University of West Hungary

Theses of PhD dissertation

The Effect of Environmental Factors on the Physiological State of Trees

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AMDG

Introduction

Based on the reflectance spectra measurements of foliage, I have worked out and tested a new method, that is capable of distinguishing various physiological states of trees (primarily drought stress),. Therefore, I extended the theory of state-dependent correlation (Németh *et al.* 2009) to spectrophotometric applications. The change of state-dependent correlations indicates the modification of the physiological control system of the plant; therefore, it can be used to detect stress in the early stages, too.

The investigation of the adaptation capacity and stress resistance of plants is crucial not only for agriculture but also for the forestry sector, which requires sensitive yet simple methods. The non-invasive spectrometric methods hold this potential but the prevailing use of Vegetation Indices has some inherent limitations, thus a new approach was needed.

Main steps in the research carried out by me

- Designing the infrastructure of the Magas-bérc Artificial Drought (MAD) Experiment.
- Designing, building and installing the measuring network and various sensors for the continuous registration of environmental parameters (Eredics 2013).
- Controlled soil moisture decrease was induced at some trees (at the construction of the roof system Ervin Rasztovits and Norbert Móricz gave assistance).
- Regularly collecting leaf samples from the tree crowns (Ervin Rasztovits and Norbert Móricz gave assistance) whose reflectance spectra were measured by the help of Rita Rákosa and Dorottya Badáczy.

- Extending the theory of state-dependent correlation to spectrophotometric applications and elaborated a method for the identification of stress (Eredics *et al.* 2014, 2015).
- Developing and implementing the data processing and analysing algorithms.
- Testing and evaluating the method on the data of the drought simulation experiment (Eredics *et al.* 2015).

Figure 1. Modular roof system for drought simulation at the MAD Experiment site.

Testing of the elaborated method

The applicability of the method was tested at adult sessile oak (*Quercus petraea*) and beech (*Fagus sylvatica*) trees, under partially controlled conditions. The test trees were located in the Sopron-mountains (Hungary) at a drought simulation experimental plot (MAD). Where precipitation was excluded with a modular roof system (*Figure 1*), thus increasing water deficit was induced at some trees (see *Figure 2.*). Although, the resultant artificial "drought" was not of great moment due to the relatively short time domain of the simulation (2.5 months), but significant systematic differences could be detected between the treated and the control trees, in accordance with the theoretical expectations.

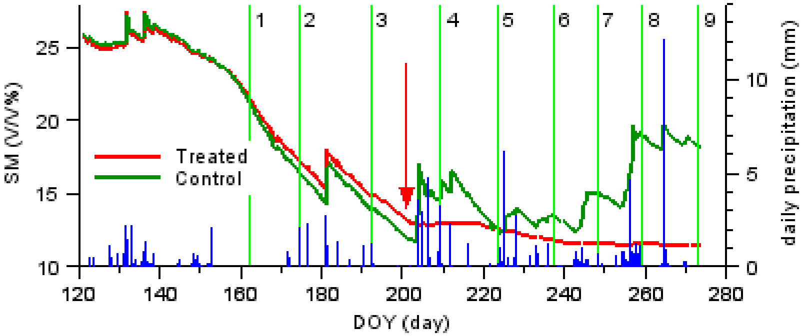

Figure 2. Soil moisture changes at the treated and control plots 1st May - 30th September 2014. (DOY 121-273). Total Soil Moisture (SM) is the thickness weighted average of 5 soil moisture sensor data of different depths. Sampling times are marked with numbered green lines; daily precipitation sums are indicated by blue columns. The construction of the roof system is indicated by a red arrow.

I evaluated the reflectance behaviour of foliage by regular leaf samplings, and monitored the environmental parameters with a measurement network, which was partially developed and built by me.

I worked out a method for the automatic identification and filtering of wavelength pairs that showed good state-dependent correlation. This method in the long run can estimate the effect of the soil moisture decrease on plants. I implemented the method with computer algorithms and tested on measured data sets.

New scientific results

Thesis 1

The concept of state-dependent correlation can be applied to reflectance intensity values of certain wavelength pairs derived from the reflectance spectra of plant foliage.

If the types of distribution of the measured absorbance values are identical, then a general equation of state-dependent correlation can be expressed with absorbance values:

$$\boldsymbol{A}_{\lambda_1} = \frac{\sigma_1}{\sigma_2}\boldsymbol{A}_{\lambda_2} + \frac{\sigma_2 \cdot \mu_1 - \sigma_1 \cdot \mu_2}{\sigma_2}$$

where μ is the expected value and σ is the standard deviation of the absorbance values $\boldsymbol{A}_{\lambda}$, measured at wavelength λ. This equation can be approximated with a linear regression of the measured absorbance values:

$$\boldsymbol{A}_{\lambda_1} = \mathrm{m} \cdot \boldsymbol{A}_{\lambda_2} + \mathrm{b}$$

where *m* is the slope and *b* is the intercept of the regression straight line.

Thesis 2

In those regulation processes where the physiological state of the plant conform not to the instantaneous environmental conditions but to the cumulative effect of a previous time period, the *time of influence* can be estimated by the extreme of correlation between the parameters of state-dependent correlation

and the characteristic time series of environmental parameters:

$$t_{infl} = \arg \max_{t \in [t_{min}, t_{max}]} \left| R_{E(t),m} \right|$$

where *R* is the Pearson's correlation coefficient:

$$R_{K_v(t),m} = \frac{cov(\boldsymbol{E}_v(t), \boldsymbol{m})}{s_{E_v(t)} \cdot s_m}$$

t_{infl} is the estimated *time of influence*, $\boldsymbol{E}_v(t)$ are the characteristic values of the *v* environmental parameter during *t* time periods before sampling (e.g. average or average change), which is analysed on the $t = [t_{min}, t_{max}]$ time period ($0 \leq t_{min} < t_{max}$), $\boldsymbol{m}$ are the parameters of the state-dependent regressions, *s* is the standard deviation.

Thesis 3

By analysing the dependence of the slope of state-dependent regressions on the influencing environmental parameter, the *sensitivity* of physiological control system can be determined. *Sensitivity* expresses the variation of state-dependent regression parameter in response to a certain change of the environment. Assuming linear relationship:

$$S_v = \frac{\Delta E_v}{\Delta m}$$

where S_v is the *sensitivity* of the state-dependent regression parameter *m* to the environmental parameter *v*, E_v is the value of the environmental parameter. S_v can be approximated with a linear regression of state-dependent regression parameters $\boldsymbol{m}$ and the corresponding characteristic values of the environment $\boldsymbol{E}_v$ before samplings:

$$\boldsymbol{E}_v = S_v \cdot \boldsymbol{m} + c$$

where $\boldsymbol{E}_v$ are the characteristic values of the examined v environmental parameter, S_v is the *sensitivity* to the environmental parameter, $\boldsymbol{m}$ are the parameters of the sate-dependent regressions, c is a regression parameter that merge the effect of the other (not investigated) environmental variables.

Thesis 4

The change of *sensitivity* in time indicates the alteration of physiological control system. This adaptation comes into existence to counteract an environmental stress factor, thus the change of *sensitivity* can be used to track the effect of this stress factor.

The *sensitivity* to an environmental factor can be regarded as an indicator of a stress factor if the following term is met:

$$\text{if } E_{stress} \text{ increases then} \rightarrow |S_v| \text{ increases}$$

where S_v is the sensitivity to the v environmental factor, E_{stress} is the value of stress factor.

Thesis 5

Certain wavelength pairs of the reflectance spectra of sessile oak and beech foliage show strong state-dependent correlations, which are influenced by the alteration of environmental factors. Such environmental factors are the air temperature and the vapour pressure deficit, which can be typified by the characteristic values of the previous period (*time of influence*), e.g., average, or average change.

This result is also the experimental proof of theses 1-3.

Thesis 6

In case of sessile oak and beech, alteration of soil moisture results in *sensitivity* variation of state-dependent regressions derived from the reflectance spectra, therefore, it can be regarded as an indicator of drought stress.

This result is also the experimental proof of thesis 4. Some examples for the sensitivity increase in response to soil moisture decrease are demonstrated in *Figure 3*.

Based on the results of the Magas-bérc Artificial Drought manipulation experiment, wavelength pairs summarised in *Table 1.* are sensitive to drought stress.

Table 1. Drought stress sensitive wavelength pairs (nm). Bandwidth 10 nm.

Sessile oak	Beech	
300-600	310-690	3650-3700
400-600	400-600	4130-5170
530-710	420-700	4140-4570
530-1150	530-980	4690-4840
720-1140	1230-1290	6930-7420
2970-3020	2800-3710	7430-7660
8980-9040	2860-3960	7870-7920
9570-9620	3420-6830	8030-8090
10740-10860	3430-3510	8390-8440

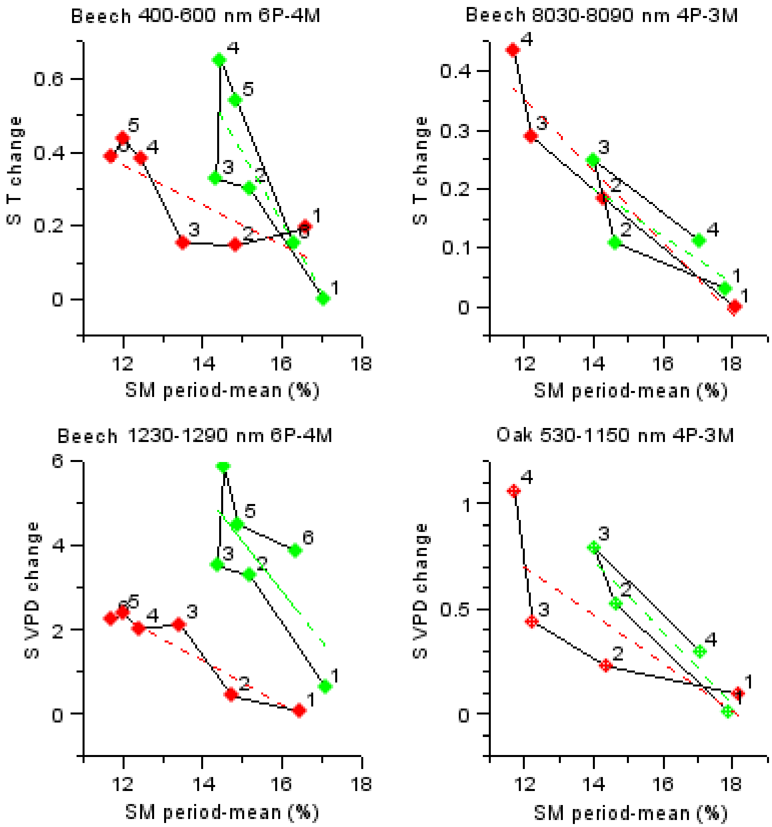

Figure 3. Some examples of the sensitivity change of state-dependent regressions, where sensitivity (S) increased due to the decreasing soil moisture ($SM_{period-mean}$), in accordance with thesis 4. $S_{Tchange}$ – sensitivity to temperature change; $S_{VPDchange}$ – sensitivity to vapour pressure deficit change; Red – treated trees; Green – control trees. The 9 samplings were divided into partially overlapping periods that are represented by the numbers. P – number of periods; M – number of samples per period.

State-dependent correlation based analysis on reflectance intensities, measured at certain wavelengths of plant foliage, can be used as an indicator of drought stress. The experimental results are in accordance with the theoretical expectations, hereby proving them.

Evaluation of the results

Calculations for *time of influence* (estimated by the change of parameters *m* of state-dependent regressions and the characteristic values of various environmental parameters) resulted mainly 2-3 days long and 5-7 days long time constants. Of course, shorter or different *times of influence* are also feasible, but estimation of shorter periods than 2 days was not possible from the data set. Nevertheless, these findings are consistent with previous studies that determined the adaptive time constants as a usually 1-2 days or maximum 1 week long period, investigating numerous biotic and abiotic environmental factors (e.g. Lichtenthaler 1996).

The elaborated method breaks with the use of average or averaged samples, which is prevailing at reflection spectra measurements of plant foliage. State-dependent correlations exploit just those small differences between individual leaves, which were usually regarded as "noise" or disturbing variability in previous studies.

The work is novel in that sense, that it does not apply the widespread Vegetation Index approach to extract the information from the reflectance spectra, but it is based on the state-dependent correlation theory. This, in turn, estimates the *state of the physiological control system* (Németh *et al.* 2009), not only the water- or other *material content* of the leaves, as several Vegetation Index do. Although, the method does not identify the materials (or properties) that determine the absorbance at the selected wavelengths, it is still based on clear physiological principles.

Compared to numerous Vegetation Indices, the investigation of state-dependent correlations proved to be a more sensitive method, which is capable of indicating smaller levels of plant stress (eustress).

Some previous investigations of the reflectance spectra have already been carried out on the theoretical basis of state-dependent correlation (Kocsis 2010, Németh *et al.* 2011, Németh – Rákosa 2013, Rákosa – Németh 2014), but in the presented work certain previously applied methods were improved on, eliminating their disadvantages. Such improvement is the automatic selection of wavelength pairs by their correlation matrix, which greatly reduces the subjective factor present in previous studies. Another development is the application of Standard Normal Variate (SNV) correction as data pre-processing, in place of the previously applied Compensation Absorption Indices, because SNV causes much less distortion to the correlations of the reflectance spectra.

The work is uncommon in the applied experimental setting: many investigations on potted seedlings in greenhouses or in gardens, orchards, or on agricultural fields are known, but *manipulation experiments on adult trees* are sporadic even on a worldwide scale (e.g. Nepstad *et al.* 2002, Lamersdorf *et al.* 1998, da Costa *et al.* 2010).

The most significant result of the work is the methodological development: the extension and definition of state-dependent correlation theory to spectrophotometric applications and the monitoring of plant stress. The theory is supported by the experimental findings.

The presented method is universal in that sense that it can be applied to different plant species, because of the similarity of control systems being present in plants (e.g.

photosynthesis), and it can be applied to measure the effect not only of drought stress but also of other biotic or abiotic stressors.

The selected wavelengths may differ by species and stressors, therefore, the applicability of presented wavelength pairs can be stated only for the investigated species at the moment. It is likely however, that such "universal" wavelength pairs may exist, which can be applied equally to different species. Therefore it might be possible to elaborate such plant diagnostic procedures, which are capable of distinguishing various adaptation and stress states of plants even on the field, by measuring the reflectance spectra of the leaves with specialised instruments. This application may be beneficial not only for the increasingly more intensive precision agricultural production, but may also help the selection of appropriate, highly resistant propagation material for forest management.

The measurement method is non-invasive, and it is based on optical sensing, therefore it might be carried out with small, portable handheld instruments. These beneficial properties may ease the widespread use of this method in the future.

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