence of non-species specific calibration strengthens the interest of the 10-min TTD method developed by Do and Rocheteau (2002*b*). In addition, the field comparison with the soil water balance method validated to a reasonable extent the absolute estimates of transpiration from the new multi-species calibration. Compared to the CTD method, the current advantages of the TTD method are (i) a more stable night-time reference (Do and Rocheteau 2002*a*) and insensitivity to a stable natural thermal gradient (Do and Rocheteau 2002*b*) and (ii) the simple linear response of the transient K_a index. Obvious drawbacks are (i) a low resolution time of measurement – with 10 min of heating and cooling: one measurement every 30 min or every 20 min at the best and (ii) a little more complex electronic circuitry required including a relay box (Lu et al. 2004). Finally, one promising new advantage of the TTD method is the applicability of its time-related signal to a single probe using exactly the same K_a index (Do unpublished) or a more sophisticated index (Mahjoub et al. 2009). The use of a single probe will further reduce problems associated with thermal gradients and make instrumentation simpler and cheaper, which may increase the current advantages of thermal dissipation methods over other thermal methods.

Acknowledgments

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Chapter IV

Result II:
Water loss regulation in mature Hevea brasiliensis: effects of intermittent drought in rainy season and hydraulic limitation

Water loss regulation in mature *Hevea brasiliensis*: effects of intermittent drought in rainy season and hydraulic limitation

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Abstract

Effects of soil and atmospheric droughts on whole-tree transpiration, leaf water potential and whole-tree hydraulic conductance were investigated for mature rubber trees (*Hevea brasiliensis,* clone RRIM 600) during the full canopy of the rainy season in a drought-prone area of North-East Thailand.

Despite well-watered soil conditions, transpiration did not follow completely evaporative demand, transpirations saturated above reference evapotranspiration (ET_0) c.a. 2.2 mm day^{-1}. Intermittent soil drought provoked a dramatic decrease of transpiration below a threshold of 50% of relative extractable water (*REW*) in the top soil which corresponded to a predawn leaf water potential ($\psi_{predawn}$) c.a. -0.45 MPa. Transpiration was reduced by 40% at 0.3 *REW* and 80% at 0.1 *REW*. The minimum leaf water potential for sunny day did not change according to soil drought and was stable around -1.95 MPa which supported an anisohydric behaviour. The decrease of transpiration was mainly due to the change of whole-tree hydraulic conductance. The results of simulation proved the ability of a simple "hydraulic limitation" model, based on evaporative demand, whole-tree hydraulic conductance, and critical minimum leaf water potential, to simulate the pattern of transpiration changes.

Finally, combination between the cohesion-tension theory, electric analogy and hydraulic limitations hypothesis provides a promising framework to analyze transpiration responses to soil and atmospheric droughts and to develop simple process-based models to compare genotypes in contrasted environments

Keywords: *Hevea brasiliensis*, water regulation, intermittent drought, tree transpiration, leaf water potential, whole tree hydraulic conductance

1. Introduction

In trees, leaf carbon gain is tightly coupled to water loss. As a consequence, to maximize their growth and productivity trees also need to maximize their transpiration. Plants respond to drought by reducing transpiration through stomatal closure which prevents the development of excessive water deficits in their tissues. Such a dilemma represents for plants facing drought the complicate trade-off between carbon gain and water losses. This probably explains why considerable attention has been given to the measurement and modeling of transpiration response to drought. A large number of empirical models have been proposed to predict transpiration. However, in order to predict tree response to environmental conditions or to evaluate the performance of new tree genotypes, it is now crucial to develop more process based models. It is then critical to identify the key processes that govern tree transpiration to properly model their function under drought and contrasted environmental conditions.

Tree transpiration (E_{Tree}) is a physical process determined by the evaporation of water molecules at the leaf surface. Hence, E_{Tree} is first governed by an evaporative demand closely linked to climatic variables like global radiation or vapor pressure deficit (*VPD*). This climatic demand sets an upper physical limit to E_{Tree}. When reference evapotranspiration (ET_0) is low, during rainy days for instance, E_{Tree} is also low. But there are a number of situations where ET_0 largely overestimates $E_{Tree.}$ In other words, under these circumstances trees operate a biological control limiting their water losses. This is usually achieved by a stomatal closure (Comstock and

Mencuccini 1998; Cochard et al. 2002; Buckley 2005). For instance, under high climatic demand (high *VPD*), several studies reported results in well-watered soil conditions where E_{Tree} does not follow evaporative demand, a saturated E_{Tree} at maximum value occurs (David et al. 2004; Bovard et al. 2005; Oguntunde et al. 2007; Bush et al. 2008). Similarly, decline of soil water content induces limitations and reductions of E_{Tree} through stomatal closure (Breda et al. 1993; Cochard et al. 1996; David et al. 1997; Irvine et al. 1998; Meinzer et al. 1999).

Water transport in trees behaves like the transport of water in a plumbing system and can be efficiently modeled by simple hydraulic analogy where flows are proportional to pressure gradients, the coefficient of proportionality being analog to a hydraulic resistance (Van den Honert 1948; Tyree and Zimmermann 2002):

$$\psi_{Soil} - \psi_{Leaf} = E_{Tree} * R_{Tree} = \frac{E_{Tree}}{K_{Tree}} \qquad (1)$$

where ψ_{Soil} is the soil water potential, ψ_{Leaf} the leaf water potential, R_{Tree} and K_{Tree} are the hydraulic resistance and conductance of the whole sap pathway, respectively.

The ψ_{Leaf} is a key physiological parameter that has strong direct of indirect impact on E_{Tree}. Direct effects of ψ_{Leaf} on E_{Tree} can be caused by its impact on leaf turgor (Cochard et al. 2002) or on the interaction with stomata sensibility to ABA (Tardieu and Simonneau 1998). More indirect effects explained by the correlation between ψ_{Leaf} and the xylem water potential (ψ_{Xylem}) and by the effect of ψ_{Xylem} on cavitation (Sperry et al. 1998; Cochard et al. 2002). Whatever the mechanisms, experimental data suggest that many tree species tend to adjust their transpiration with the effect of maintaining ψ_{Leaf} above a critical value (ψ_{crit}) which results in an isohydric behavior (Tardieu and Simonneau 1998; Franks et al. 2007; Maseda and Fernandez 2006; West et al. 2007). Conversely, the anisohydric plants are less strict control by stomata, thus no discernible threshold (West et al. 2007).

On the other hand, maximum leaf water potential or predawn ($\psi_{predawn}$) is typically used as a reliable indicator of soil water potential, which fluctuates following soil water status (Richter 1997; Donovan et al. 2001). The relative extractable water (*REW*) is also used to directly characterize soil water status in transpiration modeling (Granier et al. 1999, 2000; Breda et al. 2006). Additionally, the decrease of K_{Tree} under soil drought has been demonstrated in several temperate trees (Breda et al. 1993; Cochard et al. 1996; Lu et al. 1996; Alarcon et al. 2000; Cochard et al. 2002; Brodribb et al. 2002).

A number of mechanistic models have been proposed to predict E_{Tree} under these limiting conditions which are based on hormonal (Tardieu and Simonneau, 1998) or air humidity signals (Granier et al 1996, 2000; Ewers et al. 2001; Oguntunde et al. 2007). The 'hydraulic limitation' hypothesis (Jones 1998; Sperry et al. 1998) is an alternative and very promising way to model tree transpiration under water stress. According to Eq. 1 it is possible to compute a critical transpiration (E_{crit}) corresponding to ψ_{crit}:

$$E_{crit} = (\psi_{soil} - \psi_{crit}) * K_{Tree} \qquad (2)$$

According to this hydraulic limitation hypothesis, E_{crit} set a functional limitation to E_{Tree} that can be reached under drought conditions (low ψ_{soil} and low K_{Tree} values) but also when the evaporative demand is very high (Jones and Sutherland 1991; Sperry et al. 2002). By combining the limiting effects of ET_0 and E_{crit} on tree transpiration, it is possible to construct a simple model for E_{Tree}:

$$E_{Tree} = \quad Min(E_{crit}; ET_0) \qquad (3)$$

This approach has proven to be valid and robust for temperate angiosperms (Sperry et al. 1998; Cochard et al. 2002). As far as we know, there is little evidence so far that this approach remains valid species from other biomes, such a tropical species for instance. In wet tropical conditions, tropical species are more rarely exposed to severe water stresses than species from other biomes. Therefore, they may have developed very different mechanisms to control their water losses.

The general objective of this study was to test this approach in *Hevea brasiliensis* (rubber tree), a species native from wet tropical forests in Amazonia. To benefit from the ever-increasing demand for natural rubber, the cultivation of *Hevea brasiliensis* is extended in drought prone areas such as in the southern part of northeast Thailand. In this area, rubber tree has to face soil drought and atmospheric drought in both wet and dry seasons. Several authors have studied the influence of soil and atmospheric droughts on the water relations of mature rubber trees (Chandrashekar et al. 1990; Chandrashekar 1997; Gururaja Rao et al. 1990), however they focused on the absolute comparison of variables between seasonal dry season and wet season and on the relationships with latex yield. Two studies of hydraulic on young potted trees provide interesting insights before testing hydraulic limitation hypothesis (Ranasinghe and Milburn 1995; Sangsing et al. 2004). First, both authors found high values of xylem tension corresponding to 50% of embolism in the petioles (between -1.5 and -2.0 MPa) which suggests that rubber tree is relatively vulnerable to cavitation. Secondly, Sangsing et al (2004) found that stomatal control operates at the onset of xylem embolism in the petiole which suggests an isohydric behavior.

Our study had four detailed objectives. The first objective was to assess the response of whole-tree transpiration to intermittent drought in rainy season: we hypothesized responses to both atmospheric drought and soil drought with particular thresholds. The second objective was to study the operational range of ψ_{Leaf} water potential under soil and atmospheric drought conditions: we hypothesized sensitivity of predawn value to soil drought but a relative stability of midday value for sunny days. The third objective was to study the concurrent change of whole-tree hydraulic conductance: we expected that these changes mainly explain the reduction of transpiration. The last objective was to test the ability of a simple "hydraulic limitation" model, based on i) whole-tree hydraulic conductance (sensitive to soil drought) and on ii) a critical minimum leaf water potential, to simulate the main changes of transpiration.

To address these issues, we selected a mature and representative rubber tree stand of the main planted clone in South East Asia. It was located in the southern part of northeast Thailand where trees are regularly exposed to soil and atmospheric droughts during the full canopy in rainy season. Seasonal and diurnal variations of E_{Tree}, ψ_{Leaf} and K_{Tree} were measured with concurrent recordings of atmospheric and soil water conditions.

2. Materials and methods

2.1 Field site and plant material

The experiment was conducted in a plot of RRIM600, planted at 2.5m x 7.0m spacing and tapped for 4 years. The plantation is located at Baan Sila (N15^{o} 16′ 23″ E103^{o} 04′ 51.3″), Khu-Muang, Bureerum, northeast Thailand. The rainy season lasts approximately from April to October and the annual amount of rainfall averages 1176 mm. Six representative trees were selected. Their trunk girths, measured at 1.50 m above soil, varied from 43.3 to 58.3 cm (average: 52.5 cm). The maximum leaf area index measured by litterfall collection at defoliation time (December-January-February) was estimated c.a. 3.89 in 2007.

2.2 Climatic measurements

Local microclimate was automatically monitored in an open field, 50 m from any trees. A datalogger (Minimet automatic weather station, Skye Instruments Ltd, U.K.) recorded half hourly values of air temperature, relative humidity, incoming short wave radiation and rainfall. The reference evapotranspiration (ET_0) was calculated according to Allen et al. (1998).

2.3 Soil water content measurements

Volumetric soil water content (θ) was measured with a neutron probe (3322, Troxler, Research Triangle Park, North Carolina, USA) calibrated for the experimental soil with separated calibrations between upper (0-0.2 m) and lower (below 0.2 m) layers. Twelve tubes of 2.0 m in length were set up, six along the rows and six between the rows. Measurements every 0.2 m, from 0.1m until 1.7 m depth, were performed every two weeks from May 2007 to October 2007. According to soil water fluctuation, the soil profile was separated between two layers, a top soil (0-0.4 m) and subsoil (0.4-1.8 m). Average field capacity and permanent wilting points were equal to 19.8 and 7 $cm^3/100\ cm^3$ of soil for the top soil, and to 25.1 and 10 $cm^3/100\ cm^3$ of soil for the subsoil, respectively (Isarangkool Na Ayutthaya et al., Paper 1).

Additionally, θ was measured continuously with a capacitive probe (EnvironSCAN System, Sentek Sensor Technologies, South Australia, Australia) within a single tube close to a tube dedicated to neutron probe measurement.

Capacitive sensors were located at the same levels than neutron probe measurements. For each capacitive sensor, θ was estimated from a cross-calibration with the neutron probe measurements over the whole season range. To estimate continuous change of the average soil water profile, linear regressions were performed between θ of the average soil water profile (12 neutron probe access tubes) and θ of the profile continuously measured with the capacitive probe. For the previously defined top soil and sub soil layers, R^2 of linear regressions were 0.89 and 0.76, respectively. Relative extractable soil water (*REW*) was calculated according to Granier et al. (1999) and Breda et al. (2006).

2.4 Leaf water potential

Leaf water potential (ψ_{Leaf}) was measured on the six instrumented trees with a Scholander type pressure chamber (PMS 1000, PMS Instrument Company, Corwallis, Oregon, USA). Two trifoliate leaves with petiole were randomly selected from sunny locations on each experimental tree. ψ_{Leaf} measurements were performed in situ rapidly after cutting. Regular measurements of ψ_{Leaf} were carried out once or twice times per month, $\psi_{predawn}$, between 05:30 and 06:15 hours, and midday leaf water potential (ψ_{midday}), between 12:30 and 13:30 hours. Additionally, four diurnal kinetics of ψ_{Leaf} measurements, i.e., every 1-2 hour from predawn to sunset, were performed in contrasting periods of soil water content.

2.5 Xylem sap flow measurements and tree transpiration calculation

The measurements of xylem sap flow density were made using the transient thermal dissipation method (TTD) developed by Do and Rocheteau (2002) which is a modification of the continuous thermal dissipation method of Granier (1985). The modification avoids the influence of passive temperature gradients. The TTD method is based on the same Granier's probe design and heating power but uses a cyclic schedule of heating and cooling to assess a transient thermal index over 10 min change. The hourly sap flux density (J_s; L dm^{-2} h^{-1}) was calculated according to the empirical and non species-specific calibration assessed by Isarangkool Na Ayutthaya et al. (Paper 1):

$$J_s = 12.95 K_a \quad (4)$$

where K_a is a transient thermal index (dimensionless).

An alternate signal (ΔT_a) was defined as:

$$\Delta T_a = \Delta T_{on} - \Delta T_{off} \quad (5)$$

where ΔT_{on} is the temperature difference reached at the end of the 10 min heating period and ΔT_{off} is the temperature difference reached after 10 min of cooling,

To measure J_s every half hour with a heating period of 10 min, a cycle of 10 min heating and 20 min cooling was applied and the temperature signals were recorded every 10 min. Experience showed that averaging ΔT_{off} values (before 10 min of heating and after 10 min of cooling) improves measurement accuracy. This interpolation of ΔT_{off} at the time of ΔT_{on} measurement likely reduces sensitivity to quick changes of reference temperature or natural thermal gradients.

The transient thermal index was calculated as:

$$K_a = (\Delta T_{0a} - \Delta T_{ua}) / \Delta T_{ua} \quad (6)$$

where ΔT_{0a} is the maximum alternate temperature difference obtained under zero flow conditions and ΔT_{ua} is the measured alternate signal at a given J_s.

The zero flux signal was determined every night assuming that sap flow was negligible at the end of the night. Probes were inserted into the trunks at a height of 1.8 m above the soil. At this height, average sapwood area was estimated at 1.97 dm^2. After removal of the bark, the probes, 2-cm long probes were inserted into a hole of 2.5 cm deep within the sapwood, in such a way that the whole probe was inside the conductive sapwood. Three probes were inserted into each trunk to take circumferential variability into account. After the probe was inserted, the exposed parts of the needles were coated with silicone. The trunk area containing the probes was protected from direct solar radiation and rainfall by a deflector. Probes were connected to a data logger (CR10X, Campbell Scientific, Leicester, U.K.).

Hourly sap flow density (J_s) was cumulated over 24 h to calculate daily J_s (J_{s_daily}). For taking care of the variation of sap flux density in the depth of wood, a reduction coefficient of 0.874 was applied to the J_s measured in the outmost ring of conducting xylem (Isarangkool Na Ayutthaya et al, Paper 1). Finally, neglecting tree water storage, E_{Tree} (mm day^{-1}) was estimated according to the equation:

$$E_{Tree} = 0.874 * J_{s\_daily} * \text{sapwood area/tree spacing area} \qquad (7)$$

2.6 Whole tree hydraulic conductance

The whole-tree hydraulic conductance (g_L) was calculated from concurring measurements of sap flow rate and leaf water potential following Eq. 1. The multi-points method plotted the diurnal changes of ψ_{Leaf} versus sap flux density, the slope of the assumed linear relationships representing the hydraulic resistance, the reverse of the hydraulic conductance. The single point method applied the simplified following formula (Cochard et al. 1996).

$$g_L = J_{s\_midday} / (\Psi_{predawn} - \Psi_{midday}) \qquad (8)$$

where J_{s_midday} is the maximum sap flux density, $\psi_{predawn}$ and ψ_{midday} are predawn and midday leaf water potentials, respectively.

2.7 Hydraulic limitation model of water loss

The basis of the "hydraulic limitation" model is a critical minimum leaf water potential at the level of which, tree regulates its transpiration whatever it is induced by atmospheric drought or soil drought. The basic model used was similar to the ones developed by Tyree and Sperry (1988) and Sperry et al. (1998). Called "RER", it was developed in Microsoft Office Excel according to Cochard et al. (1996, 2002) following the simple equation:

$$J_{s\_crit} = g_L (\Psi_{predawn} - \Psi_{crit}) \qquad (9)$$

where J_{s_crit} is the critical maximum sap flux density and ψ_{crit} is the critical leaf water potential which stomata are assumed completely closed at this point (Cochard et al. 1996). Following Eq. 9, ψ_{crit} corresponds to the value of ψ_{midday} when J_{s_crit} equals actual midday sap flux density, J_{s_midday} (slope close to one).

Then, the midday or maximum sap flux density was estimated daily according to the following equation:

$$J_{s\_est} = g_L(\Psi_{predawn} - \Psi_{crit}) \tag{10}$$

where J_{s_est} is estimated maximum sap flux density, g_L and $\psi_{predawn}$ are both estimated daily from relationships with REW (input data). ψ_{crit} was assumed stable to the same value for the whole rainy season. The critical tree transpiration (E_{crit}; mm day^{-1}), was deduced from J_{s_est} according to Eq. 11. A simplified linear relationship was applied ($R^2 = 0.93$, n = 434; Figure 1):

$$E_{crit} = 0.7194 J_{s\_est} \tag{11}$$

E_{crit} defines a maximal value of daily transpiration due to the hydraulic limitations, however it may not be reached due to low evaporative demand. Hence, the final step of modeling selects the minimum value between E_{crit} and ET_0 (input data) according to Eq. 3.

2.8 Statistical analyses

Mean comparison, regression analysis and other statistics were performed using SPSS11.5 and Sigmaplot10.0. Linear slopes were compared using their confidence intervals at 95%. For the models, measured E_{Tree} and estimated E_{Tree} were compared using the root mean square error (RMSE) according to the formula:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(x_{1,i} - x_{2,i}\right)^2}{n}} \tag{12}$$

where $x_{1,i}$ and $x_{2,i}$ are measured E_{Tree} and estimated E_{Tree}, respectively, and n is the number of estimated E_{Tree}.

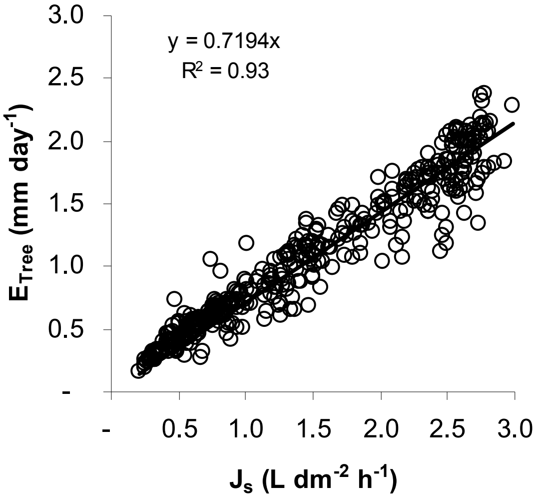

Figure 1 Daily tree transpiration (E_{Tree}) versus maximum sap flux density (J_s) in mature rubber tree (n = 434). The continuous line indicates the tendency of relationship, slope equals 0.7194 ($r^2 = 0.93$).

3. Results

3.1 Environmental conditions and seasonal changes

In 2007, rubber trees of the plantation displayed as usual a full canopy from May to November. Rainfall occurred from mid April to October. The cumulated amount of 960 mm was 20% below the long-term average in the area. After the onset of the rainy season, several drought spells, i.e., periods without significant rains from 10 to 20 days, occurred in May, June, July, September (Figure 2A). In the rainy season, the ET_0 varied largely from 0.43 to 4.17 mm day^{-1}. The values were particularly high (above 2 mm day^{-1}) in the first part of the rainy season from May to July and at the end of the rainy season in October (Figure 2A).

3.2 Transpiration

Despite full canopy, daily transpiration (E_{Tree}) showed remarkable changes along the rainy season (Figure 2A). First, there were recurring stable values around 2 mm day^{-1} (maximum = 2.38 mm day^{-1}), where the transpirations did not follow the

ET_0 increases. Secondly, there were dramatic decreases down to 0.32 mm day^{-1} (more than 80%) in June and July. This period which combined low rainfall and high ET_0, is known as the "mid-drought" period of the wet season in this area. However some peaks of transpiration decrease could also correspond to low ET_0 and rain occurrence (Figure 2A).

3.3 Soil drought

The volumetric soil water content (θ) showed marked changes in the top soil (0 - 0.4 m depth) with a continuous decrease from May to July (Figure 2B). Values ranged from 26 to 8 cm^3/100 cm^3 of soil, the minimum being reached at the same time than the minimum transpiration (Figure 2A). By contrast, θ in the subsoil (0.4 – 1.8 m depth) stayed at low values around 11 cm^3/100 cm^3 of soil, except a slight increase in October after high rains.

REW estimated from continuous soil water data (capacitive probe) confirmed the very low water availability in the sub soil (below 0.2 *REW*) except in October (Figure 2C). Above all, it showed the importance and severity of the soil drought in the top soil from June to the end of July, approximately 60 days below 0.5 *REW*. According to *REW* in the top soil, three periods of time were approximately distinguished: well-watered period (May and August to October), *REW* being above 0.5; mild-drought period (June to early July), *REW* fluctuating around 0.3; severe-drought (mid-end July), *REW* fluctuating around 0.15.

3.4 Leaf water potential and whole-tree hydraulic conductance

Leaf water potential before dawn ($\psi_{predawn}$) ranged between -0.32 and -0.44 MPa in the well-watered period (average = -0.38 MPa, Figure 2D). It slightly decreased, between -0.47 and -0.54 MPa in the mild drought period ($P<0.05$) and reached -0.83 MPa at the peak of drought ($P<0.01$). By contrast, ψ_{midday} did not appear related to soil drought. It ranged from -1.21 to -1.84 MPa when ET_0 was less than 2.2 mm day^{-1} (averaged -1.60 MPa), and ranged from -1.57 to -2.27 MPa when ET_0 was more than 2.2 mm day^{-1} (averaged -1.93 MPa). ψ_{midday} in sunny days did not significantly differ between the three periods of contrasting *REW*.

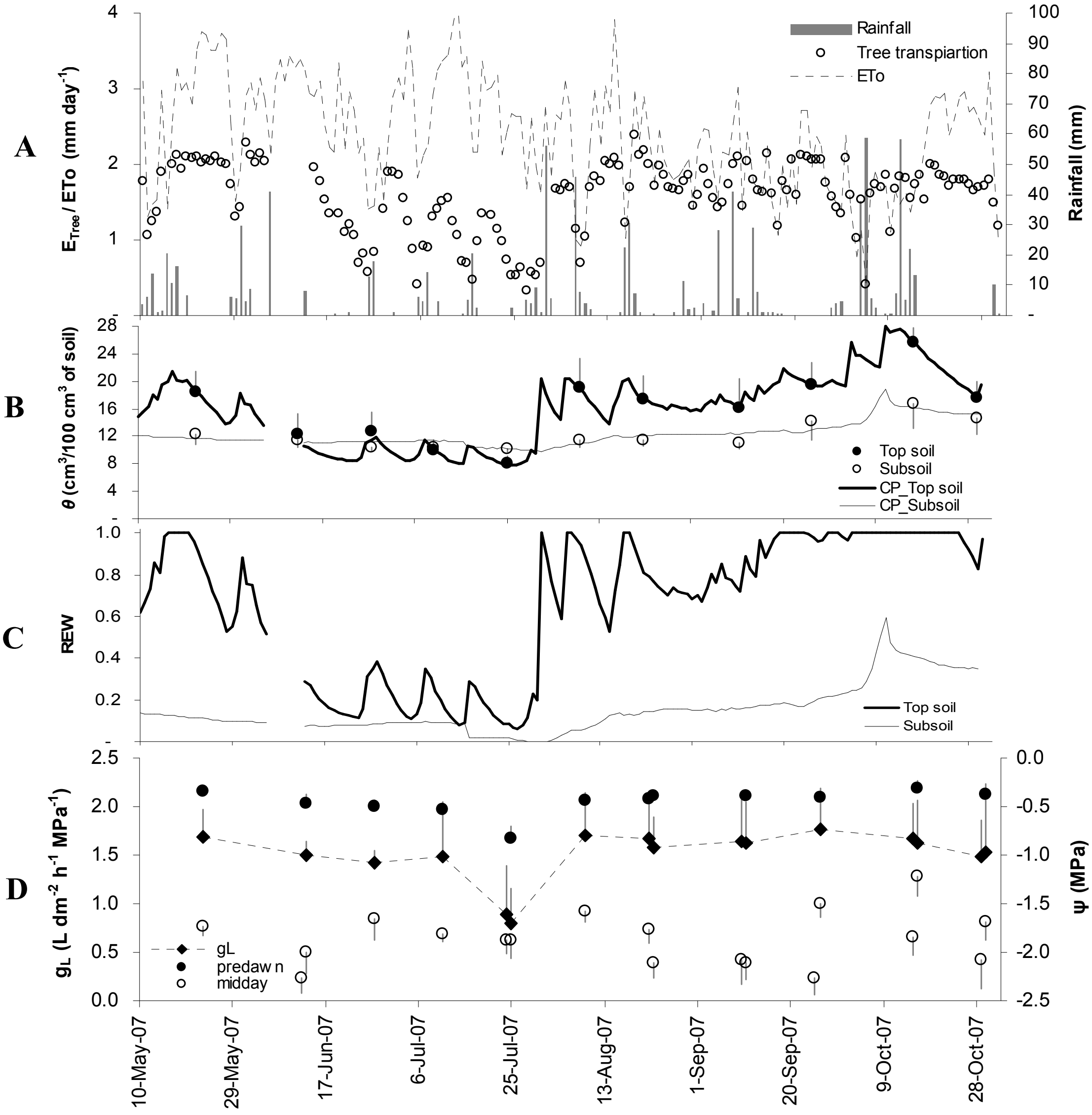

Figure 2 The environments condition and tree water parameters during mid May - October 2007: A) rainfall (columns), reference evapotranspiration (*ET_0*; dotted line) and average of 6 individual rubber trees in daily tree transpiration (*E_{Tree}*; opened circles), B) volumetric soil water content (*θ*) at top soil (closed circles), subsoil (opened circles), and recorded *θ* by capacitance probe of top soil (bold line) and subsoil (thin line), C) relative extractable soil water in top soil (*REW*) on top soil (bold line) and subsoil (thin line), and D) predawn ($\psi_{predawn}$; closed circles) and midday leaf water potential (ψ_{midday}; opened circle) and whole tree hydraulic conductance (g_L; closed rhombus with dotted line). The 2 g_L values in the parentheses were the estimated values from the relationship between g_L and $\psi_{predawn}$. The vertical bars in each picture indicate confidential interval at 95%.

g_L calculated with the single point method appeared related to soil drought. It averaged 1.63 L dm^{-2} h^{-1} MPa^{-1} in the well-watered. g_L decreased to 1.47 L dm^{-2} h^{-1} MPa^{-1} in the mild-drought period which did not significantly differ from the value in the well-watered period. At the peak of soil drought, the decrease by 50% was significant (0.89 L dm^{-2} h^{-1} MPa^{-1} in 24 July 2007 and 0.79 L dm^{-2} h^{-1} MPa^{-1} in 25 July 2007; $P<0.05$; Figure 2D).

3.5 Transpiration responses to atmospheric and soil droughts

3.5.1 Atmospheric drought

The relationship between E_{Tree} and ET_0 showed that in well-watered conditions, transpiration followed ET_0 along the 1:1 line up to a ET_0 threshold between 2 and 2.2 mm day^{-1} (Figure 3). Above this threshold the transpiration saturated. Points corresponding to mild and severe soil drought periods were logically displayed below the response in well-watered conditions.

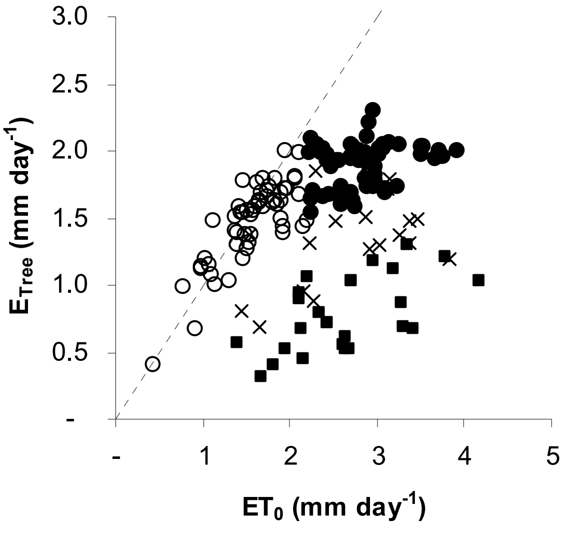

Figure 3 The relationship between tree transpiration (E_{Tree}; average of 6 rubber trees) and reference evapotranspiration (ET_0) at well-watered period ($REW > 0.5$) with $ET_0 \leq 2.2$ mm day^{-1} (opened circle), well-watered period with ET_0 more than 2.2 mm day^{-1} (closed circle), mild-moderate drought period (REW range from 0.15 to 0.5; cross), and moderate-severe drought period (REW lower than 0.15; closed square). The dotted line indicates 1:1 reference.

3.5.2 Soil drought

According to previous results of transpiration response to atmospheric drought, the values of E_{Tree} were separated depending on ET_0 threshold and transpiration was expressed relative to ET_0 (Figure 4A and 4B).

Above 0.5 *REW*, E_{Tree} and E_{Tree}/ET_0 showed scattered points without significant slope. For non-saturating conditions of ET_0 (ET_0 < 2.2 mm day^{-1}), the relative transpiration ranged between 0.69 and 1.36 and the average was closed to 1 (0.98). The scatter of relative transpiration points corresponding to ET_0 > 2.2 mm day^{-1} was logically lower than 0.5 REW. Below 0.5 *REW*, there was a significant linear decrease of the relative transpiration versus *REW* ($R^2 = 0.65$, $P<0.0001$, Figure 4B). Saturating condition of ET_0 did not affect the transpiration response at low *REW*, *REW* being the limiting factor. Considering the crossing between the decreasing line and the plateau above 0.5 *REW*, a *REW* threshold around 0.5 was estimated. This estimate was approximate due to the lack of soil data between 0.4 and 0.5 *REW*. Finally, relative transpiration had decreased by 40% at *REW* around 0.3 (mild-drought) and by 80% at *REW* around 0.1, the peak of drought.

In addition, the relationship between $\psi_{predawn}$ and *REW* confirmed a highly significant effect of soil drought upon $\psi_{predawn}$ (Figure 4C, $R^2 = 0.93$, $P < 0.0001$). The previous threshold of 0.5 *REW* for the onset of transpiration decrease corresponds to a $\psi_{predawn}$ of -0.45 MPa. The tree transpiration was also strongly related to $\psi_{predawn}$ decrease (Figure 5: E_{Tree} and E_{Tree}/ET_0), however intermediate data were missing to get an accurate relationship. The *REW* data appeared more appropriated to analyze transpiration response threshold than $\psi_{predawn}$ because their recording were continuous.

3.6 Diurnal kinetics

Bi-hourly kinetics of E_{Tree} and ψ_{Leaf} were compared for 4 sunny days of contrasted soil drought conditions (Figure 5): onset (May 23, *REW* = 0.90), mild-drought (July 11, *REW* = 0.24), peak of drought (July 25, *REW* = 0.08) and recovery period (August 22, *REW* = 0.82). Daily ET_0 significantly varied between 2.23 and 3.71 mm day^{-1}.

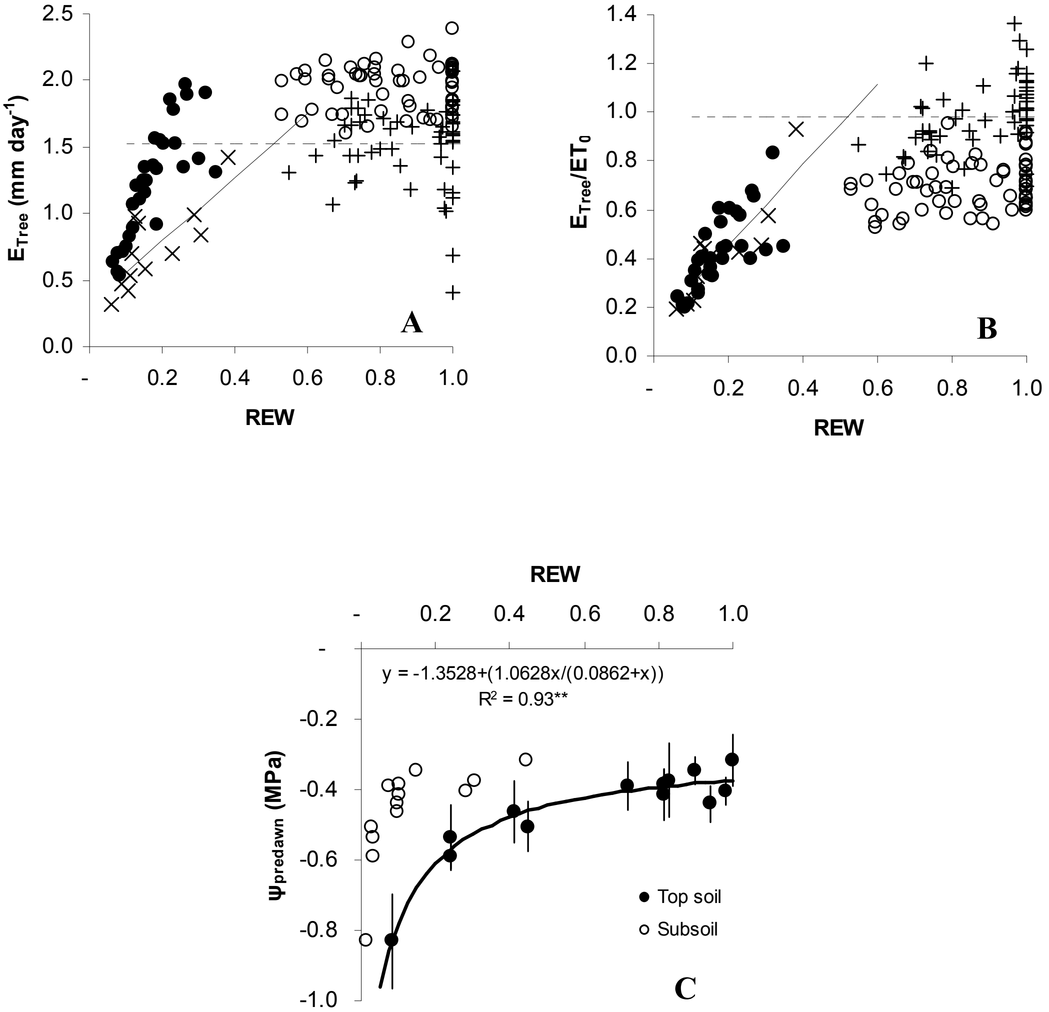

Figure 4 A) E_{Tree} versus *REW* on top soil. The continuous line indicates the tendency of E_{Tree} as related to *REW*. The dotted line shows the average of E_{Tree} at $ET_0 < 2.2$ mm day^{-1}. B) E_{Tree}/ETo versus *REW* on top soil relations. The continuous line indicates the tendency of E_{Tree}/ET_0 increasing following *REW*. The dotted line shows the average of E_{Tree}/ET_0 in non limited of ET_0 (< 2.2 mm day^{-1}). The closed circles and opened circles in Figure A and B exposed in no limited evaporative demand days ($ET_0 > 2.2$ mm day^{-1}), whereas crosses and pluses exposed in the low evaporative demand days ($ET_0 < 2.2$ mm day^{-1}). C) The relationship between $\psi_{predawn}$ and *REW* on both top soil (closed circle) and subsoil (opened circle). The vertical bars indicate the 95% confidential interval, and ** indicate the highly correlation at 99%.

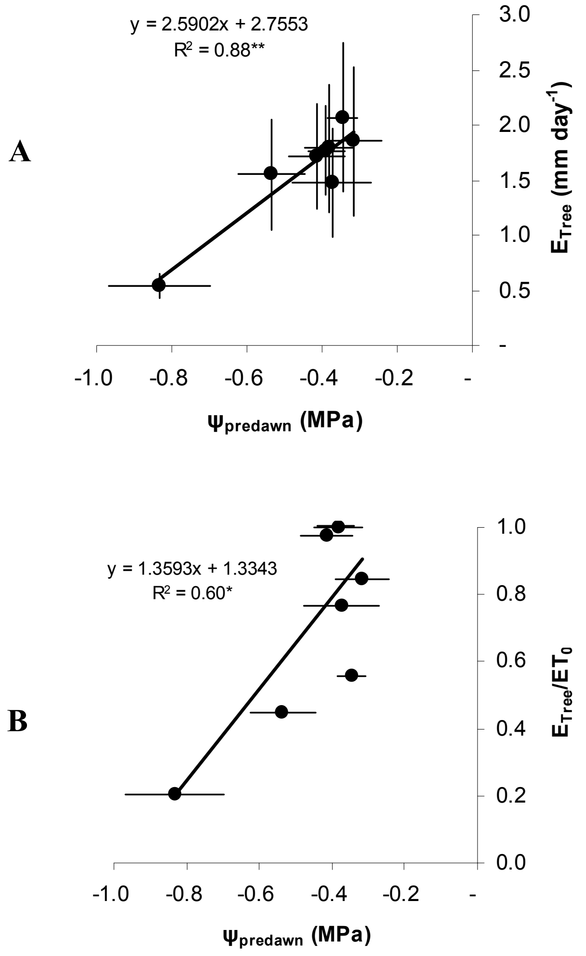

Figure 5 The relationship between A) E_{Tree} and $\psi_{predawn}$ and B) E_{Tree}/ET_0 and ψ_{predawn} in full canopy during May – October 2007. The vertical and horizontal bars indicate the 95% confidential interval. * and ** indicate the highly correlation at 95% and 99%, respectively.

In well-watered conditions, E_{Tree} exhibited a classical pattern following ET_0 increase and reaching maximum rate around midday. According to the effect of transpiration on leaf dehydration, ψ_{Leaf} showed the highest value at predawn, declined to the lowest value around midday, and increased again up to the end of daytime (Figure 6). It was noticeable that the maximum of transpiration decreased according to soil drought intensity, from 0.23 to 0.08 mm h^{-1} and reach again 0.22 mm h^{-1} at the recovery period. The curves of diurnal E_{Tree} and ψ_{Leaf} were particularly flattened during the drought period (Figure 6B and 6C).

ψ_{midday} did not change significantly, values ranging between -1.73 to -1.88 MPa. By contrast, $\psi_{predawn}$ decreased as previously shown from -0.34 to -0.83 MPa. Moreover, soil drought decreased the ψ_{Leaf} at sunset (ψ_{sunset}) not allowing the same hydration recovery at the end of day-time than in well-watered conditions (Figure 6).

3.7 g_L multipoints

Diurnal ψ_{Leaf} plotted versus hourly J_s, showed significant linear relationships ($P<0.001$, Figure 7) in which the slopes represent the reverse of *gL*. The linear form supported the hypothesis that the effect of water storage (capacitance) was negligible. It confirmed the validity of the single-point method which uses only the single gradient between $\psi_{predawn}$ and ψ_{midday} versus J_{s_midday} to calculate seasonal g_L (Figure 2D). The 3 periods of soil drought intensity were distinguished in Figure 7. It was noticeable that both Y intercepts and slopes tend to decrease according to the drought intensity. Compared with well-watered conditions, the slope differed significantly at drought peak ($P<0.05$) but not in mild drought conditions. Conversely, minimum leaf water potential (ψ_{Leaf_min}) did not change according to soil drought intensity.

3.8 g_L single point versus E_{Tree}, *REW* and $\psi_{predawn}$

As suggested in Figure 2D, plotting of E_{Tree} versus g_L confirmed that the decrease of daily E_{Tree} was mainly explained by the change of g_L following a linear response ($R^2 = 0.91$, $P< 0.001$, Figure 8A). The decrease of g_L under the effect of soil drought was confirmed by the highly significant relationships with decreasing *REW* ($R^2 = 0.91$, $P < 0.0001$, Figure 8B) and $\psi_{predawn}$ ($R^2 = 0.88$, $P < 0.001$, Figure 8C). However, intermediate values of g_L, between 1 and 1.4, were missing to provide accurate relationships.

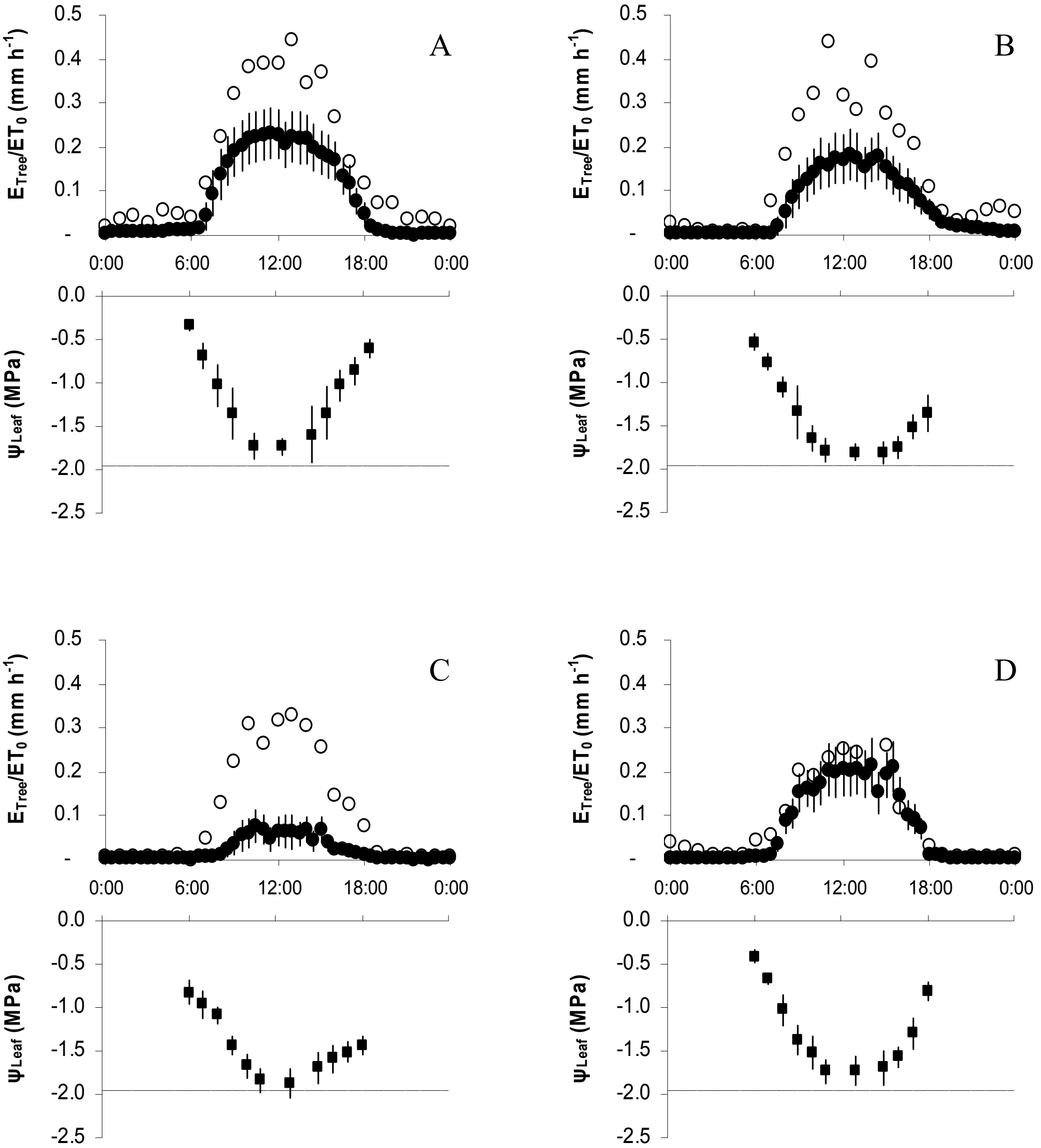

Figure 6 Diurnal reference evapotranspiration (ET_0; opened circle), tree transpiration (E_{Tree}; closed circle) and leaf water potential (ψ_{Leaf}; closed square) of rubber tree in 4 periods: A) onset (*REW* of top soil = 0.85; May 23, 2007), B) moderate drought (*REW* of top soil = 0.24; July 11, 2007), C) peak of drought (*REW* of top soil = 0.08; July 25, 2007) and D) recovery period (*REW* of top soil = 0.81; August 22, 2007). The vertical bars of E_{Tree} and ψ_{Leaf} indicate the standard deviation of 6 rubber trees. The horizontal lines in each ψ_{Leaf} illustrations indicate the critical minimum leaf water potential.

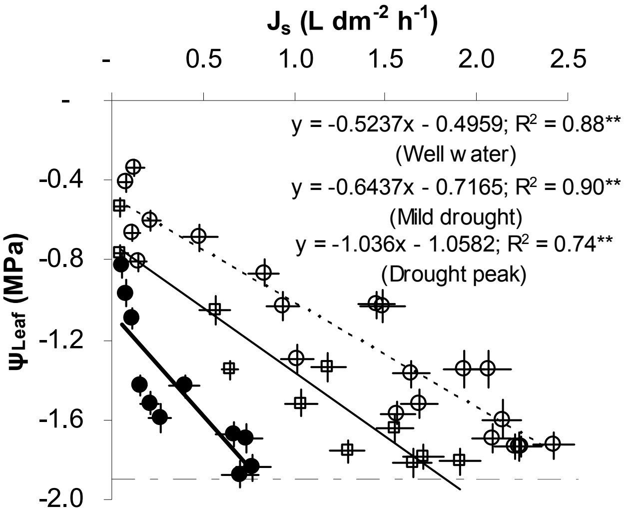

Figure 7 The pattern for daily variations of leaf water potential (ψ_{Leaf}) and sap flux density (J_s) and for well-watered in onset and recovery periods (opened circle and dotted line), started drought period (opened square and thin line) and peak of drought (closed circle and bold line). Each data point is the average of 6 rubber trees. The linear equation of well-watered period is ψ_{Leaf} = -0.5237J_s – 0.4959; R^2 = 0.88; the upper bound and lower bound of confident interval 95% are -0.61 and -0.44 for slope, and -0.63 and -0.36 for Y interception. The linear equation of mild drought period is ψ_{Leaf} = -0.6437J_s – 0.7165; R^2 = 0.90; the upper bound and lower bound of confident interval 95% are -0.81 and -0.48 for slope, and -0.92 and -0.51 for Y interception. The linear equation of peak of drought period is ψ_{Leaf} = -1.036J_s – 1.0582; R^2 = 0.74; the upper bound and lower bound of confident interval 95% are -1.50 and -0.56 for slope, and -1.28 and -0.84 for Y interception. The vertical and horizontal bars indicate the standard error, and ** are highly linear relationship. The horizontal dashed line indicated the critical minimum leaf water potential.

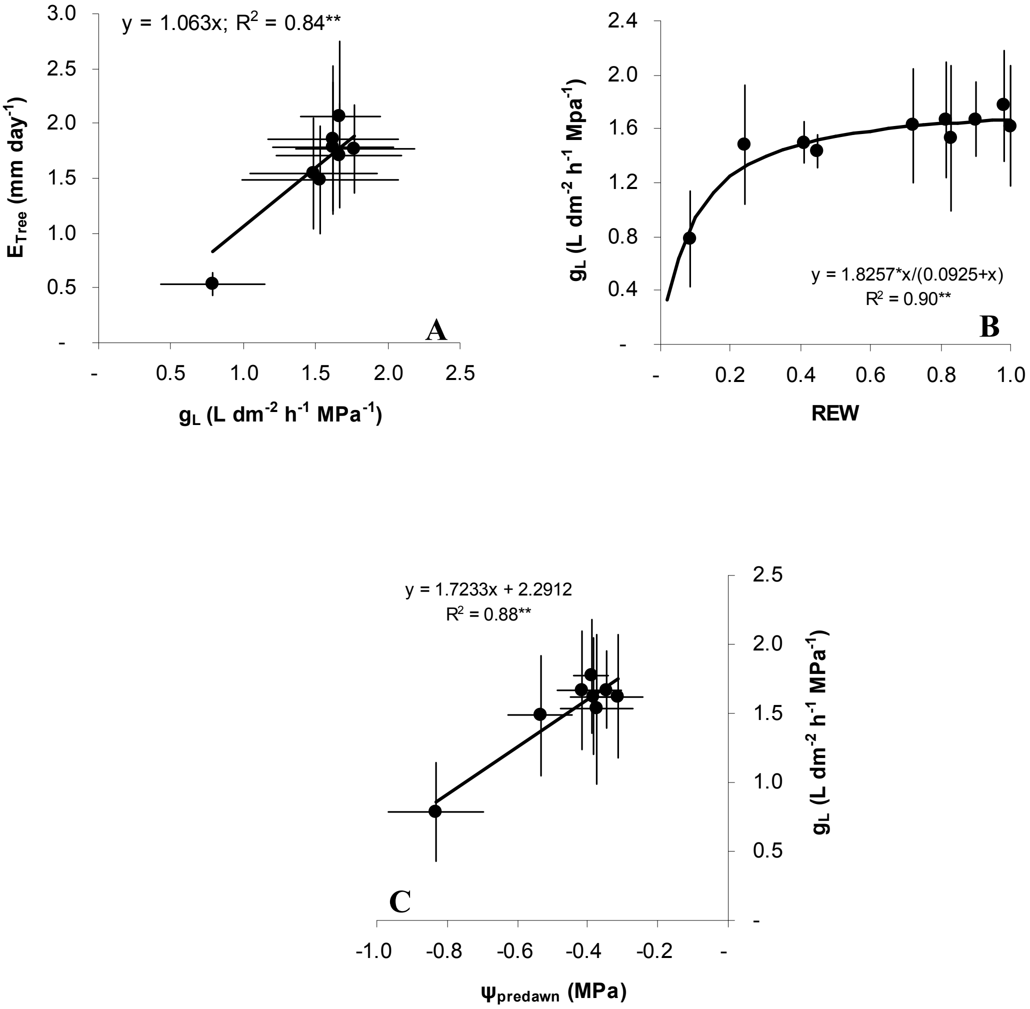

Figure 8 The relationship between A) tree transpiration (E_{Tree}) and whole tree hydraulic conductance (g_L), B) g_L and relative extractable soil water content (*REW*), and C) g_L and predawn leaf water potential ($\psi_{predawn}$) in full canopy during May – October 2007. Each data points were taken on only the sunny day. The vertical and horizontal bars indicate the 95% confidential interval, and ** indicate the highly correlation at 99%.

3.9 "Hydraulic limitation" model

ψ_{crit} was estimated according to RER model and Eq. 9. -1.95 MPa provided the best adjustment between J_{s_crit} and J_{s_midday} with a slope not different from 1 (Table 1). This simple model properly simulated both the tendencies of saturating transpiration when high ET_0 occurred in well-watered conditions and the dramatic effects of soil drought in June and July (Figure 9). In the details, the simulated saturating transpirations averaged 1.85 mm day^{-1} and were slightly lower that measured values at high ET_0 in May. However evidently the model did not simulate the decrease of transpiration related to low ET_0 in well-watered conditions (Figure 9 and 10A).

Logically, the combination between the maximum transpiration permitted by ET_0 and E_{crit} improved the simulation of transpiration (RER_ET_0) model, Figure 9 and 10B, n = 160, R²= 0.78, RMSE= 0.23). The group of points corresponding to contrasted conditions of soil and atmospheric drought are quite properly displayed along the 1:1 reference line.

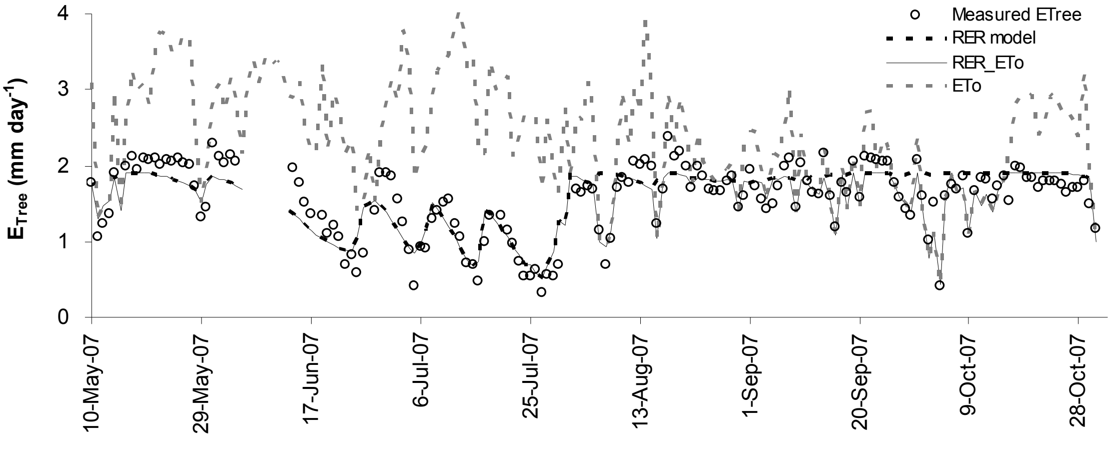

Figure 9 Comparison of measured daily tree transpiration (Measured E_{Tree}; opened circle) and estimated daily E_{Tree} of RER model (black dotted line) and RER_ET_0 model (continuous line). The bold gray dotted line indicates the daily ET_0.

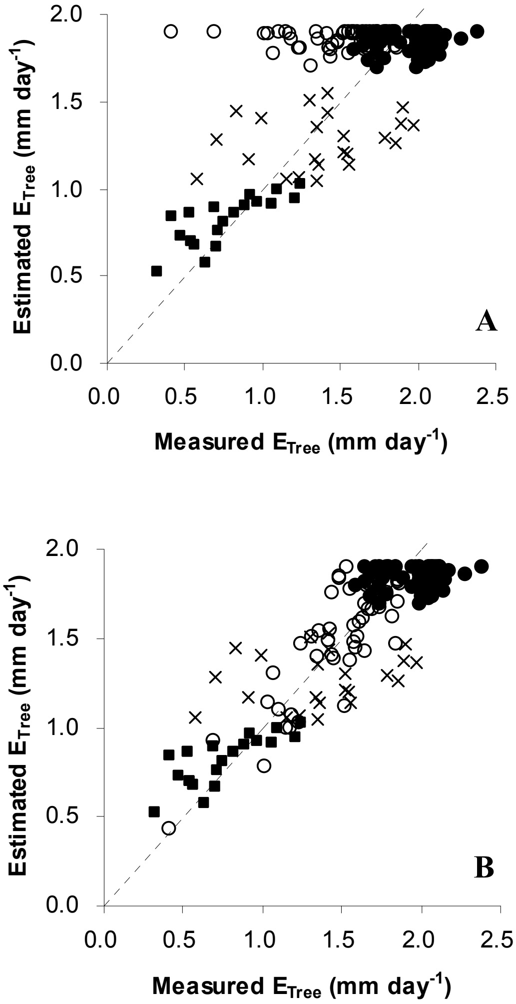

Figure 10 Relationship between measured daily tree transpiration (Measured E_{Tree}) and estimated daily tree transpiration (Estimated E_{Tree}) in the matured rubber tree during mid May – October 2007: A) RER model, and B) RER_ET_0 model. The dotted line in each pictures indicate the 1:1 reference. The meaning of each marker indicates each period: well-watered period with $ET_0 \leq 2.2$ mm day^{-1} ($REW > 0.5$; opened circle), well-watered period with ET_0 more than 2.2 mm day^{-1} ($REW > 0.5$; closed circle), mild-moderate drought period (REW range from 0.15 to 0.5; cross), and moderate-severe drought period ($REW < 0.15$; closed square). The R^2 and RSME (n=160) were 0.51 and 0.34 for RER model, and 0.78 and 0.23 for RER_ET_0 model, respectively.

Table 1 The slope with set origin at 0, statistics related to each regression (R^2 and P) and 95% confidential interval for slope (CI_95) both lower bond and upper bond of theoretical threshold sap flux density (J_{s_crit}) inducing completely stomata closure versus measured midday sap flux density (J_{s_midday}) in several given critical minimum leaf water potential (ψ_{crit_giv}).

ψ_{crit_giv}	Slope	R^2	P	CI_95		RSME
				Lower Bound	Upper Bound	
-1.70	0.851	0.979	< 0.0001	0.827	0.875	0.42
-1.80	0.916	0.980	< 0.0001	0.890	0.942	0.34
-1.90	0.981	0.980	< 0.0001	0.954	1.009	0.30
-1.95	1.014	0.980	< 0.0001	0.985	1.042	0.31
-2.00	1.047	0.980	< 0.0001	1.017	1.076	0.34
-2.10	1.112	0.980	< 0.0001	1.081	1.143	0.42
-2.20	1.177	0.980	< 0.0001	1.144	1.210	0.52

4. Discussion

4.1 Responses of transpiration to soil and atmospheric droughts

Our results demonstrate a response of transpiration to both atmospheric drought and soil drought with critical thresholds. Firstly, a critical ET_0 inducing saturating transpiration despite well-watered soil conditions was noticed between 2.0 and 2.2 mm day^{-1}. These values corresponded to maximum and average VPD of 1.6 kPa and 1.8 kPa, respectively. The maximum E_{Tree} measured was around 2.4 mm day^{-1} and corresponded to J_s per sapwood area close to 3 L dm^{-2} h^{-1}. To our knowledge, this is the first time that a saturating transpiration over a certain threshold of ET_0 is assessed for rubber tree. Continuous recording of rubber E_{Tree} with sap flow measurement are very rare. These results concern RRIM600, the most planted clone in South-East Asia. The data of Gururaja Rao et al. (1990) for mature rubber trees in India did not support saturating transpiration under high evaporative demand. They found very high transpirations in dry season, between 3.5 and 4.5 mm depending the clone, the relative transpiration E_{Tree}/ET_0 being close to 1. Transpirations were estimated by porometer which could have induced large over-estimation. Moreover, they also recorded xylem sap speed with Heat Pulse method at the same date but the corresponding sap flux densities, approximately ranged between 1.6 and 1.0 L dm^{-2} h^{-1}, appear very low for such high whole-tree transpiration. However, the studied clones, RRII 105 and RRII 118, were also different from RRIM 600 and comparative studies showed large clonal variability of water response to soil and atmospheric droughts (Chandrashekar 1997). Several recent studies quote the saturation of transpiration at high evaporative demand for other species (David et al. 2004; Bovard et al. 2005; Oguntunde et al. 2007; Bush et al. 2008; Huang et al. 2009). The thresholds of critical evaporative demand are within similar range: at 1.5 kPa of *VPD* for *Quercus rotundifolia* (David et al., 2004), around 1 kPa of *VPD* for *Acer rubrum*, *Betula papyrifera*, *Populus grandidentata*, *Quercus rubra* (Bovard et al., 2005) and around 1.5-2 kPa of *VPD* for *Gleditsia triacanthos*, *Quercus gambelii*, *Quercus rubra* (Bush et al., 2008). However, some species in ring-porous taxa do not exhibit saturating transpiration, E_{Tree} increasing almost linearly with *VPD* until 5 kPa (Bush et al., 2008).

Secondly, soil drought in rainy season induced a dramatic decrease of E_{Tree}. The relative transpiration started to decrease linearly below c.a. 0.5 *REW* in the top soil (0-40 cm) and was reduced by 40% at 0.3 *REW* and 80% at 0.1 *REW*. The threshold of 0.5 *REW* for the onset of transpiration decrease was close to the range (0.4-0.5) quoted by Granier et al. (1999, 2000), Breda et al. (2006), Domec et al. (2009), for mainly temperate trees. In our conditions the particularly low water availability in the sub soil (40-180 cm), below 0.2 *REW* at the period of the top soil drought, could have amplify the reduction of transpiration versus water availability in the top soil.

4.2 Operational range of leaf water potential

Results confirmed a significant decrease of $\psi_{predawn}$ under soil drought and demonstrate a relative stability of minimum leaf water potential ($\psi_{minimum}$) for sunny days. Well-watered soil conditions corresponded to predawn values between -0.3 and -0.4 MPa. The threshold of 0.5 *REW* for the start of transpiration decrease corresponded to a $\psi_{predawn}$ value of -0.45 MPa. The extreme soil drought (0.1 *REW*) with 80% of transpiration reduction corresponded to -0.78 MPa.

$\psi_{minimum}$ values ranged between -1.8 and -2.2 MPa for sunny days and did not decrease when soil drought occurred. The average value was -1.93 MPa. Such pattern expresses an isohydric behaviour which maintains leaf hydration above a critical $\psi_{minimum}$ through stomatal closure (West et al. 2007). Such behaviour appears also consistent with the observation of saturating transpirations at high evaporative demand.

For mature trees of the same clone RRIM 600, Chandrashekar (1997) found values of $\psi_{minimum,}$ between -1.8 and -1.9 MPa, within the low range of our results. Comparing 8 clones, this author found values between -1.75 and -2.13 MPa, within a similar range to our results. For other clones in traditional area of India, Gururaja Rao et al. (1990) found minimum values of $\psi_{minimum}$ within similar range, between -1.8 and -2.4 MPa. Comparing seasons, Chandrashekar et al. (1990) suggested an anisohydric behaviour under drought of rubber trees when $\psi_{minimum}$ decreased from -1.3 MPa in wet season to -1.8 MPa in dry season. But we are not sure that this comparison refers to similar sunny days, the difference could be related to difference of evaporative demand and magnitude of transpiration.

However, for young potted rubber trees of the same clone (RRIM600), the results of Sangsing et al. (2004) clearly did not support an anisohydric behaviour. In the latter experiment, the drought treatments provoked in four weeks a marked decrease of minimum xylem pressure from -0.8 to -1.7 MPa. Hence, the separation between isohydric and anisohydric behaviour for the same clone could be relative to the experimental conditions of study.

The decrease of $\psi_{predawn}$ under soil drought was not a surprise. It is a well known result which explains that $\psi_{predawn}$ is often used as a surrogate of soil water potential or soil water availability in the zone of root uptake (Richter 1997; Donovan et al. 2001). In the literature, the range of $\psi_{predawn}$ values quoted for mature rubber trees in well-watered conditions is very large. Chandrashekar et al (1990) and Chandrashekar (1997) found values between -0.57 and -0.68 MPa for the same clone (RRIM 600) in the wet season of a water-limited area of India. Gururaja Rao et al. (1990) found values between -0.1 and -0.3 MPa in a water-optimal area of India. Previous values between 0.6 and 0.7 appear definitively too low to represent well-watered conditions. This artifact may come from limiting conditions of soil water availability or root uptake despite wet season. Another reason can be methodological because the measurements were performed with psychrometer which could be much more difficult to apply than pressure chamber, particularly at high pressure. The range found by Gururaja Rao et al. (1990) appears more representative of well-watered conditions.

The range of our values may appear slightly too low. With the pressure chamber, if the increment of pressure is too quick the measurement of pressure in equilibrium with sap exudation can be over-estimate. It is possible because measurements done in the following year (2008) with a very low pressure increment provided predawn values between -0.2 and -0.3 MPa (unpublished data). However, this year there was more water availability in the subsoil in wet season. Finally, predawn value below -0.4 MPa appears as a clear indicator of water stress and constraint over water use for mature rubber trees.

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4.3 Whole tree hydraulic conductance and transpiration

Results demonstrate that the decrease of E_{Tree} was mainly due to the change of the g_L. And the decrease of g_L was strongly correlated to the decline of REW and $\psi_{predawn}$. Our maximum values of g_L (between 2.0 and 1.5 L dm^{-2} h^{-1} MPa^{-1}) are within the range found by previous authors on temperate trees (Cochard et al. 1996, Lu et al. 1996) and tropical trees (Do et al. 2008, O'Grady et al. 2009). The decrease of E_{Tree} mainly related to the decline of g_L has been reported for several tree species (Cochard et al. 1996; Lu et al. 1996; Irvine et al. 1998; Addington et al. 2004; Domec et al. 2009). The quick recovery of g_L after substantial rainfall in August suggests that the main control came from the change of soil-root conductance. Several studies showed that, compared to trunk and branches, roots and leaves had the highest loss of conductivity and contributed for more than 70% of the total hydraulic resistance (Cruiziat et al. 2002; Sack and Holbrook 2006; Domec et al. 2009). As rubber tree exhibited a strong control of water losses through the relative stability of $\psi_{minimum}$, we assume that during soil drought the percentage of embolism was relatively low. However, it could have increased and contributed to the decrease of g_L. The hydraulic limitation hypothesis assumes that the plant maintain $\psi_{minimum}$ above a critical value through stomatal closure to prevent the vessels from xylem tension inducing runaway embolism (Jones and Sutherland 1991; Cochard et al. 1996; Sperry et al. 2002). It was found in several species that ψ_{crit} corresponded to ca. 10% of percentage loss of hydraulic conductivity (PLC) and 90% of stomatal closure (Cochard et al 1996; Cruiziat et al. 2002). However, no study of vulnerability to cavitation has been published on mature rubber tree. Nevertheless in young potted trees, Ranasinghe and Milburn (1995) and Sangsing et al. (2004) both found values of xylem tension corresponding to 50% of embolism in the petioles between -1.5 and -2.0 MPa. Which suggests that $\psi_{minimum}$ values measured in our studies (from -1.8 to -2.2 MPa) could correspond to substantial embolism of petioles and xylem vessels. However, the operational range of $\psi_{minimum}$ values noticed by Sangsing et al. (2004) in its study on young rubber trees was higher than the one in our studies. And results of several authors support the idea that operational range of leaf water potential and vulnerability to cavitation decrease with age (Becker et al. 2000; McDowell et al. 2002; Phillips et al. 2002; Ryan and Yoder 1997). Measurements of native embolism and vulnerability to cavitation in xylem of mature rubber tree deserve further researches.

4.4 Performance of the "hydraulic limitation" model

The results proved the ability of a simple model, based on g_L and ψ_{crit}, to simulate the pattern of transpiration changes. It was remarkable to see that this model could simulate the main effects of both soil drought and atmospheric drought, i.e. the saturating transpiration at ET_0 higher than 2.2 mm day^{-1}. The change of g_L was only related to soil water availability (*REW*) which influenced also $\psi_{predawn}$. Hence the saturating transpiration in well-watered conditions was only simulated by the maximum value of g_L and by ψ_{crit} which are hydraulic traits of the tree. Moreover, there is no reason to consider that these parameters were completely stable over 6 months. They could fluctuate according to root growth, stem growth and leaf aging. These evidences could explain inaccuracies in the simulation of maximum transpiration.

The good performance of the simple model is likely related to several features. One feature is the isohydric behaviour of the clone in the experimental conditions. A comparison between 8 clones in a water limited areas of India ranked RRIM 600 with the best performance in term of control of water status and girth growth (Chandrashekar 1997). The extreme condition of soil drought could be another feature because it exacerbates the responses with little possibility of root water uptake in the subsoil. The full canopy insures a relative stability of the leaf area index which is a key factor of whole-tree transpiration. Hence it will be interesting to apply the same model and parameters in the more complicated conditions of the seasonal drought in dry season where soil water availability is different in the subsoil and leaf yellowing and shedding occur.

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5. Conclusion

We investigated for the main clone planted in Southeast Asia (RRIM600) the transpiration response of mature trees to atmospheric and soil droughts during the full canopy in the rainy season of a drought-prone area of northeast Thailand. Despite well-watered soil conditions, transpiration did not follow evaporative demand at high values and saturated above a critical value of ET_0. Soil drought provoked a dramatic decrease of transpiration below a threshold of 50% of *REW* in the top soil or -0.45 MPa $\psi_{predawn}$. And E_{Tree} reduction was mainly explained by a decrease of the g_L. Under soil drought, the relative stability of $\psi_{minimum}$ for sunny days supports an isohydric behavior of these trees. Additionally, a simple modeling approach based on the hydraulic limitation hypothesis proved to be successful to simulate the main pattern of transpiration responses to soil and atmospheric droughts.

Finally, the combination between the cohesion-tension theory, electric analogy and hydraulic limitations hypothesis had provided a useful framework to analyze transpiration responses to soil and atmospheric droughts and to develop a simple process-based models. On this basis, several development of such process-based model could be considered towards the inclusion of details about soil-root interface, vulnerability to cavitation, stomatal behaviour and consequence on carbon gain. This approach is a promising way to better evaluate the performance of genotypes in contrasted environments.

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Chapter V

Result III:
Whole-tree transpiration response to seasonal drought in mature Hevea brasiliensis: hydraulic limitation and influence of leaf phenological stages

Whole-tree transpiration response to seasonal drought in mature *Hevea brasiliensis*: hydraulic limitation and influence of leaf phenological stages

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Abstract

The changes of whole-tree transpiration under atmospheric and soil droughts were investigated for mature rubber trees, and the main clone planted in Southeast Asia (RRIM600), in the seasonally dry period where several major phenological changes occur: leaf senescence, leaf shedding and leaf flushing.

The responses were analyzed under the framework of a simple hydraulic limitation model 'RER_ET_0 model' previously assessed by Isarangkool Na Ayutthaya et al. (Paper 2) in full canopy conditions for intermittent drought in rainy season. This model is mainly based on a critical minimum leaf water potential (ψ_{crit}) and the response of the whole-tree hydraulic conductance (g_L) to the relative extractable water (*REW*) from soil.

Data were separated in three periods according to canopy phenology and soil drought: full canopy period in well-watered soil conditions (topsoil REW>0.5, September – mid November), full canopy period with starting soil drought (topsoil

REW<0.5, mid November – mid December 2007), and leaf senescence-shedding and leaf flushing conditions which corresponded to a low green leaf area, and a severe soil drought (topsoil REW < 0.25, mid December 2007 – mid March 2008).

Results proved first that the principles of RER_ET_0 model hold: stability of minimum leaf water potential ($\psi_{minimum}$) for sunny days under soil drought, decrease of tree transpiration (E_{Tree}) mainly related to change of g_L, decrease of g_L mainly related to *REW*. Secondly, the deciduous pattern showed little influence upon the parameters and the relationships of the hydraulic limitation model. The simulations demonstrated the ability the model to describe the trend of transpiration change under seasonal drought without taking into account the phenology and variation of green leaf area by contrast with the majority of transpiration models.

Finally, our results support the idea that phenological changes converged toward a same response of whole-tree water relations to soil and atmospheric drought, which was correctly described by the concepts of hydraulic limitation: ψ_{crit}, g_L decrease versus soil water availability. This convergence certainly hides complex adjustments between short term process (stomatal regulation, xylem embolism) and long term process of leaf and root phenologies which change the relative contribution of hydraulic conductances to the soil-root and canopy-atmosphere interfaces.

Key words: *Hevea brasiliensis*, rubber tree, seasonal dry period, tree transpiration, whole tree hydraulic conductance, leaf water potential

1. Introduction

Facing drought, plants have to prevent excessive dehydration and maintain the integrity of their hydraulic system. They can achieve these goals by short term reversible responses like stomatal closure and by long term responses like increasing root development in wetter soil layers or reducing transpiring leaf area (Breda et al. 2006).

Reducing leaf area is a major phenological adaptation of deciduous trees to seasonal drought in the dry tropics (Eamus and Prior 2001). Many deciduous trees drop all their leaves during seasonal drought and sprout new ones just before or after the first rains (Taiz and Zeiger 1991). Moreover, the different stages of leaf phenology may correspond to particular changes of root phenology: root decay or root growth in different soil layers according to soil water availability. Hence, environmental and hydrological models have to take into account the phenological stages and changes of leaf area and or root profile to predict the pattern of tree transpiration under seasonal drought.

The hydraulic limitation hypothesis (Jones 1998; Sperry et al. 1998) is a promising plant-based way to model transpiration under water stress. This approach has proven to be valid for several temperate angiosperms (Sperry et al. 1998; Cochard et al. 2002). Recently, Isarangkool Na Ayutthaya et al. (Paper 2) proved the validity of this approach to analyze and to simulate the change of rubber tree transpiration under intermittent drought in rainy season while trees maintained full canopy. In this simple model called 'RER_ET_0', tree transpiration (E_{Tree}) is calculated as the minimum value between the reference potential evaporative demand (ET_0) and a critical transpiration (E_{crit}) according to critical value of minimum leaf water potential (ψ_{crit}) and whole-tree hydraulic conductance from soil to leaves (g_L):

$$E_{crit} = (\Psi_{predawn} - \Psi_{crit}) \times g_L \times a \qquad (1)$$

Where $\psi_{predawn}$ is assumed to correspond to ψ_{soil} in contact with roots and a is a coefficient to transform midday sap flow density in total flow of transpiration expressed per day and unit of soil area.

The hydraulic limitation hypothesis assumes that many species (i.e., isohydric ones) tend to adjust their transpiration with the effect of maintaining ψ_{Leaf} above a critical value. In RER_ET_0 model, g_L and $\psi_{predawn}$ are calculated from relationships with the relative extractable water from soil. Hence, E_{crit} sets a functional limitation to E_{Tree} that can be reached under soil drought (low $\psi_{predawn}$, low g_L value) but also under atmospheric drought in well-watered soil conditions (g_{L_max} value, ψ_{crit}).

One question was how the changes of leaf phenological stage that occurred in seasonal dry period for rubber trees may influence the responses of transpiration to drought under this framework and by comparison with the ones assessed by Isarangkool Na Ayutthaya et al. (Paper 2) in rainy season.

Rubber tree (*Hevea brasiliensis*) is a tree member of the Euphorbiaceae family and native from the tropical rainforest of the Amazon Basin. It is a tropical brevi-deciduous tree. The rubber trees older than 3 or 4 years are subject to 'wintering', which is the term used to describe the annual senescence and shedding of leaves. The duration and intensity of deciduousness are variable depending environment, clones, and individuals. Leaf fall is quickly followed by bud bursting and expansion of new leaves within few weeks (Webster and Paardekooper 1989). In the water limited area of Northeast Thailand (the southern part), the period of leaf yellowing-leaf falling-bud bursting–leaf flushing normally lasts at its maximum extent five months, from December to April. Hence, such period of relatively low green leaf area should markedly reduce whole-tree transpiration. The dry season generally lasts from November to April.

In the same area, Gonkhamdee et al. (2010) had followed growth dynamics of fine roots of rubber trees down to 450 cm. They found that the onset of the dry season (November) corresponded to a period of active growth in the subsoil from 100 to 400 cm depth. After a rest period, root growth appeared again in the very deep soil between 300 and 400 cm around the time of massive leaf flushing in March. The onset of the rainy season (May) corresponded to an active growth in the top soil above 100 cm. The higher root length density was found above 50 cm. Root decay was observed above 100 cm at the end of the rainy season in September-October.

Guardiola-Claramonte (2008) stressed up for rubber tree the importance of including leaf phenology in soil water balance model to correctly predict the trend of water uptake in dry season. Moreover, the same authors found a shift of root water uptake from topsoil in the onset of dry season to subsoil at the end of the dry season when leaf flushing occurs.

Hence, the period of leaf senescence and shedding corresponds to several long term processes which could accelerate the decrease of g_L versus top soil *REW* compared to intermittent drought conditions in rainy season where no leaf yellowing and shedding were observed.

Leaves are assumed to contribute to between 40% and 80% of the g_L (Becker et al., 1999; Nardini and Tyree, 2000; Brodribb et al. 2002; Domec et al., 2009). Following soil drying, root decay may decrease furthermore the soil to root conductance. Reversely, root growth in the wetter subsoil could increase soil to root conductance and root conductance. At last, leaf flushing should increase the hydraulic conductance of leaves.

Therefore, this work had two objectives. The first objective was to investigate for mature rubber trees the change of whole-tree transpiration under seasonal drought within the framework of a simple hydraulic limitation model. The second objective was to assess how the deciduous phenological pattern may influence the responses to atmospheric and soil droughts under this framework and by comparison with the ones assessed by Isarangkool Na Ayutthaya et al. (Paper 2) in rainy season with full canopy condition. We hypothesized first that the principles of RER_ET_0 model apply also for seasonal drought with leaf senescence-shedding and leaf flushing period: stability of $\psi_{minimum}$ under soil drought, decrease of E_{Tree} related to change of g_L, and decrease of g_L related to *REW*. Secondly, we hypothesized that the deciduous pattern markedly changes the parameters and relationship of the model. Because of the periods of massive leaf shedding and leaf flushing correspond first to changing and low green leaf area and secondly to a possible shift of root water uptake between topsoil to subsoil.

To address these issues, we selected a mature and representative rubber tree stand of the main planted clone in South East Asia. It was located in the southern part of northeast Thailand where trees are exposed to soil and atmospheric droughts in the seasonal dry period. Seasonal and diurnal variations of xylem sap flow density, ψ_{Leaf} and g_L were measured with concurrent observation of canopy phenology and recordings of atmospheric and soil water conditions.

2. Materials and methods

2.1 Field site and plant material

The experiment was conducted in a plot of RRIM600, planted at 2.5m x 7.0m spacing and tapped for 4 years. The plantation is located at Baan Sila site (N15° 16′ 23″ E103° 04′ 51.3″), Khu-Muang, Bureerum province in northeast Thailand. The average annual rainfall in 2007 was less than 1,000 mm; the rainy season lasts approximately from April to October. Six representative trees were selected for intensive measurements. Their trunk girths, measured at 1.50 m height above the soil, varied from 43.3 to 58.3 cm. The average circumference was 52.5 cm, and the maximal leaf area index was estimated around 3.9 in 2007. The investigation was carried out from the end of the rainy season (September 2007) to the period of massive leaf flushing in March.

2.2 Leaf phenology observation

Leaf phenology or more exactly the canopy fullness was regularly assessed on the same sample of 38 trees including the six instrumented trees. A percentage of canopy fullness was allocated to each tree according to the seven following categories taking into account the percentage of twigs bearing green expanded leaves in the whole-canopy: 100, 90, 75, 50, 25, 10 and 0%.

2.3 Climatic measurements

Local microclimate was automatically monitored in an open field, 50 m from any trees. A datalogger (Minimet automatic weather station, Skye Instruments Ltd, U.K.) recorded half hourly values of air temperature, relative humidity, incoming short wave radiation and rainfall. A reference potential evapotranspiration (ET_0) was calculated according to Allen et al. (1998).

2.4 Soil water content measurements

Volumetric soil water content (θ) was measured with a neutron probe (3322, Troxler, Research Triangle Park, North Carolina, USA) calibrated for the experimental soil with separated calibrations between upper (0-0.2 m) and lower (below 0.2 m) layers. Twelve tubes of 2.0 m in length were set up, six along the rows and six between the rows. Measurements every 0.2 m, from 0.1m until 1.7 m depth, were performed every month or two weeks from September 2007 to March 2007. According to soil water fluctuations, the soil profile was separated between two layers, a top soil (0-0.4 m) and a subsoil (0.4-1.8 m). Average field capacity and permanent wilting points were equal to 19.8 and 7 $cm^3/100\ cm^3$ of soil for the top soil, and to 25.1 and 10 $cm^3/100\ cm^3$ of soil for the subsoil, respectively (Isarangkool Na Ayutthaya et al. Paper 1).

Additionally, θ was measured continuously with a capacitive probe (EnvironSCAN System, Sentek Sensor Technologies, South Australia, Australia) within a single tube close to a tube dedicated to neutron probe measurement. Capacitive sensors were located at the same levels than neutron probe measurements. For each capacitive sensor, θ was estimated from a cross-calibration with the neutron probe measurements over the whole season range. To estimate continuous change of the average soil water profile, linear regressions were performed between θ of the average soil water profile (12 neutron probe access tubes) and θ of the profile continuously measured with the capacitive probe. Relative extractable soil water (*REW*) was calculated according to Granier et al. (1999) and Breda et al. (2006).

2.5 Leaf water potential

Leaf water potential (ψ_{Leaf}) was measured on the six instrumented trees with a Scholander type pressure chamber (PMS 1000, PMS Instrument Company, Corwallis, Oregon, USA). Two trifoliate leaves with petiole were randomly selected from sunny locations on each experimental tree. ψ_{Leaf} measurements were performed in situ rapidly after cutting. Regular measurements of ψ_{Leaf} were carried out once or twice times per month, $\psi_{predawn}$, between 05:30 and 06:15 hours, and midday leaf water potential (ψ_{midday}), between 12:30 and 13:30 hours. Additionally, diurnal kinetics of ψ_{Leaf} measurement, i.e., every 1-2 hour from predawn to sunset, were performed on sunny days in contrasting conditions of leaf phenology and soil drought.

2.6 Xylem sap flux measurements and tree transpiration calculation

The measurements of xylem sap flow density were made using the transient thermal dissipation method (TTD) developed by Do and Rocheteau (2002) which is a modification of the continuous thermal dissipation method of Granier (1985). The modification avoids the influence of passive temperature gradients. The TTD method is based on the same Granier's probe design and heating power but uses a cyclic schedule of heating and cooling to assess a transient thermal index over 10 min change. The hourly sap flux density (J_s) was calculated according to the empirical and non species-specific calibration assessed by Isarangkool Na Ayutthaya et al. (Paper 1):

$$J_s = 12.95K_a \qquad (2)$$

where K_a is a transient thermal index (dimensionless).

An alternate signal (ΔT_a) was defined as:

$$\Delta T_a = \Delta T_{on} - \Delta T_{off} \qquad (3)$$

where ΔT_{on} is the temperature difference reached at the end of the 10 min heating period and ΔT_{off} is the temperature difference reached after 10 min of cooling,

To measure J_s every half hour with a heating period of 10 min, a cycle of 10 min heating and 20 min cooling was applied and the temperature signals were recorded every 10 min. ΔT_{off} values were averaged between values before 10 min of heating and after 10 min of cooling.

The transient thermal index was calculated as:

$$K_a = (\Delta T_{0a} - \Delta T_{ua}) / \Delta T_{ua} \qquad (4)$$

where ΔT_{0a} is the maximum alternate temperature difference obtained under zero flow conditions and ΔT_{ua} is the measured alternate signal at a given J_s.

The zero flux signal was determined every night assuming that sap flow was negligible at the end of the night. Probes were inserted into the trunks at a height of 1.8 m above the soil. At this height, average sapwood area was estimated at 1.97 dm^2. After removal of the bark, the probes, 2-cm long probes were inserted into a hole of 2.5 cm deep within the sapwood, in such a way that the whole probe was inside the conductive sapwood. Three probes were inserted into each trunk to take circumferential variability into account. After the probe was inserted, the exposed parts of the needles were coated with silicone. The trunk area containing the probes was protected from direct solar radiation and rainfall by a deflector. Probes were connected to a data logger (CR10X, Campbell Scientific, Leicester, U.K.).

J_s was cumulated over 24 h to calculate daily J_s (J_{s_daily}). For taking care of the variation of sap flux density in the depth of wood, a reduction coefficient of 0.874

was applied to the J_s measured in the outmost ring of conducting xylem (Isarangkool Na Ayutthaya et al, Paper 1). Finally, neglecting tree water storage, E_{Tree} (mm day^{-1}) was estimated according to the equation:

$$E_{Tree} = 0.874 * J_{s\_daily} * sapwood\ area/tree\ spacing\ area \qquad (5)$$

2.7 Whole tree hydraulic conductance

The g_L (expressed in L dm^{-2} h^{-1} MPa^{-1}) was calculated from concurring measurements of sap flow rate and leaf water potential. The multi-points method plotted the diurnal changes of ψ_{Leaf} versus sap flux density from kinetic measurements, the slope of the assumed linear relationships representing the hydraulic resistance, the reverse of the hydraulic conductance. The single point method applied the simplified following formula (Cochard et al. 1996).

$$g_L = J_{s\_midday} / (\Psi_{predawn} - \Psi_{midday}) \qquad (6)$$

where J_{s_midday} is the maximum daily sap flux density, $\psi_{predawn}$ and ψ_{midday} are predawn and midday leaf water potentials, respectively.

2.8 Hydraulic limitation model of water loss (*RER_ET$_0$* model)

The basis of the "hydraulic limitation" model is a critical minimum leaf water potential at the level of which, tree regulates its transpiration whatever it is induced by atmospheric drought or soil drought. Called '*RER_ET$_0$*', it was developed in Microsoft Office Excel according to Cochard et al. (1996, 2002) following the simple equation:

$$J_{s\_est} = g_L (\Psi_{predawn} - \Psi_{crit}) \qquad (7)$$

where J_{s_est} is estimated maximum sap flux density, g_L and $\psi_{predawn}$ are both estimated daily from relationships with *REW* (input data). ψ_{crit} was assumed stable for the whole period (-1.95 MPa; Isarangkool Na Ayutthaya et al. Paper 2).

The critical tree transpiration (E_{crit}; mm day^{-1}), was deduced from J_{s_est} with the multiplicative constant coefficient 0.7194 which transforms the midday sap flow density in total flow of transpiration expressed per day and unit of soil area (Isarangkool Na Ayutthaya et al. Paper 2).

E_{crit} defines a maximal value of daily transpiration due to the hydraulic limitations of the trees; however it may not be reached due to low evaporative demand. Hence, the final step of modeling selects the minimum value between E_{crit} and ET_0 (input data) according to Eq. 8.

$$E_{Tree} = Min(E_{crit}; ET_0) \tag{8}$$

The summary of model parameters and units has shown in Table 1.

Table 1 Summary of model parameters

Symbol	Name	Unit
E_{Tree}	tree transpiration	mm day^{-1}
E_{crit}	critical tree transpiration	mm day^{-1}
ET_0	reference evapotranspiration	mm day^{-1}
REW	relative extractable soil water	dimensionless
J_{s_est}	estimated maximum sap flux density	L dm^{-2} h^{-1}
ψ_{crit}	critical minimum leaf water potential (-1.95)	MPa
$\psi_{predawn}$	predawn leaf water potential	MPa
g_L	whole tree hydraulic conductance	L dm^{-2} h^{-1} MPa^{-1}

The previous study on mature rubber trees in rainy season and full canopy conditions (Isarangkool Na Ayutthaya et al. Paper 2) provide a first set of parameter and relationships for the value of ψ_{crit} and the non linear relationships of $\psi_{predawn}$ and g_L versus REW, which were developed in intermittent drought in rainy season as Eq. 9 ($R^2 = 0.93$, n = 12) and Eq. 10 ($R^2 = 0.90$, n = 10), respectively.

$$\Psi_{predawn} = -1.3528 + (1.0628REW/(0.0862 + REW)) \tag{9}$$

$$g_L = 1.8257REW/(0.0925 + REW) \qquad (10)$$

A second set of parameters were estimated when necessary in the current study. The whole data set comprise 196 days of automatic recordings of sap flux density, soil water and ET_0. Within this, the 12 days with measurements of leaf water potential and calculation of g_L were used to assess the parameters and relationships of the simple model. The remaining 184 days were used to test the model performances.

2.9 Data analysis

Mean comparison, regression analysis and other statistics were performed using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA) and Sigmaplot 10.0 (Systat Software, Inc., San Jose, California USA). Linear slopes were compared using their confidence intervals at 95%. For the models, measured E_{Tree} and estimated E_{Tree} were compared using the root mean square error (RMSE) according to the formula:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(x_{1,i} - x_{2,i}\right)^2}{n}} \qquad (11)$$

where $x_{1,i}$ and $x_{2,i}$ are measured E_{Tree} and estimated E_{Tree}, respectively, and n is the number of estimated E_{Tree}.

3. Results

3.1 Environmental conditions and leaf phenology

Leaf phenology showed a stage of full canopy until the end of November, one month and a half after the last significant rainfall (Figure 1A). Leaf yellowing started in mid December. The percentage of green leaves in six experimental trees steeply decreased with leaf shedding at the end December, and it reached its minimum value at the end of January (13.33%). The tendency of decreasing in percentage of green leaves exposed in the same values with 38 trees in the same 2 lines of six experimental trees. The trees were almost leafless from the end January to mid February. Flushing of new leaves occurred at the end of February (Figure 1A).

ET_0 fluctuated around 2 mm day^{-1} from September to mid October. In the dry period, ET_0 rose to reach an average value of 3.10 mm day^{-1} from November to March. The maximum value occurred in early March (5.85 mm day^{-1}).

θ in both top and subsoil reached their highest values at the end of the rainy season (Figure 1B). Top soil θ had dramatically decreased at the onset of the dry period from 25.8 cm^3/100 cm^3 of soil in mid October to 7.4 cm^3/100 cm^3 of soil in March 2008. Subsoil θ decreased more slightly from 16.7 cm^3/100 cm^3 of soil to 10.0 cm^3/100 cm^3 of soil.

REW in the top soil decreased below 0.5 (assumed soil drought) in mid November and below 0.25 (assumed severe drought) at the end December. Maximum value of *REW* in subsoil in mid October corresponded only to 0.5 and decreased below 0.2 at the end of November (Figure 1C)

3.2 Tree transpiration, leaf water potential and whole tree hydraulic conductance

At the end of the rainy season, E_{Tree} fluctuated around 2 mm day^{-1}, very close to the ET_0 values. E_{Tree} started to decline at the end October while ET_0 increased and trees were still with full canopy. Leaf yellowing started approximately 1.5 month later. Compared to the average value in September, the decrease of E_{Tree} reached approximately 45% at the end of full canopy period (end of November) and 80% at the time of maximal defoliation (end of January). Surprisingly, E_{Tree} did not increase in March according to the flushing of new leaves since February. (Figure 1A)

$\psi_{predawn}$ ranged from -0.32 to -0.41 MPa during full canopy stage from September to November (Figure 1D). Compared to the average value in September - mid November, $\psi_{predawn}$ slightly decreased in mid December ($P>0.05$) and reached -0.65 MPa at end of December ($P<0.01$). For these two periods, percentages of green leaves were 95.4 and 38.3%, while topsoil *REW* were 0.25 and 0.16, respectively (Figure 1C). The lowest value of $\psi_{predawn}$, -0.81 MPa ($P<0.01$), was noticed in mid March 2008 at the stage of leaf flushing.

For $\psi_{minimum}$, the values recorded for sunny days from September to October (full canopy, well-watered soil) averaged -2.03 MPa (Standard deviation; SD = 0.19, Figure 1D). The value recorded for sunny days in full canopy and soil drought conditions (end of November) was similar (-1.96 MPa), even slightly lower. In mid December, while leaf yellowing started and soil drought reached severe rate (REW 0.25 in topsoil and 0.08 in subsoil, the value was also similar (-1.94 MPa).

g_L was maximal and stable around 1.60 L dm^{-2} h^{-1} MPa^{-1} from September to October, in well-watered conditions (Figure 1D). It significantly decreased by 40 % at the end of November ($P<0.05$), before any visible changes of leaf phenology and $\psi_{predawn}$. The decline of g_L reached 55% when leaf yellowing started in mid December and 65% at the time of leaf flushing in March.

3.3 Relationships with evaporative demand and soil water availability

Data were separated in three periods according to leaf phenology and soil drought: full canopy period in well-watered conditions (P1; topsoil REW>0.5, September – mid November), full canopy period with starting soil drought (P2; topsoil REW<0.5, mid November – mid December 2007), and the period of leaf senescence-shedding and leaf flushing conditions (P3; topsoil REW<0.25, mid December 2007 – mid March 2008) where the green leaf area was markedly lower than in the previous periods.

In well-watered conditions, P1, E_{Tree} increased according to ET_0 increase (Figure 2A) but, it exhibited a saturating plateau for ET_0 above about 2 mm day^{-1}. The periods P2 and P3 concerned ET_0 above 1.5 mm day^{-1}. For the P2 with starting soil drought, E_{Tree} dropped towards 1.0 mm day^{-1} average value. And for the P3, E_{Tree} dropped further toward 0.5 mm day^{-1} value. When the relationship was considered with the ratio of $E_{Tree}/ET_{0,}$ it showed that the 3 data groups belong to a similar global trend driven by evaporative demand (Figure 2B).

The relationship between E_{Tree} and *REW* showed that tendency of the data of P1 which is E_{Tree} above 0.5 *REW* seemly was stable and a large variability (Figure 3A). Especially, this period in the sunny day (ET_0 > 2 mm day^{-1}) average E_{Tree} was around 1.9 mm day^{-1}. Below 0.6 *REW*, E_{Tree} decreased according to the same linear relationship ($P<0.01$) from full canopy to almost leafless canopy. The relationship expressed with E_{Tree}/ET_0 ratio displayed a similar trend with a higher variability above 0.6 *REW* and a steep early decrease below 0.6 *REW* (Figure 3B).

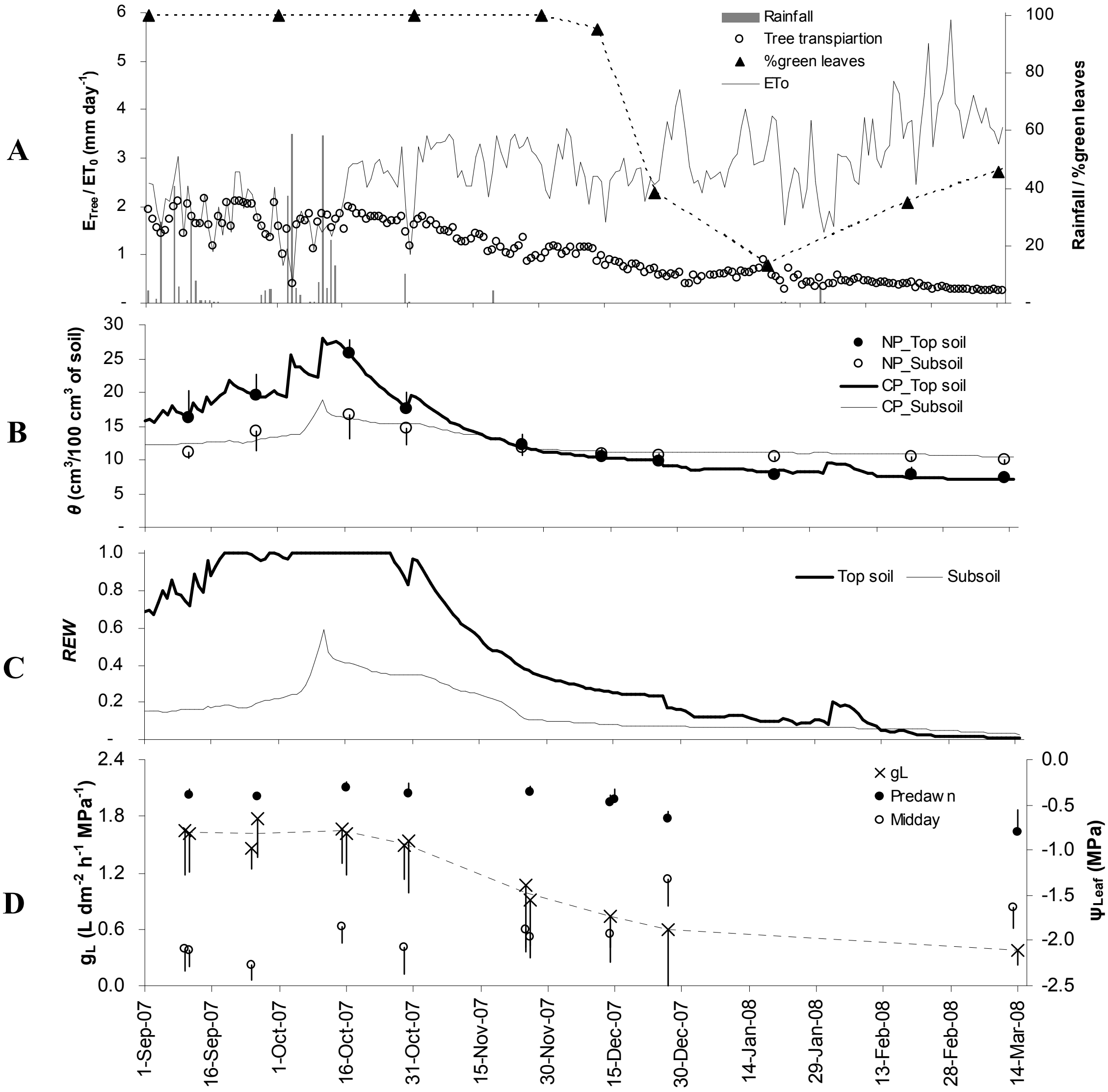


Figure 1 Changes in environments and tree physiological responses of the rubber tree during September 2007 – mid March 2008: A) percentage of green leaves (closed triangle and dotted line), daily rainfall (vertical bars), reference evapotranspiration (ET_0; continuous line) and daily tree transpiration (E_{Tree}; opened circles); B) volumetric soil water content (θ) that measured by neutron probe (NP) on top soil (closed circles) and sub soil (opened circles) and the θ that measured by capacity probe (CP) on top soil (bold line) and subsoil (thin line), C) relative extractable soil water (*REW*) on top soil (bold line) and subsoil (thin line) and D) whole tree hydraulic conductance (g_L; opened squares and dotted line) and predawn (closed circles) and midday (opened circles) leaf water potential (ψ_{Leaf}). The vertical bars in each marker indicate 95% confidential interval.

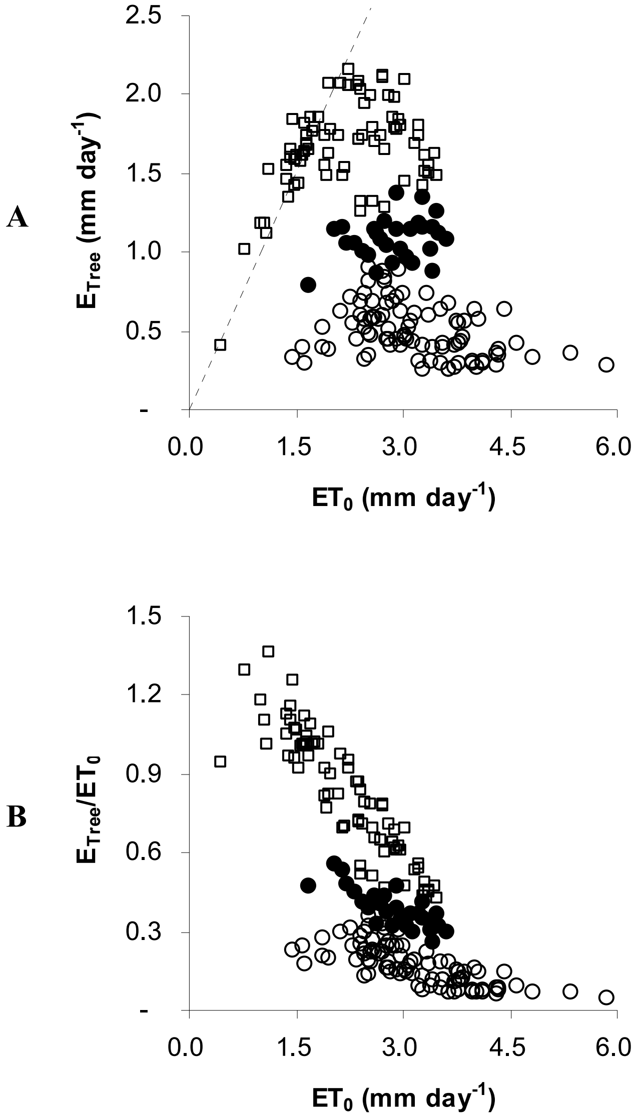


Figure 2 A) Daily tree transpiration (E_{Tree}) versus reference evapotranspiration (ET_0) and B) ratio of E_{Tree}/ET_0 versus ET_0 in 3 leaf phynology periods: full canopy period in rainy season (opened squares; September – mid November 2007), full canopy period with starting soil drought (closed circles; mid November – mid December 2007), and period of leaf senescence-shedding and flushing (opened circles; mid December 2007 – mid March 2008). The dotted line in Figure A indicates 1:1 reference.

3.4 Diurnal kinetics

Bi-hourly kinetics of E_{Tree} and ψ_{Leaf} were compared for 6 sunny days of contrasted leaf phenology and soil drought conditions (Figure 4). The daily ET_0 for sunny days increased towards the dry season from 1.76 mm day^{-1} in September 11 to 3.54 mm day^{-1} on March 13. The diurnal E_{Tree} exhibited a classical pattern in full canopy stage and well-watered soil conditions; E_{Tree} followed the steeply increase of ET_0 and reached maximum rate around midday. The pattern started to flatten on November 26 at the end of the full canopy stage (Figure 4C), while $\psi_{predawn}$ was still high (-0.35 MPa). The diurnal kinetic became almost completely flatted at on end December while percentage of green leaves equaled 38.3% (Figure 4E).

According to the effect of daily transpiration on leaf hydration, ψ_{Leaf} equated the highest value at predawn, declined to the lowest value around midday, and went back to high values at the end of the daytime (Figure 4). During the full canopy period and onset of leaf yellowing, $\psi_{minimum}$ was stable around -1.95 MPa despite soil and atmospheric drought (Figure 4A-4D). Finally, ψ_{Leaf} kinetic dramatically flattened on December 27 following the pattern change of sap flow rates.

Maximum E_{Tree} had approximately declined according to the decrease of the percentage of green leaves from 0.26 to 0.10 mm h^{-1}. However, in the period of leaf flushing, the maximum E_{Tree} was close to zero (0.06 mm h^{-1}) while the percentage of green leaves had increased to 45 % (young and light green leaves; Figure 4F).

3.5 g_L multipoints

Diurnal ψ_{Leaf} plotted versus hourly J_s, showed significant linear relationships ($P<0.0001$, Figure 5) in which the slopes represent the reverse of g_L. The linear form supported the hypothesis that the effect of water storage (capacitance) was negligible. It confirmed the validity of the single-point method which used only the difference between $\psi_{predawn}$ and ψ_{midday} versus J_{s_max} to calculate g_L (Figure 1D).

The 3 periods of contrasted conditions of phenology and soil drought were distinguished in Figure 5. The slope significantly changed between the three periods (differed in 95% confidence interval, Table 2). The change which represents a decrease of g_L was particularly remarkable between P1 and P2, before any leaf yellowing and shedding. By contrast, Y intercept which represents a theoretical $\psi_{predawn}$ did not differed between the 3 periods (Table 2).

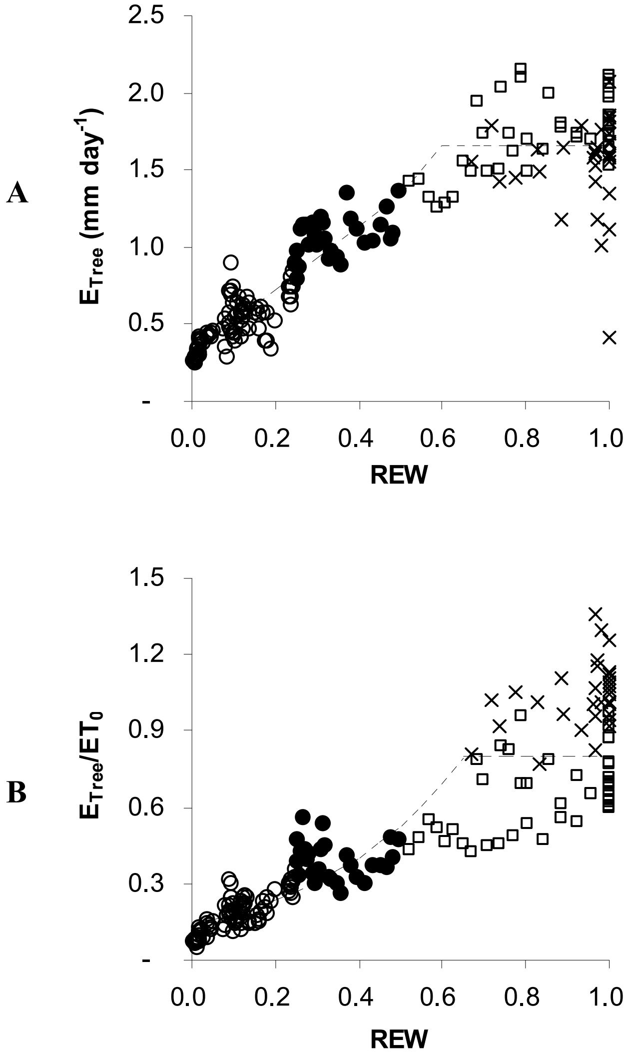


Figure 3 A) E_{Tree} versus relative extractable soil water (*REW*) and B) ratio of E_{Tree}/ET_0 versus *REW* in 3 leaf phenology periods: 1) full canopy period in rainy season during September – mid November 2007 with sunny days $ET_0>2.0$ mm day^{-1} (opened squares) and with low evaporative demand days $ET_0<2.0$ mm day^{-1} (crosses), 2) full canopy period with starting soil drought (closed circles; mid November – mid December 2007), and 3) period of leaf senescence-shedding and leaf flushing (opened circles; mid December 2007 – mid March 2008). The dotted lines indicate the tendency of the relationship in each figure.

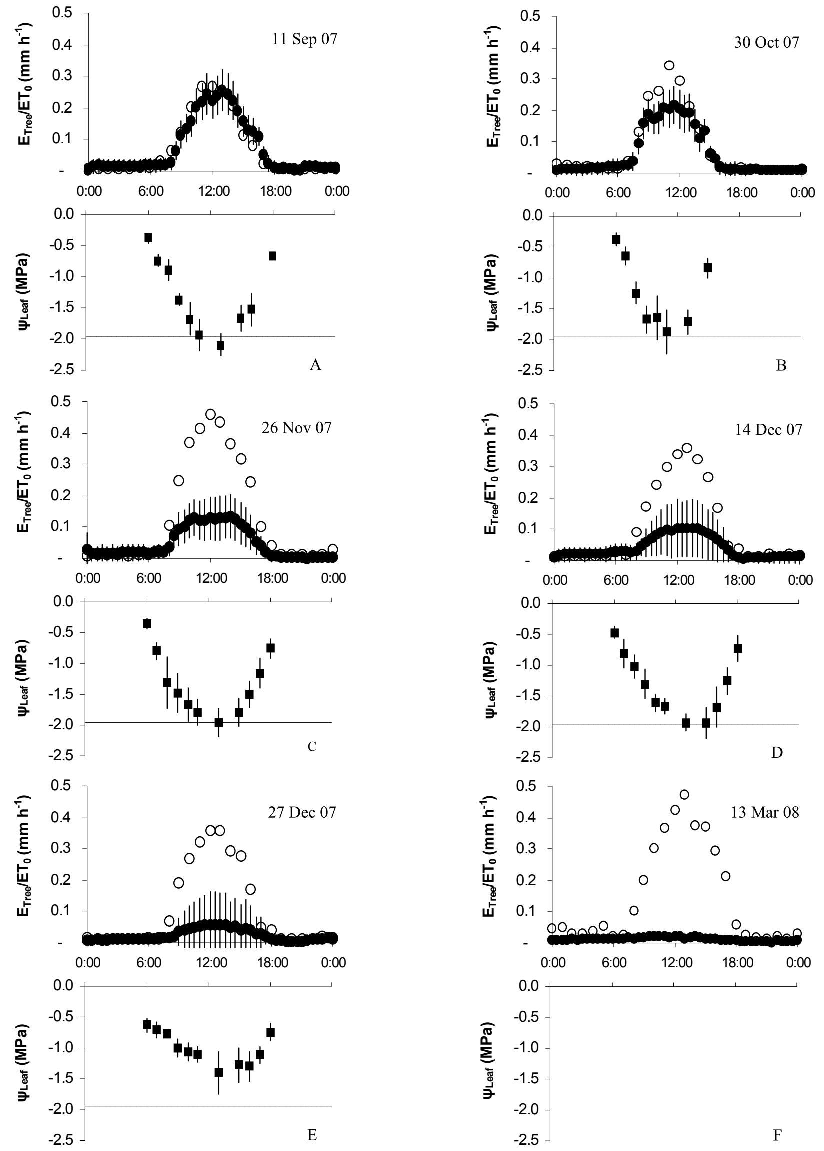


Figure 4 Diurnal reference evapotranspiration (*ET_0*; opened circles), tree transpiration (*E_{Tree}*; closed circles), and leaf water potential (*ψ_{Leaf}*; closed squares) on: A) full canopy period at mid rainy season (11 September 2007), B) full canopy period at end rainy season (30 October 2007), C) end full canopy period in started dry season (26 November 2007), D) leaves shedding periods with 95% of green leaves (14 December 2007), E) leaves shedding periods with 38% of green leaves (27 December 2007) and new leaves flushing period (13 March 2008). The horizontal line in each period indicate critical minimum leaf water potential of mature rubber tree (*ψ_{crit}* = -1.95 MPa; Isarangkool Na Ayutthaya et al. Paper 2). The vertical bars in each marker indicate standard deviation (SD).

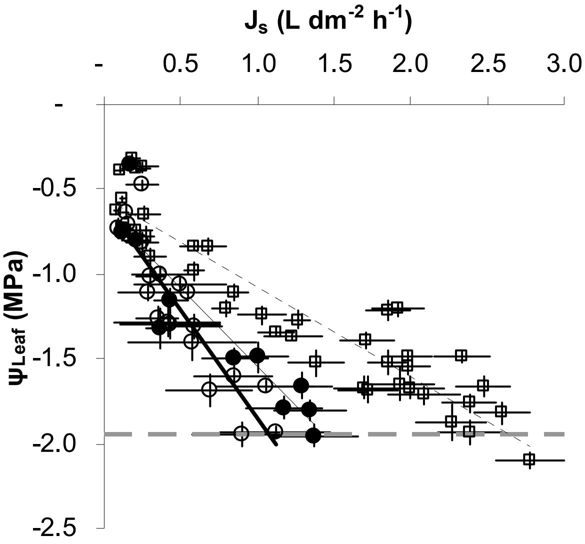


Figure 5 Hourly leaf water potential (ψ_{Leaf}) versus hourly sap flux density (J_s) in 3 periods: 4 days of full canopy period in rainy season (FLR; opened squares and dotted line), full canopy period with starting soil drought in end November (FLD; closed circles and thin line) and 2 days of leaves senescence-shedding period in December 2007 (LSS; opened circles and bold line). The vertical and horizontal bars indicate the SD. The horizontal bold dotted line indicates the critical minimum leaf water potential of mature rubber tree, which was -1.95 MPa (Isarangkool Na Ayutthaya et al. Paper 2).

Table 2 Details of regression curves between water potential (ψ_{Leaf}) and hourly sap flux density (J_s) in 3 periods: 4 days of full canopy period in rainy season (FLR), full canopy period with starting soil drought in end November (FLD) and 2 days of leaves senescence-shedding period in December 2007 (LSS).

Periods	n	Slope	95% confidence interval for slope		Interception Y-axis	95% Confidence interval for interception Y-axis	
			Lower Bound	Upper Bound		Lower Bound	Upper Bound
FLR	39	- 0.5295	- 0.5964	- 0.4626	- 0.5471	- 0.6498	- 0.4444
FLD	11	- 0.9294	- 1.2221	- 0.6367	- 0.6224	- 0.8846	- 0.3602
LSS	22	- 1.2756	- 1.5353	- 1.0158	- 0.5760	- 0.7177	- 0.4342

3.6 g_L single point versus E_{Tree}

Plotting of E_{Tree} versus g_L demonstrated that the decrease of daily E_{Tree} was mainly explained by the change of g_L following a linear response ($R^2 = 0.98$, $P<0.001$; Figure 6). The same linear trend included data of the three periods distinguished by leaf phenology and soil drought. Additionally, the slope calculated over the seasonal drought (P2 and P3) did not differed from the slope calculated over the whole data including P1 period of well-watered soil and full canopy conditions.

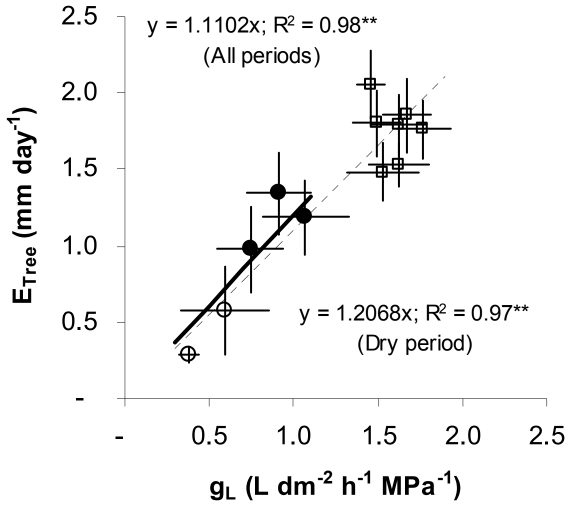


Figure 6 The relationship between tree transpiration (E_{Tree}) versus whole tree hydraulic conductance (g_L) in 3 leaf phenology periods: full canopy with well-watered condition (opened squares), full canopy period with starting soil drought (closed circles) and period of leaf senescence-shedding and leaf flushing (opened circles). The dotted lines indicate tendency in all periods, whereas bold lines indicate only tendency in seasonal drought period (mid November 2007 – March 2008). The horizontal and vertical bars indicate standard error (SE). ** indicates the significant linear relationships at 99%.

3.7 $\psi_{predawn}$ and g_L versus *REW*

Figure 7A illustrated the relationships between $\psi_{predawn}$ and *REW* in the top soil. However, the relationships between $\psi_{predawn}$ and *REW* in both top soil and subsoil are highly related. Nevertheless, the relationship with subsoil *REW* (data not shown) appears more correlative than causal because of the magnitude of the values; $\psi_{predawn}$ starts to decrease below 0.1 *REW*, a very low value. Therefore the change of $\psi_{predawn}$ appeared more related with topsoil *REW*. In details, $\psi_{predawn}$ started to decrease below 0.4 *REW* in the topsoil, which did not exclude any partial influence of *REW* in the subsoil. The simple relationships assessed were:

$$\Psi_{predawn} = 1.2487REW - 0.8514, \quad \text{for } REW = 0\text{-}0.4$$

$$(R^2 = 0.95, P<0.05, n = 4)$$

$$\Psi_{predawn} = -0.36\,\text{MPa}, \quad \text{for } REW>0.4 \qquad (12)$$

The relationship between g_L and *REW* confirmed a strong decrease of g_L at low *REW* which include in the same trend data of P2 and P3 (Figure 7B). Data of P1 defined a plateau of maximum g_L at high *REW*. However, data were missing to determine an accurate threshold which could have been between 0.7 and 0.5 *REW*. The change of g_L showed a non linear decrease, quick at the start. Relationships between g_L and subsoil *REW* showed also a significant link (R^2 = 0.63; data not shown) but the less stronger than with topsoil *REW*. The relationships assessed for modeling considered only the top soil:

$$g_L = 0.283\exp(3.3456REW), \quad \text{for } REW = 0\text{-}0.5$$

$$(R^2 = 0.95, P<0.01, n = 6)$$

$$g_L = 1.60\ \text{L dm}^{-2}\,\text{h}^{-1}\,\text{MPa}^{-1}, \quad \text{for } REW>0.5 \qquad (13)$$

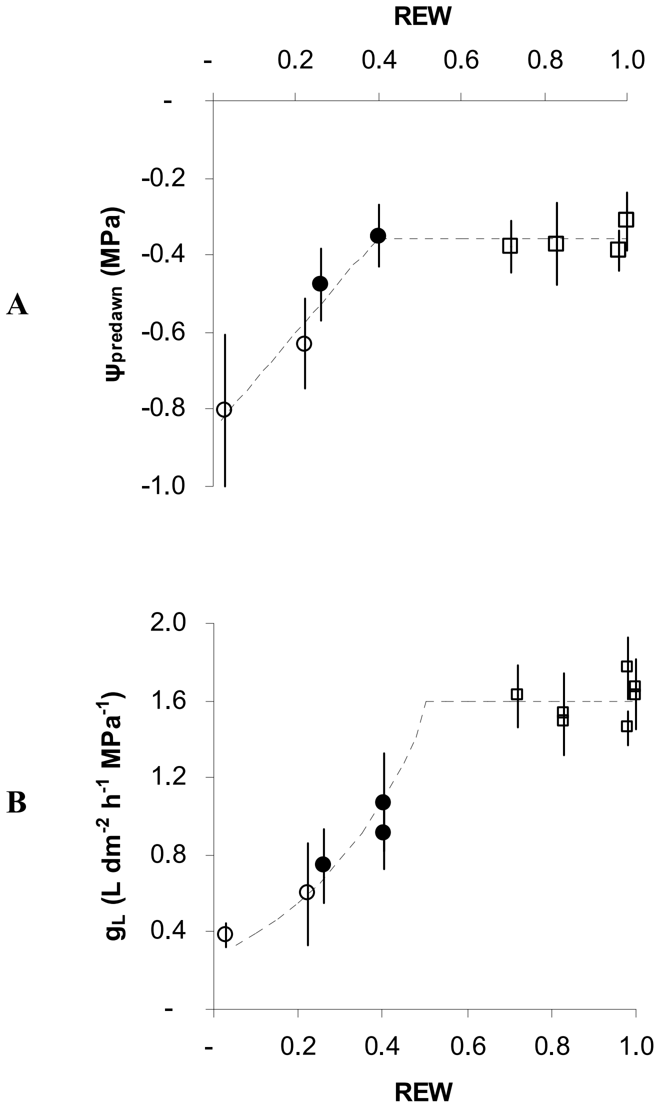


Figure 7 Relationship between A) predawn leaf water potential ($\psi_{predawn}$) and top soil relative extractable soil water (*REW*) and B) whole tree hydraulic conductance (g_L) and top soil *REW* in 3 leaf phenology periods: full canopy with well-watered condition (opened squares), full canopy period with starting soil drought (closed circles) and period of leaf senescence-shedding and leaf flushing (opened circles). The dotted lines indicated tendency of relationships. The vertical bars in Figure A and Figure B indicate SD and SE, respectively.

3.8 Test of hydraulic limitation model

RER_ET$_0$ model was applied with two sets of parameters (Figure 8). The first set corresponded to the initial parameters assessed by Isarangkool Na Ayutthaya et al. (Paper 2) for intermittent drought with full canopy conditions (Eq. 9 and Eq. 10). The second set corresponded to the parameters assessed in this study (relationship of predawn versus top soil *REW*, Eq. 12; relationship of g_L versus top soil *REW*, Eq. 13, ψ_{crit} = -1.95 MPa).

The model with initial parameters provided already the trend of transpiration decrease, however with overestimates for the starting drought and mild drought periods (Figure 8 and 9A; n = 185, RMSE = 0.33, R^2 = 0.78). The model with new parameters logically provided simulations closer to measured transpirations (Figure 8 and 9B, RMSE = 0.22, R^2 = 0.90). Nevertheless, the model with initial parameters overestimated E_{Tree} by 16%, while the model with new parameters underestimated E_{Tree} by 9%.

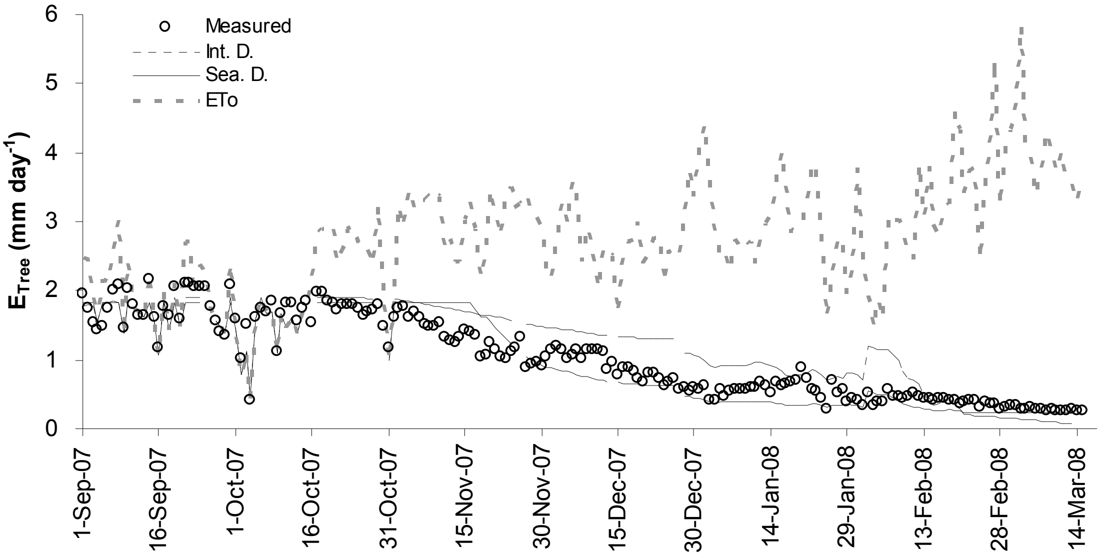
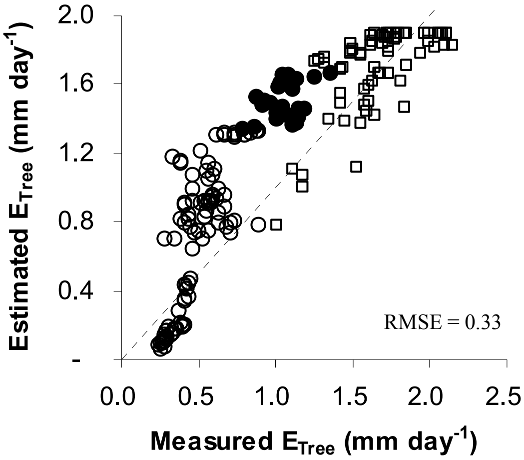


Figure 8 Comparison of *RER_ET$_0$* model that predicted tree transpiration (E_{Tree}) by developed physiological relation equations during intermittent drought in rainy season (Int. D.; black dotted line) and *RER_ET$_0$* model that predicted E_{Tree} by developed physiological relation equations during seasonal dry period (Sea. D.; continuous line). The opened circles and gray dotted line are measured E_{Tree} and ET_0, respectively.

A



B

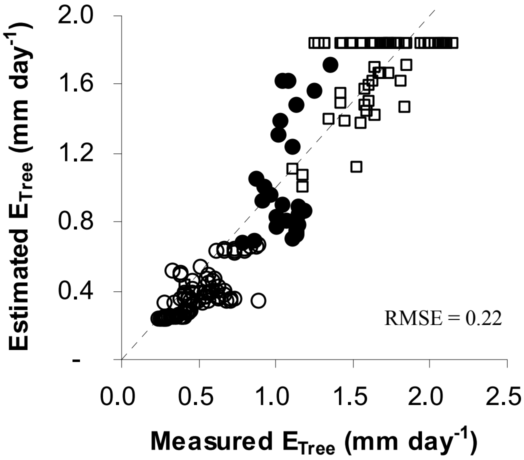


Figure 9 Relationship between estimated daily tree transpiration (Estimated E_{Tree}) and measured daily tree transpiration (Measured E_{Tree}) during leaf senescence-shedding-new flushing period. A) RER_ET_0 model that predicted tree transpiration (E_{Tree}) by developed physiological relation equations during intermittent drought in rainy season and B) RER_ET_0 model that predicted E_{Tree} by developed physiological relation equations during seasonal dry period during seasonal dry period with leaf senescence-shedding and leaf flushing occurrence. The dotted line in each figure indicates 1:1 reference. The meaning of each marker indicates each leaf phenology period: full canopy period in rainy season (opened squares; September-mid November 2007), end full canopy with starting soil drought (closed circle; mid November – mid December 2007) and period of leaf senescence-shedding and leaf flushing (opened circle; mid December –mid March 2008).

4. Discussion

4.1 Principles of a simple hydraulic limitation model

Results confirm the hypothesis that the principles of RER_ET_0 model hold to describe transpiration response to seasonal drought.

First, $\psi_{minimum}$ for sunny days did not decrease under soil drought and atmospheric drought while maintaining full canopy for two months after the last significant rains. Secondly, the decrease of E_{Tree} was mainly explained by the change of g_L. The data corresponding to the period of massive leaf shedding and leaf flushing are fully included in the same linear trend. Thirdly, the decline of g_L was strongly related to *REW* decrease.

It was expected that the basis of RER_ET_0 model applied similarly for transpiration response under seasonal drought at least with full canopy conditions. The principles of the "hydraulic limitation model" are assumed to be clonal or species-specific characteristics; such as the isohydric behaviour of mature trees for ψ_{crit}, or the negligible quantitative effect of tree capacitance on daily transpiration. Hence, these properties are not expected to change depending drought conditions. These conclusions confirm the principles of water losses regulations and the isohydric behaviour previously assessed for mature rubber trees under intermittent drought and for this clone in particular (Isarangkool Na Ayutthaya et al. Paper 2).

4.2 Influence of the deciduous pattern

Results do not confirm the hypothesis of a marked influence of the deciduous pattern on the parameters and relationships of RER_ET_0 model. The changes were lesser than expected despite the conditions of changing and low transpiring leaf area and the possible shifts of root water uptake from topsoil to subsoil. The model with previously assessed parameters for intermittent drought in rainy season provides the trend of transpiration decrease under seasonal drought. The newly assessed parameters and relationships were not very different from initial parameters assessed in intermittent drought and full canopy conditions (Isarangkool Na Ayutthaya et al. Paper 2). In the details, there were similarities and differences.

4.3 Value of $\psi_{minimum}$ under drought

The average $\psi_{minimum}$ for sunny days under drought (end of November an December) was very close to the value measured in intermittent drought conditions. ψ_{crit} equated -1.95 MPa in intermittent drought of rainy season (Isarangkool Na Ayutthaya et al. Paper 2).

4.4 $\psi_{predawn}$ response to drought

The late change of $\psi_{predawn}$ compared to the decreases of transpiration and g_L was the main difference with the responses noticed for intermittent drought in rainy season. The decrease of $\psi_{predawn}$ occurred at least one month after the decrease of g_L, at the same time than the onset of leaf yellowing and shedding in mid December. For the intermittent drought, $\psi_{predawn}$ had decreased at the same time with E_{Tree} and g_L (Isarangkool Na Ayutthaya et al. Paper 2). Comparison based on the relationships versus *REW* shows that both $\psi_{predawn}$ are still mainly related to *REW* in the topsoil (Figure 7A). However, $\psi_{predawn}$ started to decrease at a lower threshold value than in the intermittent drought (Figure 10A). This result suggests that $\psi_{predawn}$ was complementary controlled by other parameters than *REW* in the top soil. By contrast with the drought in rainy season (Isarangkool Na Ayutthaya et al. Paper 2), more water was available in the subsoil at the onset of the seasonal drought. Average *REW* in the subsoil (40-180 cm) was between 0.2 and 0.3 in November against <0.1 in June-July (intermittent drought). Moreover, root growth was observed in the same experimental field at depths between 1.25 and 4.25 m in November (Gonkhamdee et al., 2010). Hence, root water uptake in the subsoil could have influenced $\psi_{predawn}$ at this period.

In the literature, it is known that the interpretation of $\psi_{predawn}$ is complicated in conditions of large development of roots and heterogeneous soil water content (Richter 1997; Ameglio et al. 1999). Moreover reduction of transpiration through stomatal regulation and leaf senescence could have decrease daily dehydration and facilitate nighttime rehydration of remaining green leaves, which may have delayed the decrease of $\psi_{predawn}$.

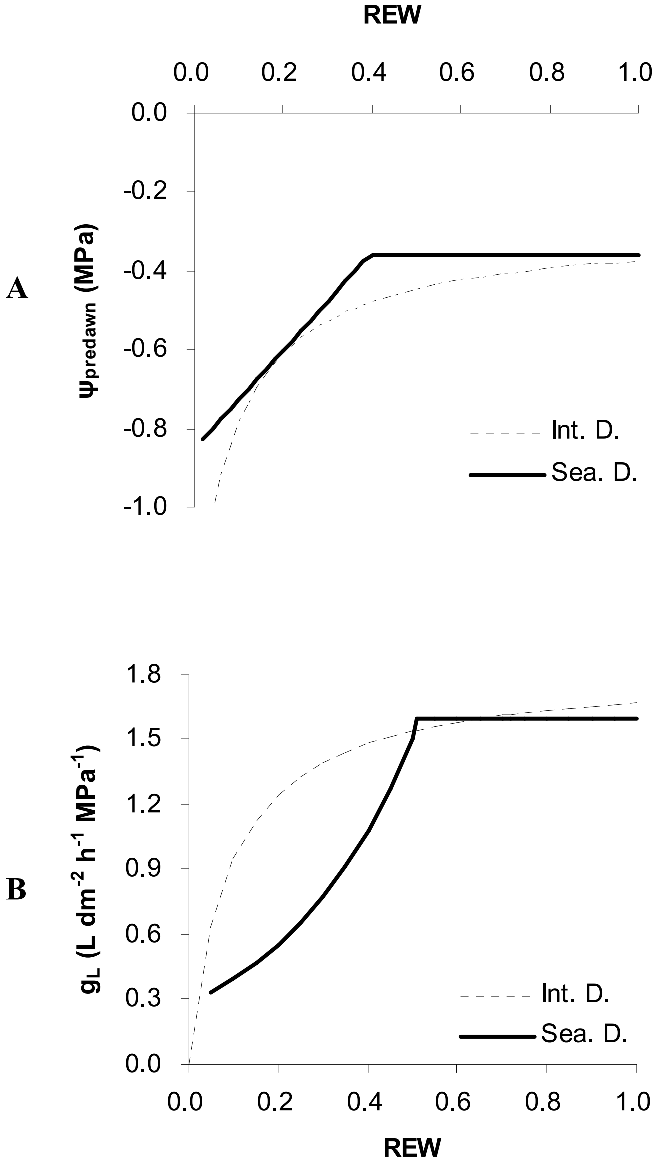


Figure 10 Relationship between A) predawn leaf water potential ($\psi_{predawn}$) and top soil relative extractable soil water (*REW*) and B) whole tree hydraulic conductance (g_L) and *REW* in intermittent drought in rainy season (Int. D., dotted line; Isarangkool Na Ayutthaya et al. Paper 2) and seasonal dry period (Sea. D., bold continuous line).

4.5 g_L response to *REW*

The relationship of g_L versus topsoil *REW* yielded for seasonal drought showed similarities and differences with the one obtained for intermittent drought in full canopy conditions (Isarangkool Na Ayutthaya et al. Paper 2). By comparison with subsoil, *REW* in the top soil still appeared as the main driver of g_L decrease from the full canopy stage to the leaf shedding and leaf flushing stages (Figure 7B). The relationships showed both a plateau and a critical threshold around 0.5 *REW* for the onset of g_L decrease. However after the plateau, the decrease of g_L differed between the two periods of drought (Figure 10B). It was quicker in the seasonal drought where 40% of g_L decrease corresponded approximately to 0.4 REW of top soil compared to 0.10 REW in intermittent drought.

4.6 Processes of g_L decrease and increase

Such as in mid-drought conditions, the decline of the g_L can be mainly explained by the decrease of the soil to root conductance. The decrease of the soil to root conductance is logically attributed to the decrease of the soil water availability (measured) and the declining contact between soil and roots. Moreover a certain percentage of xylem embolism in the petioles and branches may have contributed to the decrease of g_L. For example, $\psi_{minimum}$ compared to 50% loss of hydraulic conductivity in young potted trees that assessed by Ranasinghe and Milburn (1995) and Sangsing et al. (2004) found values of xylem tension corresponding to 50% of embolism in the petioles between -1.5 and -2.0 MPa. Moreover, a higher sensitivity to xylem embolism is expected with leaf aging (Cochard et al. 1992; Cruiziat et al. 2002).

The period of leaf senescence and shedding also corresponds to several long term processes which can explain the quicker decrease of g_L versus top soil *REW* compared to intermittent drought conditions in rainy season where no leaf yellowing and shedding were observed (Isarangkool Na Ayutthaya et al. Paper 2). At the stage of leaf senescence preceding leaf shedding, there is the formation of an abscission zone in the petiole (Taiz and Zeiger 1991). This abscission zone should logically decrease the hydraulic conductance between xylem of twigs and leaves. Additionally, several studies on temperate species have shown decrease of g_L before leaf senescence

(Salleo et al. 2002; Brodribb and Holbrook 2003). Later on, leaf yellowing and shedding should reduce drastically the total hydraulic conductance of leaves. Leaves are assumed to contribute to between 40% and 80% of the whole-plant hydraulic conductance (Becker et al. 1999; Nardini and Tyree 2000; Brodribb et al. 2002; Domec et al. 2009).

However, two other phenological long term processes in the seasonal drought may have a reverse effect by increasing g_L. The significant root growth observed in the subsoil (Gonkhamdee et al., 2010) could have increased soil to root conductance and K_{root}. Later on, in February and March, the observed leaf flushing should have increased the hydraulic conductance of leaves. Surprisingly, the leaf flushing observed in February and March was not followed by an increase of g_L and transpiration (Figure 1A). This suggests a strong stomatal regulation adjusted to the very low g_L likely mainly controlled by low soil water availability and soil to root conductance. To maintain steady-state water status, stomatal conductance is assumed to adjust the water losses in gas phase to the change of g_L which controls the water transfer from soil to leaf in liquid phase (Whitehead, 1998). The large number of processes involved reflects the complexity of the regulation of g_L and transpiration in the seasonal drought.

4.7 Convergence of whole-tree responses and modeling

The simulations confirm the ability of RER_ET_0 model to simulate the trend of transpiration change under seasonal drought without taking into account the phenology and variation of green leaf area by contrast with the majority of transpiration models. It was particularly remarkable to see that transpirations simulated by the model with simple parameters issued from full canopy conditions meet the values measured in January with only 15 % of green leaves. The results imply that in our experimental conditions the simple principles of the hydraulic limitation model were enough to simulate the influence of the deciduous pattern of the trees facing climatic and soil droughts.

This conclusion supports the idea that phenological changes converge toward a same response of whole-tree water relations to soil and atmospheric drought, which is correctly described by the concepts of hydraulic limitation: g_L decrease versus soil water availability and ψ_{crit} stability. This converging response of transpiration to droughts despite contrasted conditions of leaf phenology and, i.e. green leaf area, was particularly illustrated by Figure 2 and Figure 3 where the data corresponding to leaf shedding and leaf flushing periods fit the same tendencies against ET_0 and *REW* than the ones of the full canopy period.

Finally, this convergence certainly hides complex adjustments between short term process (stomatal regulation, xylem embolism) and long term process of leaf and root phenologies which change the relative contribution of hydraulic conductances to the soil-root and canopy-atmosphere interfaces.

The results suggest that in our conditions the deciduousness of rubber tree was completely driven by drought. However it may be not always the case even for the same rubber tree stand. We noticed in the following year (2008/2009), a deciduous pattern despite a large soil water availability. However, the duration of the period of changing and low leaf area was particularly shortened compared to 2007/2008. The next interesting step will be to apply RER_ET_0 framework in various environmental conditions and with different clones.

5. Conclusion

In this study, we investigated for the main clone planted in Southeast Asia (RRIM600) the transpiration response of mature trees to atmospheric and soil droughts in the seasonally dry period where several phenological changes occur: leaf senescence and massive leaf shedding, leaf flushing. The results were compared with responses to intermittent drought in rainy season with full canopy conditions (Isarangkool Na Ayutthaya et al. Paper 2)

The results proved first that such as for intermittent drought the principles of a simple hydraulic limitation model hold: stability of $\psi_{minimum}$ for sunny days under soil drought, decrease of E_{Tree} mainly related to change of g_L, decrease of g_L mainly related to *REW* in the top soil. Secondly, the deciduous pattern showed little influence upon the parameters and the relationships of the hydraulic limitation model.

Finally, our results support the idea that phenological changes converged toward a same response of whole-tree water relations to soil and atmospheric drought, which was correctly described by the concepts of hydraulic limitation: g_L decrease versus soil water availability, while ψ_{crit} stable. This simple convergence certainly hide complex adjustments between short term process (stomatal regulation, xylem embolism) and long term process of leaf and root phenologies which change the relative contribution of hydraulic conductances at the soil-root and canopy-atmosphere interfaces (Domec et al. 2009).

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Chapter VI

General conclusion

GENERAL CONCLUSION

This general conclusion is developed according to the objectives set in the general introduction.

1. Experimental conditions

Before to draw the general conclusion it is of value to recall that the conditions of droughts were particularly extreme compared to the other areas of rubber growing. Before the onset of the rainy season in May, the soil water availability was very low in the subsoil, below 0.2 REW up to 1.8 m. Moreover, deep profiles up to 5 meters have shown that the soil water availability was still low up to this depth (data not shown). The annual amount of rainfall in 2007 was less than 1,000 mm, 15 % lower than the average in the area and 45% lower than the optimum amount of 1,800 mm. The evaporative demand was particularly high, ET_0 above 2 mm day^{-1} when soil drying occurred in both intermittent drought and seasonal dry period. In the seasonal dry period (2007/2008), the duration of the period of low leaf area, (including leaf senescence, massive leaf shedding and leaf flushing) was maximum c.a. four months. However these conditions of drought occur regularly in this large area of rubber growing. They represented a suitable environment to study water relations under drought.

2. Accuracy of transpiration estimate

The first step of the study was to test and validate the estimate of whole-tree transpiration by sap flow measurement. Paper 1 assessed a reliable average calibration for rubber tree wood (and other woody species) to estimate xylem sap flux density from very low rates (0.3 L dm^{-2} h^{-1}) to high rates (5 L dm^{-2} h^{-1}). And the field comparison with soil water balance in the dry season validated the absolute estimates of whole-tree transpiration at a reasonable extent. The number of replicate measurement in the field (11 trees and 3 probes per tree) insured a good accuracy in the determination of the average sap flux density in the outer xylem. The determination of the sapwood area was facilitated by a very limited non conductive heartwood and a strong relationship between trunk diameter and bark thickness.

However, the average decreasing radial profile of sap flux density was possibly a source of inaccuracy at medium and low flow. Because the decreasing radial profile within the xylem was assessed with 4 replicates over 7 days for medium-high flow rates in the outer xylem in well soil watered conditions. It is possible that the current reduction coefficient (0.87) was slightly underestimated for medium-low flows. This possibility may explain some overestimate values of whole-tree transpiration which were substantially over ET_0 (around 10 %). However, the overestimation occurred in only low evaporative demand or cloudy day (ET_0 < 2.0 mm day^{-1}). Therefore, another cause for this overestimation could be occurred by water storage in the top part canopy above sap flow probe installation area.

3. Thresholds of transpiration change

Results in papers 2 and 3 have shown particular thresholds of transpiration change for evaporative demand, soil water availability and tree water status. The whole-tree transpiration of the studied trees saturated when ET_0 increased over 2.0 – 2.2 mm day^{-1} or 1.6 – 1.8 kPa of vapour pressure deficit (*VPD*) despite soil water availability. And values of relative extractable soil water (*REW*) below 0.5 corresponded clearly too constraining conditions for tree water uptake. Moreover, predawn leaf water potential ($\psi_{predawn}$) below -0.4 MPa indicated water stress and the onset of transpiration decrease. However, in paper 3 it was shown that values above $\psi_{predawn}$ threshold do not prove that there is no water stress. Because of $\psi_{predawn}$ was very late indicator of transpiration decrease, it decreased only after the tree exhibited leaf fall.

There are no reasons to think that these observations might not be general. As detailed in the discussion of papers, threshold *REW* of 0.4-0.5 is an agreement with literature references on other woody species yielded mainly in temperate conditions. It is also known that $\psi_{predawn}$ could be a very late indicator of soil water constraint because it is influenced by features of the plant itself such as the relative equilibrium of the root profile with a heterogeneous soil water profiles or the rehydration allowed by reducing transpiring leaf area. The saturating transpiration at high evaporative demand is a new and important insight for rubber tree water relations. It has consequences on the modeling of tree transpiration and on the reduction of carbon gain through the induced stomatal closure. This behaviour has been recently observed in several temperate species with different thresholds of evaporative demand. However, it is fairly possible that the threshold differ between clones of rubber trees as it differs between species.

4. Framework of the hydraulic limitation model

The framework of a simple hydraulic limitation model was successful to describe the main change of whole-tree transpiration under both atmospheric and soil drought. Firstly, the saturating of transpiration at high evaporative demand was explained by plant hydraulics. The combination of a critical minimum leaf water potential (ψ_{crit}) and of the maximum value of whole-tree hydraulic conductance (g_L) defines a maximal transpiration that cannot be over-passed despite well soil watered conditions. Secondly, the decrease of transpiration under soil drought was correctly described by the decrease of g_L versus *REW*. Thirdly, paper 3 has shown that the simple model globally applied also for the seasonal drought where the leaf area was dramatically reduced and root growth was noticed in the subsoil. This result implies complex adjustment between short term process (stomatal closure, xylem embolism) and long term process (canopy area, root dynamic) at the different parts of the soil-plant-atmosphere continuum that converge toward an apparently simple relationship of g_L versus soil drought.

5. Limits of modeling framework

The conditions of very marked droughts were likely favourable to the test of this approach particularly for the results with changing leaf area. The model RER_ET_0 was used as an exploring model. It has obviously several over-simplicity and lack of general appliance. For instance, ET_0 appeared correct here to define directly the maximum transpiration without plant constraint. Hence, the maximum transpiration allowed by climatic demand is certainly smaller for a young plantation where the transpiration is limited by the size of the canopy and the sapwood area. However, the model assessed represents an interesting base for further improvement.

6. Limits of the experimental approach

As many works in the field, this experiment in 2007 has studied change of tree transpiration under drought without factor experimentally controlled (like with irrigation or rainfall restriction). In this case, one difficulty is to separate causal from correlative relationships. It was done by several ways. Firstly, the relationships are assessed from a framework with mechanistic basis. Secondly inside the relationships, the points corresponding to contrasted conditions of soil water availability, evaporative demand or leaf phenology were clearly identified and compared. Thirdly, the soundness of the observed thresholds was considered. However, some correlative contribution may not be completely excluded. For example, the relationship of g_L versus *REW* assessed in the seasonal drought with 0-40 cm topsoil, was likely not completely causal, because a significant co-variance of *REW* in the subsoil was noticed. It is likely that a relationship considering *REW* for 100 cm instead of 50 cm could have improved the analysis. In 2008, we set up new experimental design comparing rainfed trees with trees which were complementary irrigated. But these data were not usable to complete the study of soil drought effects because 2008 was an exceptional year with high rainfall and no natural water shortage and likely several period of water stress due to excess of water. However, the saturating transpiration was confirmed.

7. Isohydric behaviour

Our experimental and modeling results demonstrate the isohydric behaviour of the studied mature rubber trees in both intermittent drought and seasonal drought. Despite soil or atmospheric droughts, trees maintained the minimum leaf water potential ($\psi_{minimum}$) between -1.8 and -2.2 MPa, -1.95 MPa being the critical value estimated. In the hypothesis of the hydraulic limitation, these trees control leaf water potential above this critical value through a strict stomatal closure to prevent the occurrence of runaway embolism. For several temperate species, the critical minimum leaf water potential corresponds to 10% of percentage loss of hydraulic conductivity (PLC) and 90% of stomata closure (Cochard et al, 1996; Cruiziat et al., 2002). However, these related parameters are not known for mature trees and this clone in particular. It is possible that values of $\psi_{minimum}$ values at the lower range of -2.2 MPa, corresponded to a substantial embolism. One next step of research on this topic is to analyze for the mature trees the xylem embolism occurrence (native embolism and vulnerability curve) and the response of stomatal closure to decreasing leaf and xylem water potential.

8. Summary of physiological responses values

Thresholds of atmospheric and soil droughts limitation and the value of important physiological responses are shown in Table 1.

Table 1 Conclusion of several physiological parameters

Name	Value	Unit
Threshold of saturated ET_0	2.0 - 2.2 or 1.6 - 1.8	mm day^{-1} kPa
Threshold of soil drought in *REW*	0.5	
Threshold of soil drought in $\psi_{predawn}$	-0.45	MPa
Ψ_{crit}	-1.95	MPa
Maximum E_{Tree}	2.4	mm day^{-1}
Maximum J_s	3.0	L dm^{-2} h^{-1}
Maximum g_L	1.7	L dm^{-2} h^{-1}MPa^{-1}

9. Clonal variability

Literature results yield on young and mature trees support the existence of substantial variability of water relations within rubber clones (Gururaja Rao et al. 1990, Sangsing 2004). RRIM600 was ranked as a drought tolerant clone with the best water status and long term growth. Hence, it is possible that the ψ_{crit} may vary according to the clone. One major interest of the hydraulic limitation model is that it will allow to compared clone performance in contrasted environments

10. Consequence on potential growth and production

Hence, both soil and atmospheric drought decrease or limit the transpiration of mature rubber trees of RRIM600 clone. Such regulation implies a strong stomatal closure which should decline CO_2 diffusion and rate of photosynthesis. Then, the final impact of these droughts is the inhibition of growth and production. As these droughts certainly occur more often in sub-optimal area than in traditional area, it may partly explain the difference of growth and production between the areas. Irrigation is one obvious solution to improve growth and production in water limited areas (Vijayakumar et al. 1998). However our results suggest that the gain will be not maximum because the negative effect of high evaporative demand remains. One solution is likely to adapt the clone choice to the agricultural practice. For instance, a “water saver” as it is assumed for RRIM600 is may be a good choice for rainfed growing, but it is likely that a clone with less strict control of stomata will be more adapted to irrigation practice.

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ABSTRACT

The changes of whole-tree transpiration under atmospheric and soil droughts were investigated for mature rubber trees (*Hevea brasiliensis,* clone RRIM 600) in a drought-prone area of North-East Thailand. The changes were analyzed and compared between intermittent drought in the rainy season and seasonal drought where occur periods of lower leaf area (leaf senescence, massive leaf shedding and leaf flushing) and subsoil root growth.

The physiological responses were analyzed under the framework of a simple hydraulic limitation model called 'RER_ET_0'. This model is mainly based on a critical minimum leaf water potential (ψ_{crit}) and the responses of whole-tree hydraulic conductance (g_L) and predawn leaf water potential ($\psi_{predawn}$) to relative extractable water (*REW*). Continuous and accurate estimate of whole tree transpiration over a whole year was a key measurement of this study with concurring measurement of environmental conditions and leaf water potential (ψ_{Leaf}). We applied the transient thermal dissipation method (TTD) developed by Do and Rocheteau (2002b) which has several advantages but which was never tested in rubber wood

The TTD method was calibrated in the laboratory with cut stems of *Hevea brasiliensis* and two other species (*Mangifera indica* and *Citrus maxima*). The results provided a simple linear calibration which was independent of the woody species tested ($R^2 = 0.88$, $n = 276$, $P<0.0001$). Field comparison with soil water depletion in the dry season validated to a reasonable extent the absolute estimates of transpiration.

In rainy season, the first remarkable result was the assessment of saturating transpiration above reference evapotranspiration (ET_0) c.a. 2.2 mm day^{-1} in period of well soil watered conditions. Intermittent soil drought provoked a dramatic decrease of transpiration below a threshold of 50% *REW* in the top soil which corresponded to a $\psi_{predawn}$ c.a. -0.45 MPa. Transpiration was reduced by 40% at 0.3 *REW* and 80% at 0.1 *REW.* The minimum leaf water potential ($\psi_{minimum}$) for sunny day did not decrease according to soil drought and was stable around -1.95 MPa which supported an isohydric behaviour. The decrease of transpiration was mainly due to the change of g_L. Results in the seasonal drought showed that the principles of the hydraulic limitation model hold despite changing phenology and leaf area. Moreover, the deciduous pattern showed little influence upon the details of parameters and relationships. Finally, the framework of a simple hydraulic limitation model was successful to describe the main change of whole-tree transpiration under both atmospheric and soil drought. Both our experimental and modeling results demonstrate the isohydric behaviour of the studied mature rubber trees.

Key words: *Hevea brasiliensis*, xylem sap flow calibration, tree transpiration, hydraulic limitation model, soil drought, atmospheric drought

Résumé

Les variations de transpiration totale sous contraintes hydriques, à la fois atmosphérique et édaphique, sont étudiées pour des arbres matures d'*Hevea brasiliensis* (clone RRIM600) dans une zone limitée en eau du Nord-Est de la Thaïlande. Les variations sont analysées et comparées entre une sécheresse transitoire en saison des pluies et la sécheresse saisonnière caractérisée par des fluctuations de la surface foliaire (sénescence foliaire, chute des feuilles and re-feuillaison) et de la croissance racinaire en profondeur.

Les réponses physiologiques sont analysées dans le cadre d'un modèle hydraulique simple appelé "RER_ET_0". Ce modèle est principalement basé sur une valeur critique du potentiel hydrique foliaire (ψ_{crit}) et sur les réponses, de la conductivité hydraulique totale de l'arbre (g_L) et du potentiel hydrique de base ($\psi_{predawn}$), à la disponibilité en eau du sol (*REW*). Une estimation précise et en continue de la transpiration totale des arbres durant une année complète était une mesure clé de cette étude avec des mesures concomitantes des conditions environnementales et du potentiel hydrique foliaire (ψ_{Leaf}). Nous avons appliqué la méthode à dissipation thermique transitoire (TTD) développée par Do et Rocheteau (2002b) qui a plusieurs avantages mais n'avait pas été testée sur bois d'hévéa.

La méthode TTD a été calibrée en laboratoire sur des segments de branches d'*Hevea brasiliensis* et de deux autres espèces (*Mangifera indica* and *Citrus maxima*). Les résultats fournissent une calibration linéaire unique indépendante des espèces étudiées ($R^2 = 0.88$, $n = 276$, $P<0.0001$). Une comparaison en plantation via un bilan hydrique du sol en saison sèche valide les ordres de grandeurs de transpiration ainsi estimés par les flux de sève.

Les résultats démontrent une remarquable saturation de la transpiration au dessus d'un certain seuil d'évapotranspiration de référence (ET_0), environ 2.2 mm day^{-1}, indépendamment de la disponibilité en eau du sol (*REW*). Ensuite, le dessèchement du sol en saison des pluies provoque une chute marquée de la transpiration en dessous d'un seuil de 50% du REW dans l'horizon de surface du sol, ce qui correspond à $\psi_{predawn}$ autour de -0.45 MPa. La transpiration est réduite de 40% à REW 0.3 et 80% à REW 0.1. La valeur minimale de potentiel hydrique foliaire ($\psi_{minimum}$) en journée ensoleillée ne décroit pas en fonction du dessèchement du sol mais est stable autour de -1.95 MPa ce qui suggère un comportement iso-hydrique. La décroissance de la transpiration est essentiellement liée à la variation de g_L. Les résultats durant la sécheresse saisonnière montrent étonnamment que les principes du modèle à limitation hydraulique tiennent malgré les variations de phénologie et de surface foliaire. De plus, les variations phénologiques ont peu influencé sur les détails des paramètres et relations du modèle. Finalement, le cadre du simple modèle hydraulique a été suffisant pour décrire correctement les principales variations de transpiration de l'arbre sous contrainte hydrique édaphique comme climatique. Un comportement iso-hydrique des arbres matures étudiés est démontré à la fois par les résultats expérimentaux et la modélisation.

Mots clés: *Hevea brasiliensis*, étalonnage des mesures des flux de sève brute, transpiration totale, modèle à limitation hydraulique, sécheresse du sol, sécheresse atmosphérique