Phyton (Austria) Special issue: "Global change"	Vol. 42	Fasc. 3	(215)-(221)	1.10.2002

Multivariate Analyses of Tree Physiological Attributes - Applications in Field Studies

By

M. TAUSZ[1)], A. WONISCH[1)], C. RIBARIČ-LASNIK[2)], F. BATIČ[3)] & D. GRILL[1)]

Key words: Principal component analysis, cluster analysis, multivariate statistics, explorative statistics, antioxidants, stress.

Summary

TAUSZ M., WONISCH A., RIBARIČ-LASNIK C., BATIČ F. & GRILL D. 2002. Multivariate analyses of tree physiological attributes - application in field studies - Phyton (Horn, Austria) 42 (3): (215) - (221).

Multivariate explorative statistics is a tool to analyze the structure of complex datasets. In tree physiological field studies relations of multivariate biochemical plant responses to complex environmental impacts are of interest for bio-indication purposes. The present paper gives a short review of statistical techniques, in particular principal component analysis (PCA) and cluster analysis (CA), to datasets consisting of stress-related biochemical variables measured on tree foliage in field studies. Special attention is paid to the physiologically meaningful interpretation of analysis results, shown at examples.

Introduction

Multivariate analyses are a widely accepted tool in the treatment and exploration of complex datasets. In general, their aim may be a reduction of the complexity, but also a direct knowledge gain due to pattern recognition techniques. In plant ecophysiology field studies, the investigation of morphological, physiological, and biochemical variables results in complex datasets related to complex environmental impacts. The use of such multivariate plant responses for

[1)] Institut für Pflanzenphysiologie, Karl-Franzens-Universität Graz, Schubertstraße 51, A-8010 Graz, Austria.

[2)] ERICO Velenje, Institute for Ecological Research and Industrial Cooperation, Koroška 58, SI-3320 Velenje, Slovenia.

[3)] Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia.

bio-indication purposes (= relation to distinct impacts) without the application of multivariate techniques is limited.

In the mid-nineties, first suggestions to use multivariate statistics (such as factor analysis) on physiological field study data were published. In a short review paper on bio-indication, WILD & SCHMITT 1995 suggested the application of factor analytical techniques to biochemical datasets. About at that time, a first application to stress-physiological data from a forest decline field study was conducted in Austria (TAUSZ & al. 1996). GRULKE & LEE 1997 related a number of morphological canopy attributes of Californian pines to ozone damage. Consequently, the application of principal component analysis and cluster analysis lead to new insight in complex biochemical responses of conifers to environmental conditions in temperate (TAUSZ & al. 1996, 1998b, WONISCH & al. 1999) and mediterranean (TAUSZ & al. 1998a, 2001) climates. Recently, a number of other studies on biochemical plant defense used multivariate grouping of response patterns (GARCÍA-PLAZAOLA & al. 2000) and the treatment of tissue chemistry data of tree foliages was also evaluated by multivariate treatments (SEIDLING 2000).

The present paper gives a review of statistical techniques recently applied to stress-related biochemical data-sets of tree physiological field studies and discusses particular aspects relevant to the use in stress indication.

Principal Component Analysis (PCA)

Principal component analysis (PCA) is used to group variables into subsets that are relatively independent from each other. The so produced new, accumulated variables (= principal components, PCs) represent underlying processes responsible for inter-correlations of variables in the original dataset. A practical overview of theory and basic assumptions is given in STATSOFT 2001.

In plant physiological field studies, this technique is mainly used to (1) reduce the number of variables in an original data-set, to (2) explore inter-correlations among original variables and learn more about the possibly underlying physiological relations grouping the variables together, and (3) to facilitate following analyses (e. g. classifications, relation to environmental data etc.).

In particular the point (2) can contribute directly to our knowledge about complex processes: The following example (Fig. 1) shows part of the results of a PCA applied to a dataset of stress-physiological variables measured on field grown pines (for the complete information see TAUSZ & al. 1998). The principal components generated are characterized by the loadings of the input variables. A higher absolute loading value means a more important contribution to the new PC. Besides statistical correctness, the plant physiologically meaningful interpretation of the PCs is decisive. To illustrate this, the exemplary PCs characterized in Fig. 1 are related to the physiological-biochemical stress responses sketched in Fig. 2. PCs are named according to the content of physiological variables: In our example, PC 1 has high loadings on ascorbate and tocopherol concentrations and strong negative loadings on V/(V+A+Z) and α-carotene concentrations. This fits in our current picture of the biochemistry of a photo-oxidative stress situation in the chloroplast (Fig. 2): Under excess light the xanthophyll cyle, a process connected to energy dissipation, would be less epoxidized (the V/V+A+Z value will drop, because zeaxanthin will be formed), and the plant would adjust to the generation of toxic reactive oxygen species (ROS) by increasing the scavenger potential: α-tocopherol is the major lipophilic antioxidant protecting the thylakoid membranes, and ascorbate is the major water soluble antioxidant required for both the action of the xanthophyll cycle and the regeneration of α-tocopherol. Another PC in this study was loaded on the proportion of oxidized ascorbate (dehydroascorbate), glutathione, and the proportion of oxidized glutathione (GSSG). Since ascorbate as the main cellular antioxidant in aqueous phase is regenerated by the glutathione system the combination of these variables to one PC is physiologically meaningful (Fig. 2). Higher scores of this PC would indicate a higher pressure on this regeneration system (smaller pool sizes of the metabolites and a more oxidized state). Hence, the statistical results leading to the PCs corroborates the conceptual model of plant stress responses given in Fig. 2.

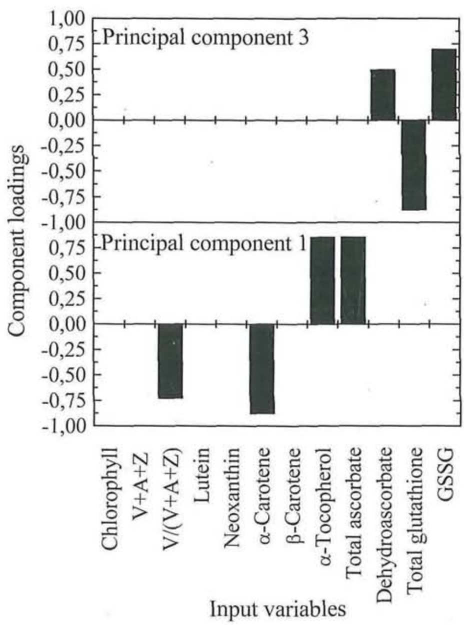

Fig. 1. Loadings of biochemical input variables on two selected principal components. The analysis was run on data of field grown *Pinus canariensis* needles, the complete results (4 PCs were extracted in total, but only 2 are discussed here) and study details are given in TAUSZ & al. 1998a. Loadings with an absolute value smaller than 0.5 are not shown.

The scores (values) of the PCs can further be used to explore relations to environmental factors, which are not detectable using the original input variables.

A good example for this application is given in WONISCH & al. 1999, where a relation of stress responses of spruce trees to small scale site factors (water supply) could be established using a PCA.

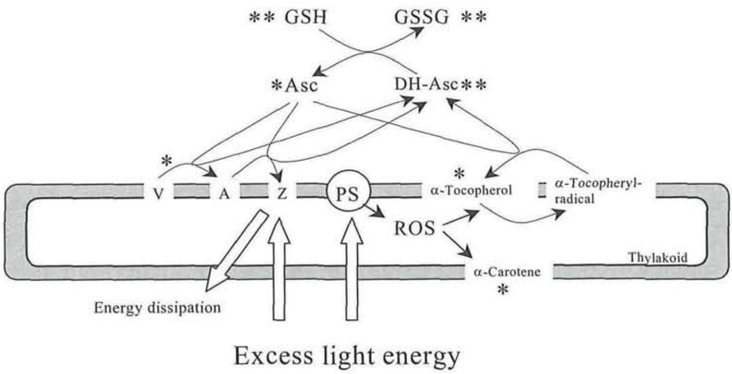

Fig. 2. Scheme of some stress-dependent biochemical reactions in plant chloroplasts related to the prinicipal component characteristics in Fig. 1. Excess light leads to de-epoxidation of violaxanthin (V) via antheraxanthin (A) to zeaxanthin (Z) which confers light protection. Under stress conditions, reactive oxygen species (ROS) are produced in photosystems (PS) and may cause oxidation of pigments, the most sensitive is a-carotene. Protection from ROS is supplied by tocopherol which is regenerated by ascorbate (Asc). Ascorbate is oxidized itself yielding dehydroascorbate (via mono-dehydroascorbate, not shown) and has to be regenerated by reduced glutathione (GSH) which is in turn oxidized (GSSG). * denotes variables contributing strongly to PC1 (Fig. 1) and ** variables contributing strongly to PC2.

Cluster Analysis (CA)

Cluster analysis is a technique used to classify objects according to similarities of multivariate patterns (STATSOFT 2001). It serves well as a tool for pattern recognition, an important task in field studies.

The result of a cluster analysis is often shown as a tree diagram, the branches depicting the similarities between the objects. The researcher has to decide at which similarity level to accept groups as homogenous, e.g. by testing between group differences of input variables. The original dataset of the following example belongs to a study of stress-physiological variables in spruce needles collected in the vicinity of a thermal power station (details about the sites and the situation there in RIBARIČ-LASNIK & al. 1999). Biochemical response patterns of individual trees were classified through a cluster analysis performed on the scores of PCs generated in a preceding PCA as described above. More details of this study will be given in a separate paper (in preparation). The use of PC scores guarantees low intercorrelations between the input variables used to cluster the tree response patterns, which improves the stability of the cluster schedule. The differences of the average input variable scores (PCs) between resulting clusters were tested by an

ANOVA and only clusters that differed significantly from each other in at least one attribute (PC) were accepted.

The response patterns in each cluster can be interpreted according to their average scores in the input variables and named correspondingly (Fig. 3). Needles of trees grouped in the "sulfur stress" cluster had high sulfur, low pigment and low antioxidant response - this pattern corresponds to trees directly impacted by sulfur emissions. Trees in the "oxidatively stressed" cluster had low sulfur, high antioxidant, and low pigment which corresponds to tree response to oxidative stress, whereas "unstressed" trees had high pigment concentrations and low sulfur and antioxidant scores. In this case, one distinct cluster could not be interpreted based on that response pattern, hence it is preliminarily named "unidentified stress" in Fig. 3.

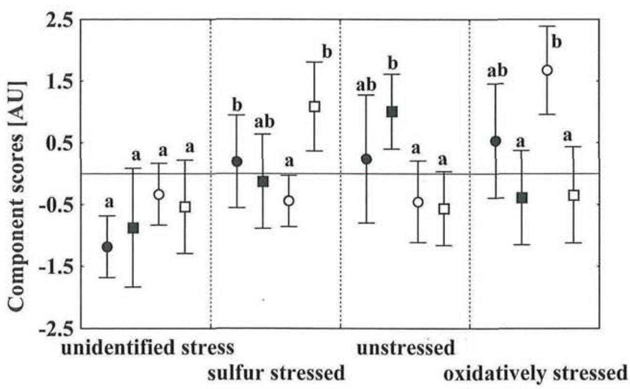

Fig. 3. Cluster analysis solution on four principal components generated from a data-set of stress-physiological variables of field grown spruce around a thermal power plant. Principal components (PCs) were named according to their physiological significance: ● pigment ratios; ■ pigment concentrations; ○ antioxidant responses; □ sulfur impact. Differences between clusters were tested with ANOVA followed by LSD-post-hoc-tests. Significantly different component scores have no letter in common. Means and SDs of average PC scores in each cluster are shown. AU arbitrary units, 0 is the mean of all trees in the study. For more details on the field study refer to RIBARIČ-LASNIK & al. 1999.

Based on the cluster membership of the trees, the study sites could be related to specific stress situations. The high-elevated sites had more oxidatively stressed trees, whereas sulfur stressed trees were confined to the closer vicinity to the power plant. Such a data treatment takes into account individual differences among the sample trees - it is possible that under moderate impacts not all trees show distinct response patterns (Fig. 4); such a situation adversely biases interpretations of average values for each site. It is obvious that trees belonging the cluster named "unidentified stress" grow mainly at one site. A more detailed investigation of site factors suggested that nutrient imbalances caused this response pattern (data in preparation).

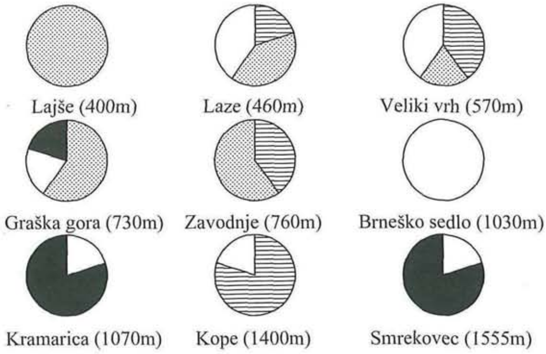

Fig. 4. Frequency (proportion of the sampled population) of trees belonging to the four physiological response pattern clusters characterized in Fig. 3 at field study sites around a thermal power plant in Slovenia. Signatures (see clusters in Fig. 3): , ▤ 'unidentified stress', ▢ 'sulfur stressed', ▢ 'unstressed trees' ■ 'oxidatively stressed'. For more details on the field study refer to RIBARIČ-LASNIK & al. 1999.

Conclusions

The multivariate approaches shown in this paper illustrate the potential for enhanced interpretation of complex field data and significant gain of knowledge. PCA and CA discussed in our paper are not the only methods applicable to multivariate physiological datasets, other statistical techniques, e.g. multiple regression approaches, discriminant analyses, canonical correlations, neuronal networks etc. may also serve. However, an important prerequisite which is often not easy to meet in plant physiological field studies, is data quality and sample size. In future studies the possibilities of multivariate statistics should be taken into account already in planning of experimental design.

Acknowledgement

Parts of this work were supported by the FIW II (Forschungsinitiative gegen das Waldsterben), by the TEMPUS program, by the Wissenschaftlich-Technische Zusammenarbeit Österreich-Spanien (Acciones integradas), and by grants of the Fulbright Commission, the Dr. Heinrich-Jörg Stiftung, and the Universidad de La Laguna with the Banco Santander to M. TAUSZ.

References

GARCÍA-PLAZAOLA J. I., HERNANDEZ A. & BECERRIL J. M. 2000. Photoprotective responses to winter stress in evergreen Mediterranean ecosystems - Plant Biol. 2: 530 - 535.

GRULKE N. E. & LEE E. H. 1997. Assessing visible ozone-induced foliar injury in ponderosa pine. - Can. J. For. Res. 27: 1658 - 1668.

RIBARIČ-LASNIK C., TURK B., BATIČ F. & GRILL D. 1999. Antioxidants in Norway spruce needles at field plots in the vicinity of a thermal power plant in Slovenia. – Phyton 39(4): 175 - 182.

SEIDLING W. 2000. Multivariate statistics within integrated studies on tree crown condition in Europe - an overview. - UN/ECE & European Commission, Geneva, Brussels.

STATSOFT 2001. Electronic Statistics Textbook. - StatSoft Inc., Tulsa, OK. Website http://www.statsoft.com/textbook/stathome.html

TAUSZ M., GRILL D. & GUTTENBERGER H. 1996. The use of physiological parameters of spruce needles as a bioindication tool. - Phyton 36(3): 31 - 34.

— , JIMENEZ M. S. & GRILL D. 1998a. Antioxidative defence and photoprotection in pine needles under field conditions - a multivariate approach to evaluate patterns of physiological responses at natural sites. - Physiol. Plant. 104: 760 - 764.

— , STABENTHEINER E., WONISCH A. & GRILL D. 1998b. Classification of biochemical response patterns for the assessment of environmental stress to Norway spruce. - ESPR Environ. Sci. Poll. Res. Special Issue No 1: 96 - 100.

— , BYTNEROWICZ A., ARBAUGH M. J., WONISCH A. & GRILL D. 2001. Multivariate patterns of biochemical responses of *Pinus ponderosa* trees at field plots in the San Bernardino Mountains (Southern California). - Tree Physiol. 21: 329 - 336.

WILD A. & SCHMITT V. 1995. Diagnosis of damage to Norway spruce (*Picea abies*) through biochemical criteria. - Physiol. Plant. 93: 375 - 382.

WONISCH A., TAUSZ M., HAUPOLTER M., KIKUTA S. & GRILL D. 1999. Stress-physiological response patterns in spruce needles relate to site factors in a mountain forest. - Phyton 39: 269 - 274.

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2002

Band/Volume: 42_3

Autor(en)/Author(s): Tausz Michael, Wonisch Astrid, Ribaric-Lasnik C., Batic Franc, Grill Dieter

Artikel/Article: Multivariate Analysis of Tree Physiological Attributes - Applications in Field Studies. 215-221