Near Infrared Spectroscopy as a Discriminate Approach to Classify Eucalyptus Tissues According to their Physiological Status

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Introduction: Studies involving highthrougput approaches, such as metabolomic, are very expensive and heavy to perform, especially for trees in the field. Indeed the control of the physiological status of material is crucial to improve the sampling procedure as well as to control the homogeneity of the material collected. The objective of this study is to evaluate if the near infrared spectroscopy (NIRS) can be used to discriminate physiological status in a rainfall exclusion x fertilisation design of Eucalyptus grandis conducted in Brazil, and could help to select the samples to analyze. To investigate the feasibility of the use of NIRS to discriminate different tissues, the leaves and wood samples of E. grandis trees, submitted to water stresses and fertilizations, were considered. For this first approach we used Linear Discriminant Analysis (LDA) applied to the NIR data.
Experience: A split-plot experimental design was set up in June 2010 with an E. grandis clone from the Suzano Company (São Paulo, Brazil). Six treatments (3 fertilization regimes crossed to 2 water regimes) were applied. 1,5 months leaves were collected on 4 trees per treatment during dry and rainy seasons from 1 to 4 year old tree. Wood disks were collected on 8 trees per treatment on 4 year-old trees. NIRS data were performed (5 leaves per tree, 2 spectra per leaves; 1 wood disk per tree, 6 spectra per disk) under diffuse reflection using integration sphere of a Bruker MPA spectrometer. Spectral analysis was performed within the 12,500–3,500 cm^{-1} range at 8 cm^{-1} resolution (each spectrum consisted of 32 scans). The temperature and humidity remained constants (20°C, 55%) throughout the experiment. We used Unscrambler software 10.3 (Camo, AS, Norway) for Nirs data analysis. LDA was used to classify leaf and wood samples according to the water supply factor, the fertilization factor and combination of the two. On the same leaves collected from 2 year-old-tree, we performed an untargeted metabolomic analysis by HLPC-MS.
Results and discussions: According to the sampling period of the leaves (dry and rainy periods), for LDA model obtained from SNV and derivative 2 spectra pre-treatments, the correct classification of leaves were from 83 % to 100% for water supply factor, from 78 % to 100% for fertilization factor, from 93 % to 100% for the 6 treatments. For wood samples, the correct classification was 68% for fertilization factor, 77 % for water supply factors and 77% for the 6 treatments. The accuracy for wood was lower than for leaves because we measured spectra at different positions, from the pith to the cork. This was the result of included annual and seasonal variability. To validate our result, we compared the discrimination obtained on the 2 year-old tree leaves by FT-NIRS with one obtained by performing an untargeted metabolomic analysis. The similar results demonstrate the feasibility of the FT-NIR spectroscopy to discriminate leave samples. Results presented here could help us to discriminate samples according to their status, and to select extreme samples for chemical and metabolomics studies.

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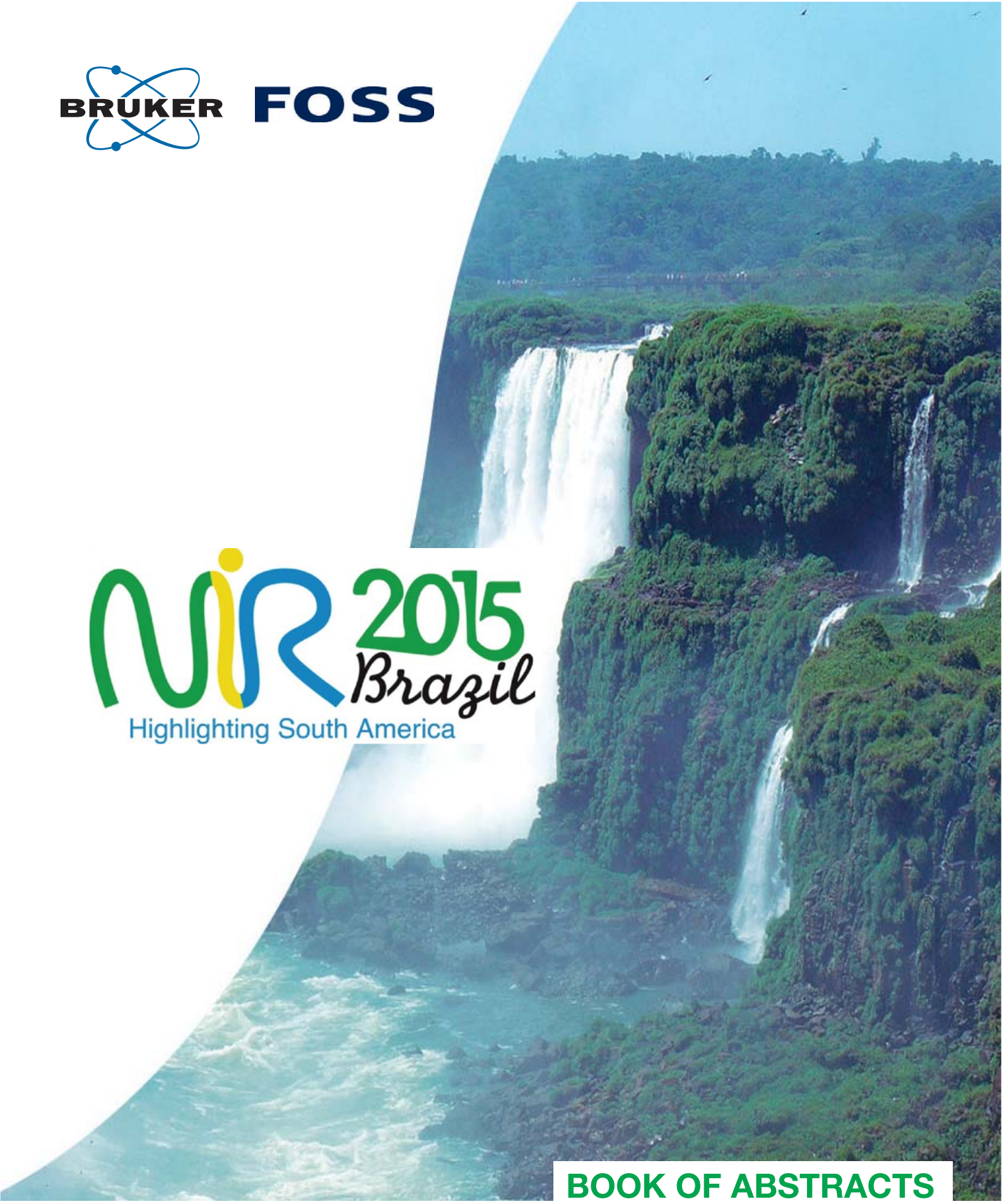

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