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DEFICIT

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## Approximate Entropy as a measure of complexity in sap flow temporal dynamics of two tropical tree species under water deficit

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### ABSTRACT

Approximate Entropy (ApEn), a model-independent statistics to quantify serial irregularities, was used to evaluate changes in sap flow temporal dynamics of two tropical species of trees subjected to water deficit. Water deficit induced a decrease in sap flow of *G. ulmifolia*, whereas *C. legalis* held stable their sap flow levels. Slight increases in time series complexity were observed in both species under drought condition. This study showed that ApEn could be used as a helpful tool to assess slight changes in temporal dynamics of physiological data, and to uncover some patterns of plant physiological responses to environmental stimuli.

**Key words:** Approximate Entropy, complexity, sap flow, time series analysis, tropical ecophysiology, water deficit.

### INTRODUCTION

Time series analysis of plant physiological data has been used to assess the effects of environmental disturbances on plant metabolism (Lüttge and Beck 1992, Krempaský et al. 1993, Shabala et al. 1997, Hütt et al. 2002, Souza et al. 2004). There are, however, several critical statistical issues in the proper application of complexity measures, both theoretically and especially to real data (Pincus 2000, Poon and Barahona 2001).

Therefore, it is proposed to use Approximate Entropy (ApEn) to measure complexity in time se-

ries of plant physiological data. ApEn is a model-independent statistic defined in Pincus (1991), with further mathematical properties and representative biological applications (Fleisher et al. 1993, Pincus and Singer 1996, Pincus et al. 1998, Pincus and Singer 1998). ApEn is robust or insensitive to artifacts or outliers: extremely large and small artifacts have small effect on the ApEn calculation, if they occur infrequently. ApEn assigns a non-negative number to a sequence or time-series, with larger values corresponding to greater apparent process randomness or serial irregularity, and smaller values corresponding to more instances of recognizable features or patterns in the data. Two input parameters, a run length  $m$  and a tolerance window  $r$ , must be specified to compute ApEn. This parameter measures

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the logarithmic likelihood that runs of patterns that are close (within  $r$ ) for  $m$  contiguous observations remain close (within the same tolerance width  $r$ ) on next incremental comparisons. Finally, further technical discussion of mathematical and statistical properties of ApEn, including mesh interplay, relative consistency of  $(m, r)$  pair choices, asymptotic normality under general assumptions, and error estimation for general processes can be found elsewhere (Pincus and Huang 1992, Pincus and Goldberger 1994). To develop a more intuitive, physiological understanding of the ApEn definition, a multistep description of its typical algorithmic implementation, with figures, is developed in Pincus and Goldberger (1994).

In this study ApEn was used for the first time to quantify changes in the regularity of temporal dynamics in sap flow of two tropical tree species subjected to water deficit.

#### MATERIALS AND METHODS

Seedlings of two tropical species of Brazilian trees of different ecological groups were evaluated. Three 9 month-old seedlings of each species were grown in plastic pots with 10 kg of an oxisoil, and daily irrigated. *Cariniana legalis* (Lecythidaceae) and *Guazuma ulmifolia* (Sterculiaceae) are semi-deciduous trees typical of semi arid regions, the former species is late successional and the latter one is early successional (Lorenzi 1992).

#### TREATMENTS

Seedlings were grown in a greenhouse at the Department of Biological Sciences, ESALQ/USP, Piracicaba, Brazil (22°42'S, 47°38'W, 546 m of altitude). Plants were subjected to water deficits by withholding irrigation during 72 hours. Water deficits induced in the plants similar leaf water potentials around -2.55 MPa at noon. Afterwards, the plants were irrigated for 48 hours (recovery) until water potentials reached values similar to the initial condition (control), around -1.32 MPa. The experiment was carried out in January 2003 (summer).

#### SAP FLOW MEASUREMENTS

Sap flow rates of three seedlings of each species were measured simultaneously by the heat balance technique devised by Sakuratani (1981), using stem flow gauge models SGA5 and SGA10 (Dynamax Inc., Houston, TX/USA), depending upon stem diameter. Stem segments were slightly sanded and a thin layer of silicone grease (Dow Corning 4, Dow Corning Corp., MI/USA) was applied on the surface to improve contact between the tissue and the heater. The gauges were installed and then covered with three layers of aluminum foil. Data were collected using a datalogger model CR7X (Campbel Sci., Logan, UT/USA). The datalogger was programmed to sample the voltage signals from the gauges every 1 minute. The voltage data were converted to sap flow rate and filtered in a spreadsheet using the formulae and restrictions provided by Van Bavel (1999). After the experimental period, sap flow measurements were halted and leaf area was determined for each plant using an electronic planimeter, model LI-3000A (Li-Cor Inc., Lincoln, NE/USA) to calculate sap flow rate per unit leaf area.

#### DATA ANALYSIS

Approximate entropy (ApEn), as defined in Pincus (1991), was used to quantify serial irregularity. For the study discussed below, ApEn values were calculated with widely established parameter values of  $m = 2$ , and  $r = 20\%$  SD (standard deviation) of the sap flow time series. Normalizing  $r$  to each time-series SD in this manner gives ApEn a translation- and scale-invariance (Pincus et al. 1993), in that it remains unchanged under uniform process magnification, reduction, or constant shift either higher or lower. Several previous studies that included both theoretical analysis (Pincus 1991, Pincus and Goldberger 1994) and clinical applications (Pincus et al. 1993, Christen et al. 1998, Pincus et al. 1998, Bruhn et al. 2000) have demonstrated that these input parameters produce good statistical reproducibility for ApEn for time series of the lengths considered herein.

Before ApEn calculation, previous time series analysis using autocorrelation coefficients (data not shown) indicated that the sap flow series were markedly nonstationary. Therefore, ApEn was applied to the first-differenced time series. This transformation, which is a standard statistical method to stationarize time series and it is applicable to a very broad class of models (Chatfield 1989, Williams 1997), was suitable in order to remove the non-stationary factors, according to the correlogram performed after series transformation (data not shown).

Spectral density analyses by Fast Fourier Transform were also performed in order to evaluate possible oscillatory behavior in the sap flow dynamics as observed by Rose et al. (1994) in other plant species.

## RESULTS AND DISCUSSION

Sap flow differences developed during stress period were observed mainly in *G. ulmifolia* (Figure 1b and c). Under water deficit, daily integrated sap flow decreased 45% in *G. ulmifolia*, whereas *C. legalis* maintained their sap flow levels stable. After the recovery period, however, both species showed similar sap flow reductions (-29%) in relation to control condition. These sap flow reductions could be partially explained by the milder environment during the measurements in the recovery condition (Figure 1a), demanding lower plant water loss.

Stomatal closure and consequent transpiration decrease are ubiquitous plant responses to water deficit in order to avoid excessive water loss (Nobel 1999). Thus, the presented results suggested that *C. legalis* was not affected by the drought treatment as *G. ulmifolia* was.

According to the spectral analyses, no single periodic behavior was observed in both species under any tested environmental condition (Figure 2). These analyses indicate that the signals were composed by more than one dominant frequency, mainly under water deficit, although different behaviors can be observed.

Water deficit tended to increase the complexity of the sap flow time series in both species, as indicated by ApEn values (Figure 2), although no significant ( $p > 0.05$ ) differences have been observed. This complexity increase tendency was also observed in the stomatal conductance dynamics of *Beta vulgaris* and *Helianthus annuus* under water deficit (Souza et al. 2004). Unpublished data from Souza et al. seems to support a hypothesis that environmental perturbations tend to increase the temporal complexity of physiological processes. According to Van Voris et al. (1980) and Souza and Oliveira (2003), complexity and stability are positively correlated. Thus, the complexity increase in the sap flow dynamics could be an adaptive response in order to hold stable the transpiration process. ApEn values tended to decrease after re-hydration, mainly in *G. ulmifolia*. In this species, the sap flow dynamics at recovery condition showed, significantly ( $p < 0.05$ ), more regularity than at control condition. The changes in ApEn values can be visually verified in the graphs of spectral analyses (Figure 2).

This study showed that ApEn could be used as a helpful tool to assess slight changes in temporal dynamics of physiological data, and to uncover some patterns of plant physiological responses to environmental stimuli.

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## RESUMO

Entropia Aproximada (ApEn), um modelo estatístico independente para quantificar irregularidade em séries temporais, foi utilizada para avaliar alterações na dinâmica temporal do fluxo de seiva em duas espécies arbóreas tropicais submetidas à deficiência hídrica. A deficiência hídrica induziu uma grande redução no fluxo de seiva em *G. ulmifolia*, enquanto que na espécie *C. legalis* manteve-se estável. A complexidade das séries temporais foi

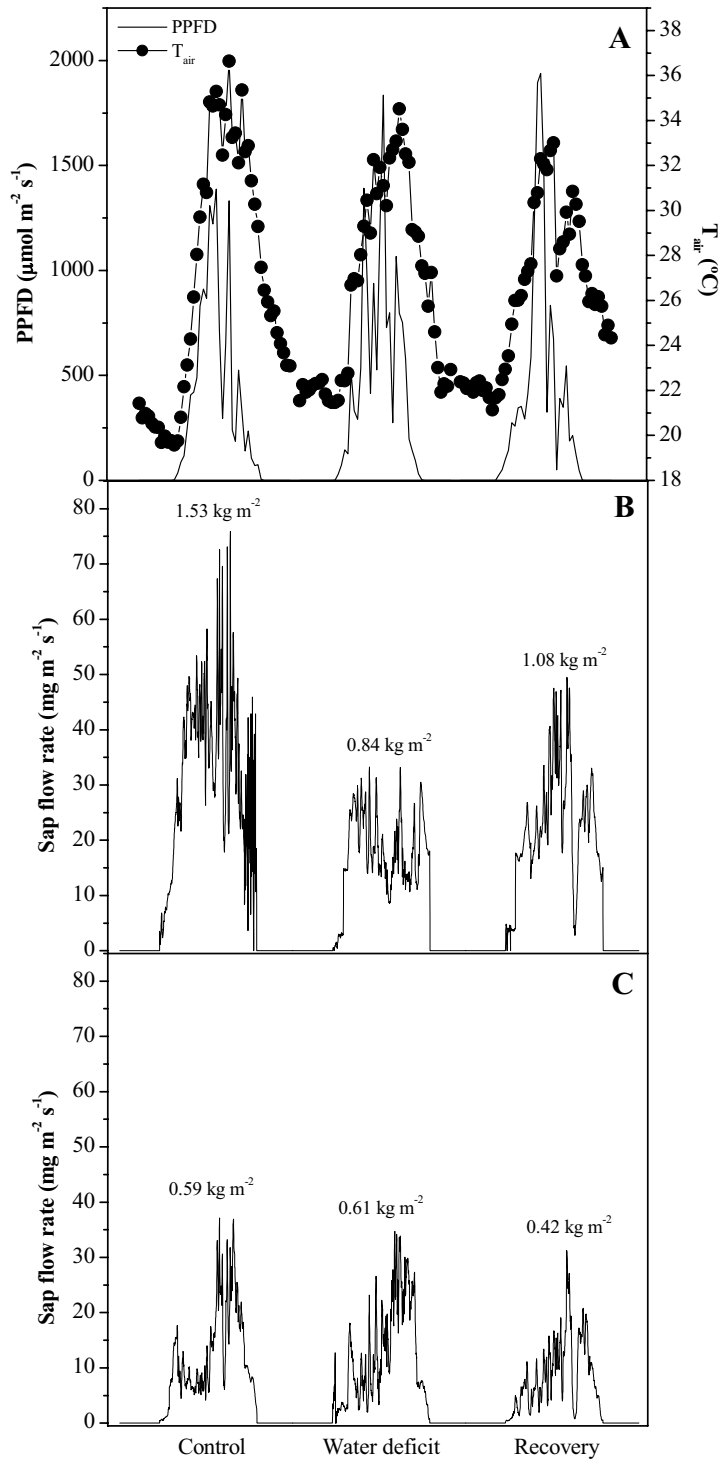


Fig. 1 – Daily changes in photosynthetic photon flux density (PPFD) and air temperature ( $T_{\text{air}}$ ) (A), and mean sap flow of three seedlings of *G. ulmifolia* (B) and *C. legalis* (C) at each experimental condition: control, water deficit, and recovery. Values of daily-integrated sap flow ( $\text{kg m}^{-2}$ ) are shown for each condition.

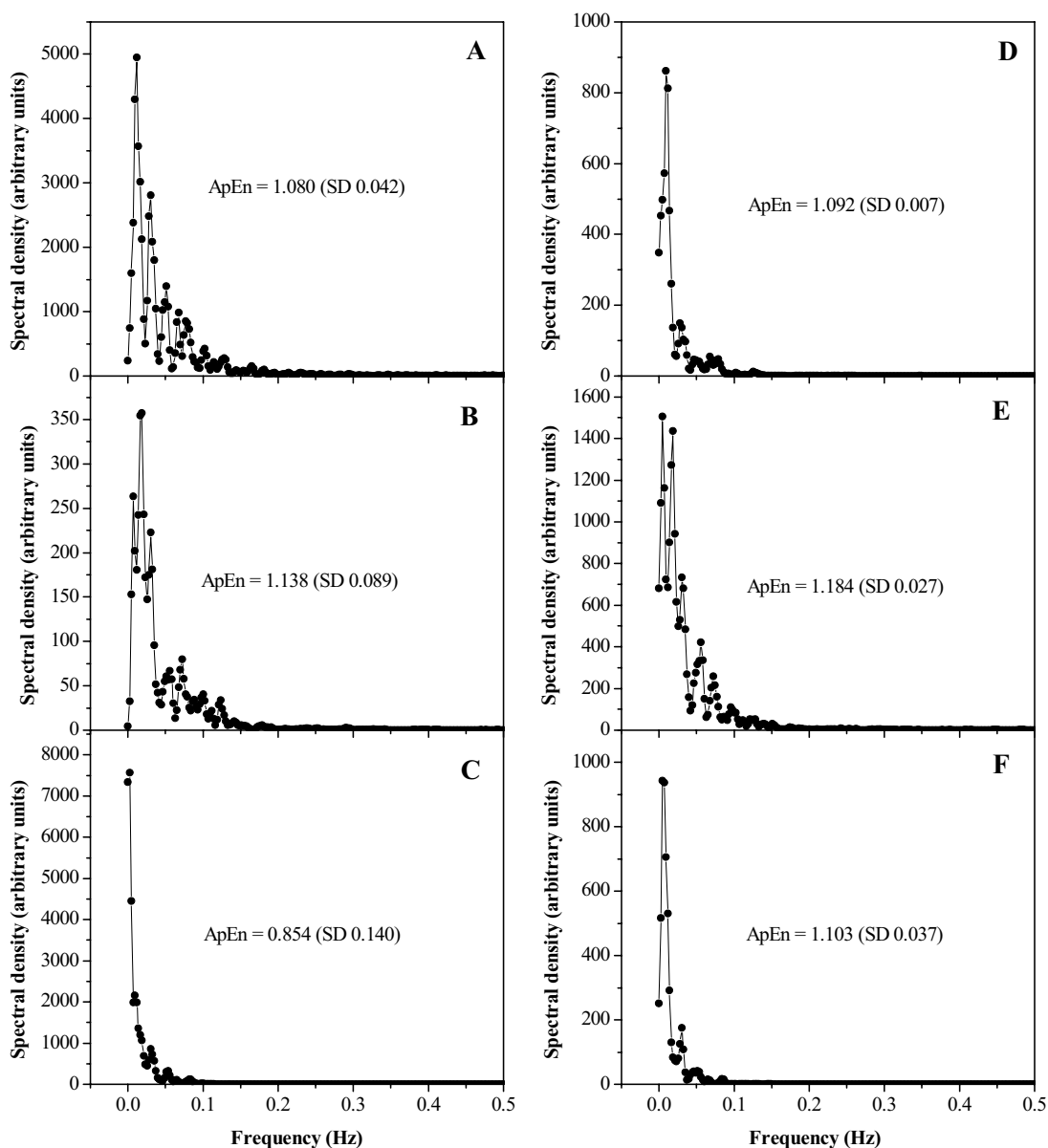


Fig. 2 – Spectral analysis by FFT of sap flow time series of *G. ulmifolia* (A, B, C) and *C. legalis* (D, E, F) at control (A, D), water deficit (B, E), and recovery (C, F) conditions. Mean values of Approximate Entropy (ApEn) are shown inside each figure ( $n = 3$ , SD = standard deviation).

levemente aumentada sob deficiência hídrica. O estudo mostrou que ApEn pode ser usada como um método para detectar pequenas alterações na dinâmica temporal de dados fisiológicos, e revelar alguns padrões de respostas fisiológicas a estímulos ambientais.

**Palavras-chave:** Entropia Aproximada, complexidade,

fluxo de seiva, análise de séries temporais, ecofisiologia tropical, deficiência hídrica.

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