



## **Institut für Landschaftsökologie**

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# **Detection of Vegetation Stress using Imaging and Non-Imaging Spectrometer Data in the Laboratory**

vorgelegt von  
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## 1 Abstract

In order to systematically explore vegetation stress-related effects on the relationship between hyperspectral data and plant physiological parameters at a canopy scale, a test series was conducted at the UFZ research laboratory in Bad Lauchstädt over a four month period. Here, ash (*Fraxinus excelsior* L.) seedlings forming a canopy were grown in a shade house and exposed to controlled drought stress and flooding treatments.

Hyperspectral measurements were performed in a dark room in artificial illumination conditions using AISA Eagle imaging spectrometer and non-imaging ASD Field spectrometer on a semi-weekly base. For determination of plant physiological status, leaf chlorophyll content, leaf area index (LAI), plant height, leaf water content, C and N content of leaves and soil moisture measurements were conducted along with hyperspectral data acquisition. A number of 34 vegetation indices known to be sensitive to plant stress were calculated from AISA and ASD data. Since most of the indices were found to perform very similar, subsequent statistical analysis focussed on a selection of four evidently different indices, namely NDVI, PRI, Vogelmann 2 and WI.

Results suggest that implementation of drought stress failed while inundation lead to leave shedding. Additionally, a combination of length of experimental period and limited number of sampling data resulted in an excess of confounding factors and thus inconsistent correlations between hyperspectral data and chlorophyll, leaf water content and C/N values. Percentage of green biomass, in part represented by Leaf area index (LAI), was found to be the dominant control on canopy reflectance. Although a confirmation of relationships reported in literature was only achieved in parts, results are consistent for AISA and ASD data. Since the ASD Field spectrometer is a well-established instrument regarding detection of plant stress and foliar chemistry in the laboratory this indicates a general correctness of experimental setup in the dark room.

## 2 Introduction

“Remote sensing” is defined as the science of obtaining information about an object without being in physical contact with it (Kappas 1994, Albertz 2007). „Hyperspectral remote sensing”, also called “imaging spectrometry” means the acquisition of images in a large number of narrow, contiguous spectral bands, thus enabling extraction of reflectance spectra at each picture element (Van der Meer & de Jong 2006). Its objective is the derivation of a quantitative measure of the interactions between matter and electromagnetic radiation in order to identify properties and processes of the Earth System (Ustin et al. 2004). Non-imaging spectrometers acquire average reflectance spectra of surface materials in a similarly large number of narrow, contiguous bands and are widely used in laboratory studies and for collecting ground truth data during airborne hyperspectral campaigns. (Analytical Spectral Device 2006)

Remote sensing technologies have been available for landscape scale mapping for several decades. In forest science, hyperspectral remote sensing imagery has been used to map forest productivity, species, foliar chemistry and tree health at the landscape scale (e.g. Sampson et al. 2000; 2003, Niemann & Goodenough 2003, Pontius et al. 2005; 2008). Until recently, the main limitation of remote sensing was that surface information lacked detail due to the broad bandwidth of sensors available, resulting in a loss of (plant) reflectance data due to averaging (Van der Meer et al. 2006). Already, data derived from hyperspectral sensors has been found to be superior in detecting vegetation health and cover compared to data obtained from broad band sensors (e.g. Collins et al. 1983, Boochs et al. 1990, Peñuelas et al. 1993, Carter 1994a, Asner 1998).

One of the benefits of hyperspectral data over traditional detection and monitoring techniques in vegetation science is its ability to detect very *early* signs of stress - stress that may not yet be visible on the ground or in aerial photography (Haller et al. 2005). However, as with any new technology, it takes time to develop new methods to fully utilize the large information content of imaging spectrometers (Kumar et al. 2006).

Traditional approaches of monitoring vegetation by remote sensing comprise the use of spectral indices, e.g. for assessing vegetation cover (De Jong & Epema 2006). Vegetation indices are intended to isolate the vegetation signal from background and other materials while minimizing solar irradiance effects (Jackson & Huete 1991, Asner et al. 2003). Further methodologies have been developed including radiative transfer modelling and inverse modelling. The different methods offer advantages and disadvantages that are related to the complexity of the modelling approach selected and the degree of



general or local applicability (Zarco-Tejada et al. 2001). While the use of vegetation indices is considered site and species specific, modelling approaches are computational intensive and many of the input parameters required remain speculative or are not fully understood yet.

Overall, hyperspectral remote sensing techniques offer rapid, comprehensive and labour saving means of assessing vegetation changes at the landscape scale. In recent years, much research has been conducted trying to link physiologically based indicators of vegetation stress to spectral indices (e.g. Vogelmann et al. 1993, Gitelson & Merzlyak 1994a;b; 1995, Carter & Miller 1994, Gitelson et al. 1996a, Zarco-Tejada et al. 1999; 2000b; 2001; 2002; 2004, Pontius et al. 2005; 2008) and to develop practical and objective measures of forest condition (Sampson et al. 2000; 2003), since stressed vegetation may be subject to consequential damage (e.g. insect calamities). Thereby, a diagnosis of the stress agents by remote sensing remains difficult due to the variety of stressors affecting vegetation simultaneously. Rock et al. (1988) suspects potential characteristic spectral signatures helpful in identifying specific kinds of damage caused by specific stress agents, whereas Carter and Knapp (2001) consider a diagnosis of stress factor impossible in many cases because of the generality of leaf optical responses to stress. Sampson et al. (2000; 2003) propose incorporation of spatial data such as soil type, terrain, insect and disease surveys and dendrochemical analysis in order to correctly identify stress factors. Thereby, one has to differentiate between detection of stress on one hand and assessment of clear damage symptoms on the other (Lichtenthaler 1996). Estimates of plant vigour traditionally used in forestry such as crown condition, foliage or transparency assessment, and biomass and height increment are not always suitable for remote sensing applications as they do not necessarily alter reflectance spectra. Indicators of plant health that were reported to be observable from spectrometer data are amount of green biomass, pigment content (especially chlorophyll), photosynthetic activity, plant water content as well as carbon and nitrogen content.

## 2.1 Hypothesis and Objective

Among others, Liew et al. (2008) conclude that research is still needed in order to define species-specific reflectance properties of unstressed plants and to distinguish stress-related effects that may be attributed to specific stress factors. In order to systematically explore relationships between hyperspectral imaging data and single stress parameters at the canopy scale, a test series using common ash seedlings was conducted at the UFZ research laboratory in Bad Lauchstädt. The selection of *Fraxinus excelsior* resulted from several criteria: First, ash is one of the dominant species in riparian forests, which are subject to intensive research at the Helmholtz centre of environmental research (UFZ), thus permitting a transfer of results derived from the laboratory study to well established sampling sites in the field. Secondly, ash is an important timber species (Leonhard et al. 2009) but in recent years has been subject to increasing damage and mortality at different site conditions (Schumacher et al. 2007) which is by now attributed to fungal infections by *Chalaria fraxinea* T. KOWALSKI (Leonhard et al. 2008; 2009). Finally, the general experience of Bad Lauchstädt laboratory staff is that ash is relatively easy to establish, compared with e.g. oak (cf. Kerr & Cahalan 2004). Since literature gives evidence of only one comparable one-day experiment with similar setup (Zarco-Tejada et al. 2000b), this study is regarded to be the first attempt of monitoring vegetation stress reaction over a longer period of time by the use of an imaging spectrometer in the laboratory. Here, stress parameters (flooding and drought stress) can be induced systematically while other environmental conditions are controllable at all times and sampling conditions are stable over the entire experimental period. Additionally, by the use of a dark room facility, hyperspectral data collection is independent of potentially unfavourable weather conditions, enabling sampling of a regular test series. The aim of this study was to assess the potential to separate the effects of different stress agents on hyperspectral reflectance data. Specific objectives of this work were (1) the attempt to establish quantitative relationships between plant physiological parameters and hyperspectral indices over a longer period of time, (2) separation of specific stress factors from hyperspectral signals, and (3) a comparison of results derived from hyperspectral imaging data and hyperspectral non-imaging data, both subject to laboratory conditions.

### 3 Theory

#### 3.1 Distribution and Ecology of Common Ash

Common ash (*Fraxinus excelsior* L.) is a member of the *Oleaceae* family of plants, which comprises 27 genera and approximately 600 species in the tropical and temperate zone (Rittershofer 2001). Thereby, common ash is the most widely distributed ash species in temperate Europe, extending from the Atlantic coast in the west to continental Russia in the east and from central Norway in the north to the north of the Mediterranean states in the south (Marigo et al. 2000, Fraxigen 2005). Its distribution is limited by its sensitivity to winter cold, late spring frosts and dry, hot summers (Fraxigen 2005). *Fraxinus excelsior* is a deciduous tree species, reaching heights of up to 40 m and up to 2 m in diameter at breast height, depending on site conditions (Marigo et al. 2000). Owing to its low branch order, its crown is usually rather transparent (Rittershofer 2001). Ash leaves are imparipinnate, with 7-13 leaflets sessile on the leaf rachis (Hofmeister, 2004). The flowers are hermaphroditic and wind-pollinated. Common ash is shade tolerant in the juvenile state, while in subsequent age states it becomes light-demanding (Marigo et al. 2000).

Furthermore, common ash is described as a very site-demanding species, requiring nutrient rich, base-saturated and moist but well-drained soils (e.g. Kölling & Walentowski 2002, Kerr & Cahalan 2004, Weber-Blaschke et al. 2008). These conditions are met by calcareous soils of mountainous sites but also by alluvial stands with fresh to wet soils, which are regularly fertilized by flooding events (e.g. Rittershofer 2001). Kerr and Cahalan (2004) found that besides fresh soil moisture and a rich soil nutrient status the early growth of ash is positively affected by warm climate. While they describe ash to be sensitive to a water deficit, Marigo et al. (2000) and Rittershofer (2001) attribute ash to be highly drought tolerant. Its resistance to flooding is variable and highly dependent on age and site conditions. Diester (1981) reports an average flood tolerance of 35-40 days for ash growing in the Rhine valley.

Overall, ash shows a large ecological amplitude which is reflected by the wide range of habitats populated and also by its numerous communities. *Fraxinus excelsior* commonly occurs in groups within mixed forests whereas pure stands are rather rare (Fraxigen 2005). Despite its large ecological amplitude, the occurrence of ash is limited by the dominance of beech (*Fagus sylvatica* L.). Typical communities with ash as a main tree species are *Carici remotae-Fraxinetum*, *Pruno-Fraxinetum*, *Quercu-Ulmetum* and

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*Adoxo-Aceretum* on moist sites along watercourses and in floodplain areas and *Fraxino-Aceretum* on mountainous sites (Kölling & Walentowski 2002).

### 3.2 Stress Reaction in Plants

Lichtenthaler (1996) defines stress as “any unfavourable condition or substance that affects or blocks a plant's metabolism, growth or development”. Consequences of such conditions are reversible at first while when long-lasting or excessive they might result in permanent damage (Larcher 1987). Coordination of stress responses are principally controlled by phytohormones (Lerner 1999). The responses can be stressor specific but often are a rather general reaction to adverse conditions. Such general responses comprise e.g. changes of enzymatic activity and membranous characteristics, accumulation of antioxidants and stress metabolites, occurrence of stress hormones (e.g. ethylene), inhibition of photosynthesis, disturbance of growth, and premature senescence (Larcher 2003).

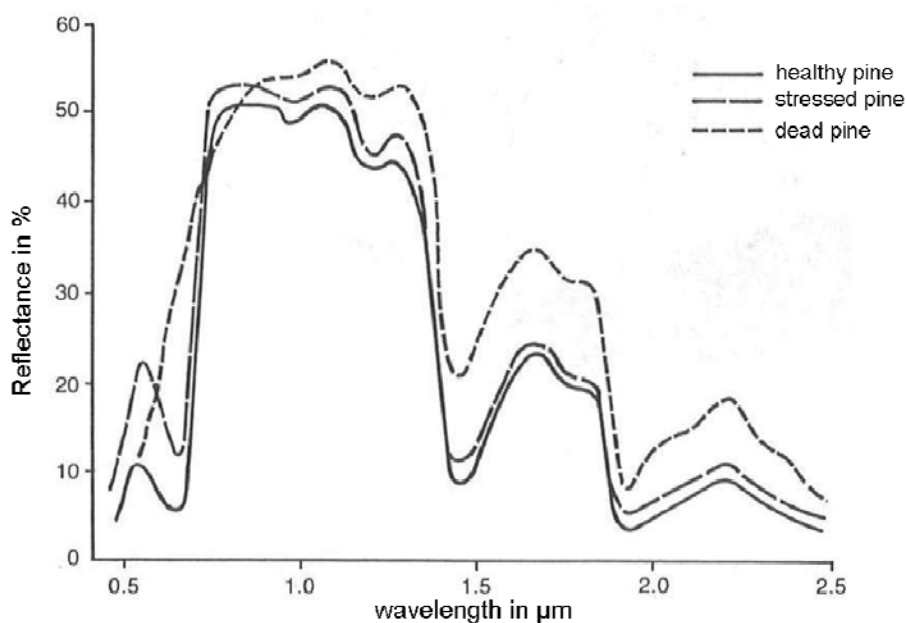
A plants short-term reaction to drought stress is the reduction of stomatal conductance and resulting inhibition of photosynthesis (Mohr & Schopfer 1995). Longer-term responses to drought stress include decreasing plant water status and resulting hardening of the cell wall (Marigo et al. 2000) or wilting, accumulation of osmotica and a certain “stress hormone” (abscisic acid), decreasing chlorophyll synthesis, stimulation of root growth while shoot growth is inhibited, reduction of protein synthesis, and finally decelerated vegetative growth (Mohr & Schopfer 1995). Another typical drought stress related mechanism is the accumulation of stress metabolites, such as proline (Lichtenthaler 1998). Due to its relatively high rate of transpiration, Kerr and Cahalan (2004) identify the growth of ash as very sensitive to drought stress.

Similar to drought stressed plants, one of the earliest symptoms of plants flooded and thus exposed to root hypoxia is a marked closure of leaf stomata (Jackson 2002), resulting in reduced rates of photosynthesis. Additionally, concentrations of chlorophyll and proteins in leaves have been found to decrease (Jäger 2008), and translocation of photoassimilates seems disturbed (Kreuzwieser 2004). Due to disturbed physiological functioning, vegetative growth is reduced and overall vitality decreases, resulting in structural damage and increased mortality rates (Kozłowski 1984; 2002). Lichtenthaler (1996) describes the accumulation of polyols (e.g. mannitol, sobitol) as characteristic at water stress conditions. For ash, Jäger (2008) found that seedlings subject to flooding

tended to shed their leaves within 10 to 14 days of inundation, and that proceeding inundation resulted in partial to total loss of green biomass whereas re-growth was reduced.

### 3.3 Spectral Reflectance of Vegetation

Radiation reaching the surface of a material may be reflected, transmitted, or absorbed (Albertz 2007). Remote sensing usually employs information derived from reflectance (or reflected radiance) properties of surface materials. Reflectance properties of vegetation are described in detail by various authors (e.g. Jackson 1986, Knapp 1994, Hildebrandt 1996, Treitz & Howarth 1999, Carter & Knapp 2001, Van der Meer et al. 2006, Kumar et al. 2006). In general the reflectance of vegetation in the visible region (400-700) is small and reflectance in the near-infrared (700-1300) is large (De Jong & Epema 2006) (see Figure 3.1).



**Figure 3.1:** Reflectance spectra of pine trees (modified from ESA Land Application Working Group (1987) in Kappas (1994))

Differences exist in reflectance at leaf level compared to reflectance at canopy scale. The most important components affecting leaf spectral properties are

- Pigmentation (chlorophyll, carotenoids)
- Internal leaf structure (arrangement of cells and aerial interspaces)
- Water content
- Surface roughness and cover (waxes, leaf hairs)

In the visible (VIS) region, leaf level reflectance is low due to strong absorptions by foliar pigments, especially chlorophyll, which absorbs violet-blue and red light for photosynthesis (Kumar et al. 2006).

Since green light is not absorbed for photosynthesis a characteristic “green peak” can be readily observed around 550 nm on reflectance spectra of healthy vegetation. Reduced absorption in the green part of the spectrum is also the reason for the green appearance of most plants. In stressed vegetation, the total chlorophyll content of leaves decreases, thus changing the proportion of light absorbing pigments and resulting in less overall absorption (Zarco-Tejada et al. 2001). While chlorophyll content is the primary factor affecting leaf reflectance in the VIS, scattering from internal leaf structure is the dominant factor controlling the spectral response of plants in the near-infrared (NIR) (Treitz & Howarth 1999). Changes in chlorophyll concentration produce spectral shifts of the green peak and of the absorption edge near 700 nm: the red edge. The red edge (680-750 nm) is a unique feature of green vegetation, resulting from the two special optical properties of plant tissue: chlorophyll absorption giving low red reflectance and high internal leaf scattering causing large NIR reflectance (Horler et al. 1983). It is considered the region most sensitive to stress induced changes, constituting a pre-visual indicator of stress (e.g. Horler et al. 1983, Rock et al. 1988, Boochs et al. 1990, Vogelmann et al. 1993, Treitz & Howarth 1999, Zarco-Tejada et al. 2001). With loss of chlorophyll pigments the red edge shifts towards the blue part of the spectrum. Dominating reflectance properties in the visible region, chlorophyll content is considered a key indicator in assessing vegetation status by many authors (e.g. Curran 1990, Carter 1993, Filella & Peñuelas 1994, Gitelson & Merzlyak 1995, Zarco-Tejada et al. 2000, Pontius et al. 2005)

Many characteristics such as carbon (sugars, starch, cellulose, and lignin), water and nitrogen mainly affect absorbance features between 1000 and 2500 nm, a spectral region not covered by the AISA Eagle sensor used in this study. Few absorption features due to

bound and unbound water occur at 970 nm. In addition to the reflection properties of leaves, reflectance of vegetation canopies is highly affected by

- Spatial distribution of vegetated and non-vegetated areas (amount of foliage)
- Leaf area index (LAI)
- Leaf angle distribution (LAD)
- Canopy geometry

This results in differing reflectance properties for different ecosystems, even though reflectance features of individual leaves are usually quite similar across species (Asner 1998). Additionally, much of the variation in spectral properties of vegetation can be attributed to viewing geometry, including angle of incidence, angle of reflection and the phase angle (Van der Meer 2006).

## 4 Material and Methods

### 4.1 Laboratory Experiment

In order to explore relationships between hyperspectral data and vegetation physiological parameters on the canopy scale a test series was conducted at the UFZ research laboratory in Bad Lauchstädt over a four month period from May 14, 2009 to September 17, 2009. Plants were grown in a shade house and hyperspectral measurements were performed in a dark room in artificial illumination conditions. This approach offered the advantages of controlled environmental conditions and constant sampling and illumination conditions over the entire experimental period.

### 4.2 Plant and Soil Material

Loamy soil material was taken from the Mulde floodplain near Sollnitz, dried to 15% water capacity and homogenised. The soil material was then filled in nine 80x80x50 cm steel containers. During filling, two Theta probes ML2x (DELTA-T DEVICES, CAMBRIDGE, UK) were placed in six of the containers in a depth of 30 cm and 15 cm, respectively.

Two year old *Fraxinus excelsior* seedlings with a size of approximately 30 to 50 cm were purchased on May 7, 2009 and planted in the containers prepared previously. Nine seedlings per container formed a vegetation canopy of approximately 60 x 60 cm. The containerized seedlings were placed in the outdoor shade house of Bad Lauchstädt research laboratory, where they were sheltered from rain by an automatically extendable roof.

### 4.3 Treatment Levels

All containers were watered to a water capacity of 60 % which is considered as optimum condition for a period of four weeks. Water capacity was calculated from soil dry weight and soil weight after 24 hours of water saturation. From June 6, two stress scenarios were applied to six of the containers whereas three drought stress containers were left without watering for four weeks and three flooding containers were watered to a water capacity of 120 % (flooded) for three weeks. Three containers were kept at 60 % water capacity as controls for the entire experimental period (see Table 4.1).



After ending the stress treatments the seedlings were left to recover for a period of five weeks (drought stress) and eight weeks (flooding) before the same stress conditions as described above were applied again (see Table 4.1).

**Table 4.1:** Water conditions in stress scenarios during experiment(\*data in percent water capacity)

Month	Week	Control	Drought stress	Flooding
May	20	60*	60	60
	21	60	60	60
	22	60	60	60
June	23	60	60	60
	24	60	-	<b>120</b>
	25	60	-	<b>120</b>
	26	60	-	<b>120</b>
July	27	60	-	60
	28	60	60	60
	29	60	60	60
	30	60	60	60
August	31	60	60	60
	32	60	60	60
	33	60	-	60
	34	60	-	60
September	35	60	-	<b>120</b>
	36	60	-	<b>120</b>

#### 4.4 Measurement Procedures

##### 4.4.1 Vegetation Parameter Sampling

For determination of plant physiological status, chlorophyll content, leaf area index (LAI), plant height, leaf water content, C and N content and soil moisture measurements were conducted on a semi-weekly base.

Relative chlorophyll content was measured using Minolta SPAD 502 Chlorophyll meter (SPECTRUM TECHNOLOGIES, PLAINFIELD, IL, USA) on a number of 3 mature leaves per container per sampling day. Dimensionless SPAD-502 values were calibrated to total chlorophyll content (mg / g), following an approach by Markwell et al. (1995). Total chlorophyll content was determined according to a method described by Lichtenthaler (1987) on a number of 50 leaf samples collected from *Fraxinus excelsior* trees in Leipzig on four occasions from June to September, 2009. Three LAI values per container were measured using LAI 2000 Plant Canopy Analyzer (LI-COR, LINCOLN, NE, USA). Plant height was measured from the top of the highest and smallest plant per container using a folding rule. Additionally, average height of the canopy was estimated. Five

volumetric soil moisture values were measured in the top 5 cm using Infield 7 (UMS, MUNICH, GERMANY). Soil moisture data provided by the ML2x probes was logged hourly. All single values measured were averaged to one value per sampling day.

Three containers – one per scenario - were used for biomass sampling only, in order to assess leaf water content and C and N content. Three leaf samples per sampling container were collected weekly and dried at 105 °C. Percent Leaf water content was calculated by the difference of fresh and dry leaf weights. Total contents of C and N were determined from the dried, ground leaf samples using an elemental analyzer (TRUSPEC CHN, LECO INSTRUMENTS LTD., ST JOSEPH, MI, USA).

#### **4.4.2 Hyperspectral Data Sampling and Processing**

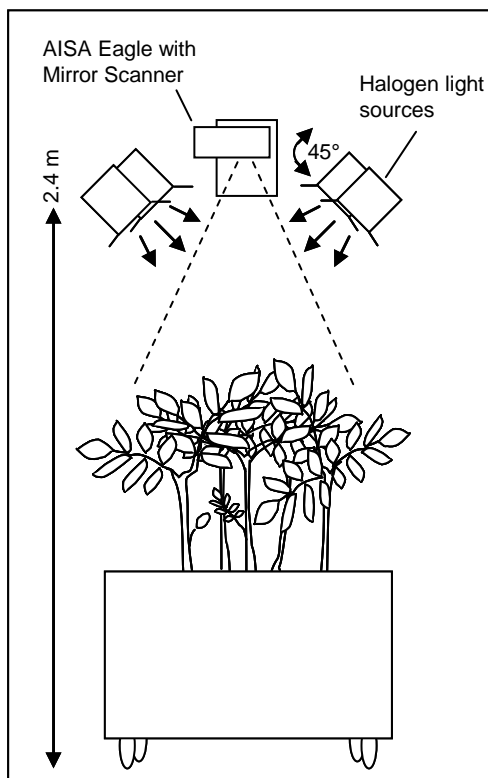
Hyperspectral canopy reflectance measurements were conducted in a dark room facility in Bad Lauchstädt research laboratory along with vegetation parameter acquisition. Illumination was provided by four 2000 W quartz tungsten halogen lamps (KAISER STUDIOLIGHT, KAISER FOTOTECHNIK, BUCHEN, GERMANY) installed at the altitude of 2.4 m above ground level at 45° inclination to both sides of the AISA sensor. The AISA Eagle sensor (SPECTRAL IMAGING LTD., OULU, FINLAND) is a hyperspectral pushbroom type imaging spectrometer and commercially available. The sensor system was installed at a height of 2.4 m and equipped with a mirror scanner to allow for hyperspectral scanning over a stationary target in the laboratory (see Figure 4.1). An arrangement of four fans provided cooling of the illumination-sensor-unit.

AISA Eagle was operated in a hyperspectral mode at spectral and spatial binning 2, thus collecting 252 spectral channels in the visible and near infrared range from 400-970 nm with a bandwidth of 2.5 nm and 3 mm spatial resolution.

In addition to AISA imaging spectrometer data, non-imaging hyperspectral data was collected using ASD FieldSpec 3 (ANALYTICAL SPECTRAL DEVICE, INC., BOULDER, CO, USA). The ASD spectrometer acquires 2150 channels in the 350-2500 nm portion with a bandwidth of 1.4 nm in the 350-1050 nm region and 2 nm in the 1000-2500 nm region. Three reflectance spectra, and from June 18<sup>th</sup> additionally three radiance spectra consisting of 25 samples each were taken from a height of 50 cm above canopy level (with a FOV of 25°), thus representing a plot of 160 cm<sup>2</sup>. White reference alignments using a White Spectralon Panel (LABSPHERE INC., NORTH SUTTON, NH, USA) were re-

peated prior to every reflectance measurements in order to account for differences in instrument or light conditions over time.

In order to check for effects of changing canopy architecture and shadows, a special test was conducted with AISA Eagle on August 13, where all containers were sampled in additional positions. These positions included displacement of the container (20 cm and 40 cm, respectively), tilting of the container (15 cm and 20 cm, respectively) and turning of the container (90°) as compared to the standard orientation.



**Figure 4.1:** Experimental setup in the dark room

Imaging AISA raw data was calibrated to spectral radiance using SPECIM CaliGeo 4.9.5, a software package for radiometric correction of AISA raw data provided by SPECTRAL IMAGING LTD. to run under ENVI 4.6.1 (ITT VISUAL INFORMATION SOLUTION, BOULDER, CO, USA). Radiometric correction is carried out to account for the dark current of the instrument, define the spectral separation of the channels, and to translate raw radiance to spectral radiance. Often, the process of radiometric correction is used to derive spectral reflectance, defined as the ratio of the radiant energy reflected from a surface to the radiant energy incident on the surface (Analytical Spectral Device 2006). Reflectance is a dimensionless measure independent of changing illumination and atmospheric conditions (Kumar et al. 2006) and thus a good measure for comparing

hyperspectral data taken at differing environmental conditions. Each AISA image was then co registered to a master image using ENVI 4.6.1 to ensure the comparability of image sections for subsequent analysis. A 50 x 50 cm spatial subset was cut from the centre of each co registered image and a K-means unsupervised classification with two classes was performed to obtain subsets consisting of vegetation pixels only, thus minimizing background and shadow effects. Both spatial and classified subsets were used for subsequent analyses to allow for investigation of these effects.

For ASD data, the instrument automatically performs radiometric correction and relative reflectance is automatically calculated from reflectance of the White Spectralon Panel. ASD spectra per plot were averaged using ViewSpecPro 4.05 software (ANALYTICAL SPECTRAL DEVICE, INC., BOULDER, CO, USA) and spectrally resampled to AISA spectral range (400-970 nm) and bandwidth (2.5 nm) using ENVI 4.6.1.

A number of 34 vegetation indices known to be sensitive to plant stress and according changes of various vegetation parameters were calculated for both types of AISA subsets as well as for spectrally resampled ASD data (see Table 4.2). For AISA subsets, the index value used for subsequent analysis is an average of all pixels of a sub scene.

**Table 4.2:** List of existing vegetation indices included in the analysis that are known to have relationships with (stress-specific) physiological responses

Index-Name	Formula	Citation
<b>Greenness indices - Canopy Level</b>		
NDVI 800/680	$(R800-R680)/(R800+R680)$	<i>Rouse 1974; Pontius et al. 2005</i>
NDVI 800/670	$(R800-R670)/(R800+R670)$	<i>Rouse 1974; Haboudane et al. 2004</i>
NDVI 858/648	$(R858-R648)/(R858+R648)$	<i>Chen et al. 2005</i>
NDVI 750/705	$(R750-R705)/(R750+R705)$	<i>Gitelson &amp; Merzlyak 1994; Sims &amp; Gamon 2002</i>
mND	$(R750-R705)/(R750+R705-2R445)$	<i>Datt 1999; Sims &amp; Gamon 2002</i>
RNDVI	$(R780-R670)/(R780+R670)$	<i>Raun et al. 2001; Babar et al. 2006</i>
RDVI	$(R800-R670)/\sqrt{(R800+R670)}$	<i>Rougean &amp; Breon 1995; Haboudane et al. 2004</i>
DVI	$R800/R680$	<i>Jordan 1969; Sims &amp; Gamon 2002</i>
SR 900/680	$R900/R680$	<i>Aparicio et al. 2000</i>
SR 750/705	$R750/R705$	<i>Sims &amp; Gamon 2002</i>
MSR	$((R800/R670)-1)/\sqrt{((R800/R670)+1)}$	<i>Chen 1996; Haboudane et al. 2004</i>
GNDVI	$(R780-R550)/(R780+R550)$	<i>Gitelson et al. 1996b; Babar et al. 2006</i>
mSR 680	$(R800-R445)/(R680-R445)$	<i>Datt 1999; Sims &amp; Gamon 2002</i>
mSR 705	$(R750-R445)/(R705-R445)$	<i>Datt 1999; Sims &amp; Gamon 2002</i>
<b>Water indices</b>		
WBI	$R970/R900$	<i>Penuelas et al. 1993; Penuelas et al. 1992</i>
WI	$R900/R970$	<i>Penuelas et al. 1995; Penuelas et al. 1997b</i>
NWI 1	$(R970-R900)/(R970+R900)$	<i>Babar et al. 2006</i>
NWI 2	$(R970-R850)/(R970+R850)$	<i>Babar et al. 2006</i>

**Table 4.3** (continued from page 14)

<b>Index-Name</b>	<b>Formula</b>	<b>Citation</b>
<b>Light use efficiency and senescence</b>		
PRI	$(R531-R570)/(R531+R570)$	<i>Gamon et al. 1992; Penuelas et al. 1997a</i>
SIPI	$(R800-R445)/(R800+R680)$	<i>Penuelas et al. 1995</i>
NPCI	$(R680-R430)/(R680+R430)$	<i>Penuelas et al. 1992</i>
PSRI	$(R680-R500)/R750$	<i>Merzlyak et al. 1999; Sims &amp; Gamon 2002</i>
<b>red edge indices - very sensitive to stress, less influenced by differences in green leaf biomass and background</b>		
Curvature Index	$R683^2/(R675 \cdot R691)$	<i>Zarco-Tejada et al. 2001; Pontius et al. 2005</i>
R750/R710	$R750/R710$	<i>Zarco-Tejada et al. 2001</i>
Vogelmann 1	$R740/R720$	<i>Vogelmann et al. 1993, Zarco-Tejada et al. 1999, 2001</i>
GM 2	$R750/R700$	<i>Gitelson&amp;Merzlyak 1994a,b, Gitelson et al. 1996a</i>
Vogelmann 3	$(R734-R747)/(R715+R720)$	<i>Vogelmann et al. 1993, Zarco-Tejada et al. 1999, 2001</i>
Vogelmann 2	$(R734-R747)/(R715+R726)$	<i>Vogelmann et al. 1993, Zarco-Tejada et al. 1999, 2001</i>
GM 1	$R750/R550$	<i>Gitelson&amp;Merzlyak 1994a,b, Gitelson et al. 1996a</i>
CMS	$R695/R760$	<i>Carter &amp; Miller 1994, Zarco-Tejada et al. 1999, 2001</i>
R695/R670	$R695/R670$	<i>Carter 1993</i>
R605/R760	$R605/R760$	<i>Carter 1993; Pontius et al. 2008</i>
R710/R760	$R710/R760$	<i>Carter 1993; Pontius et al. 2008</i>

#### 4.5 Statistical Analysis

There is no standard method to deal with hyperspectral data and the complex experiment design further complicated statistical analysis. Since all data was sampled in the form of a time series reusing the same sampling containers, the assumption of independence of observations was violated, leading to temporal autocorrelation. Unconsidered (temporal) autocorrelation may result in inefficiency of analysis (Backhaus et al. 2008) and misinterpretation of relationships between variables (Bahrenberg et al. 1992). In addition, the experiment design with 6 sampling containers and 33 repeated measures excluded conventional analytical methods on repeated measures data, such as SPSS functions “General Linear Model – repeated measures” or “Linear Mixed Model – repeated measures”, as with only 6 sampling containers the number of repeated measures to be entered is limited to 4 by the number of degrees of freedom. Separate analysis of 4 repeated measures at a time is not reasonable with regard to the content and would only contain very limited data anyway.

To still detect some basic structure of the data it was visualized over the course of the experiment. As it became evident that the unclassified subsets of the AISA data were dominated by the proportion of soil in the subset they were excluded from further analysis.

As the visualization of vegetation indices further suggested a high degree of redundancy, a hierarchical cluster analysis was performed using varclus procedure in “R”. The hierarchical cluster analysis is counted among exploratory procedures in multivariate data analysis (Backhaus et al. 2008). It can be used for assessing redundancy and for separating variables into clusters so that a variable resigned to a cluster is representative of that group. Thus, it results in data reduction. The clustering was based on squared Spearman correlation coefficients as similarity measures, and agglomeration was performed using complete linkage method. Due to the resulting clustering of indices in 3 major groups and a few “single” indices, four indices determined to be most pertinent to the current study – one representative of every group and one “single” index - were selected and analysed in greater detail. These indices are NDVI 670, PRI, Vogelmann 2 and WI.

To compare the variation among scenarios and containers, plant physiological and soil moisture data was further examined graphically using Box plots and statistically by conducting analysis of variance using PASW Statistics 17 and STATISTICA. It should be noted that due to the facts that ANOVA compares means and variances of a dataset and that its assumption of independence of observations is violated, it is no ideal method in this case and may only provide a rough idea of variation. Parameters meeting the assumptions of normal distribution and homogeneity of variance were analysed using a one-way ANOVA and following Turkey-Test ( $p < 0.05$ ). Parameters violating the assumption of homogeneity of variance were tested using Brown & Forsythe and Welch-Test (Fields 2006) and following Games-Howel-procedure (Fields 2006) alternatively. For a parameter meeting none of the assumptions mentioned above (plant height), a non-parametric Kruskal-Wallis-H-Test followed by multiple comparisons using Man-Whitney-U-Test was performed. All tests were conducted at a significance level of  $p < 0.05$ .

Relationships between plant physiological parameters and vegetation indices were analysed using Spearman’s rank correlation (STATISTICA). Furthermore, the relationships were analysed using a Generalized Additive Model (GAM) in “R”. The Generalized Additive Model is an extension of the Generalized Linear Model (GLM) that incorpo-

rates the flexibility of nonparametric regression (Hastie & Tibshirani 1990) by the use of smooth functions. A smooth function is a tool for summarizing the trend of a response measurement  $Y$  as a function of one or more predictor measurements  $X_1, \dots, X_p$ , producing an estimate of the trend that is less variable than  $Y$  itself (Hastie & Tibshirani 1990). Permission of absolutely any smooth functions in model fitting would invariably result in complex overfitting. Thus, Generalized Additive Models are usually controlled by adding a “wiggleness” penalty to the fitting objective (Wood 2006). Using the `mgcv` implementation of GAM in “R”, smooth terms are represented using penalized regression splines. Thereby, the degree of smoothness of model terms is estimated as part of the fitting, with the number of smoothing parameters (“least complicated model with best fit”) being selected by Generalized Cross Validation.

Since no reasonable relationship was expected for vegetation indices and plant height, this parameter was excluded from analysis using GAM. Another parameter removed from analysis was soil moisture due to suspected cross-correlation effects.

Additionally, effects of time, treatment and container on the relationship between plant physiological parameter and vegetation indices were determined using GAM. Statistically significant improvement of one model over the former was tested using ANOVA in “R”.

The ability of vegetation indices to detect differences between treatment groups was not tested due to several reasons. At first, there exists no adequate model to account for treatment level separation yet. Secondly, the examination of soil moisture values provided by the ML2x probes suggests that the plants were not exposed to drought stress to any time of the experiment. Plants exposed to flooding shed most of their leaves, an effect that dominates all vegetation indices calculated. There is no purpose in attempting to detect differences between treatment levels in this context.

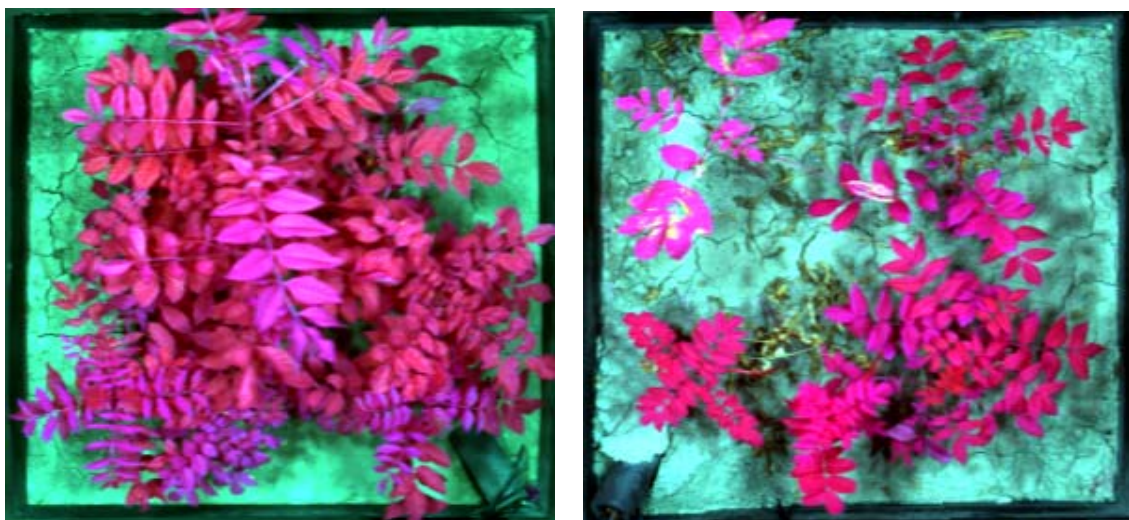
## 5 Results

### 5.1 Plant Physiological and Soil Moisture Analysis

#### 5.1.1 Optical Observations during the Course of Experiment

Observation of sampling containers during the course of experiment revealed no optical differences between control and drought stressed plants. In all of the 4 containers, growth rates of up to 50 cm, increasing development of the canopy and optically high vitality of plants were recorded. Weighing of the plants before and after the experiment indicated a weight gain of approximately 115 % to 170 % for both, control and drought stressed plants. Excavation of the plants after finishing the experiment revealed differences in root development though. The root system of drought stressed plant appeared to be more intense and deeper rooted than the root system of control plants.

In contrast, the plants exposed to water stress shed most of their leaves within 14-20 days of flooding (see Figure 5.1) and recovered only slowly and incomplete during progression of the experiment. The average weight gain per container of water stressed plants was only 24 % to 34 %. When excavated, the roots of flooded plants exposed signs of oxygen deficiency such as a loss of fine roots and dead coarse roots. By the end of experiment, most plants were developing new leaves as well as new roots and adventitious roots within the flooded stem section to compensate for the loss of original roots.



**Figure 5.1:** Colour-infrared (CIR) images taken with AISA Eagle on August 20, 2009. Left: control container. Right: flooding container after loss of foliage



### 5.1.2 Soil Moisture

Figure 5.2 depicts soil moisture values measured in the course of the experiment. Values taken in the top 5 cm clearly indicate drought and water stress scenarios with corresponding water ratios. Variations during the recovery period arise from irregularities in time span between watering and sampling. Regarding water stress, the two repeated inundations are well detectable in a depth of 15 cm and 30 cm, respectively. Drought stress is less well developed in a depth of 15 cm, and in a depth of 30 cm, the containers left without watering are obviously more humid than controls for most of the experimental period. Apparently, only a different stratification of soil moisture could be realized in the unwatered containers. Implementation of drought stress failed, which is primarily attributed to the very loamy consistence of the soil material.

### 5.1.3 Plant Physiological Parameters

In addition to soil moisture values, Figure 5.2 shows plant physiological data sampled in the course of the experiment.

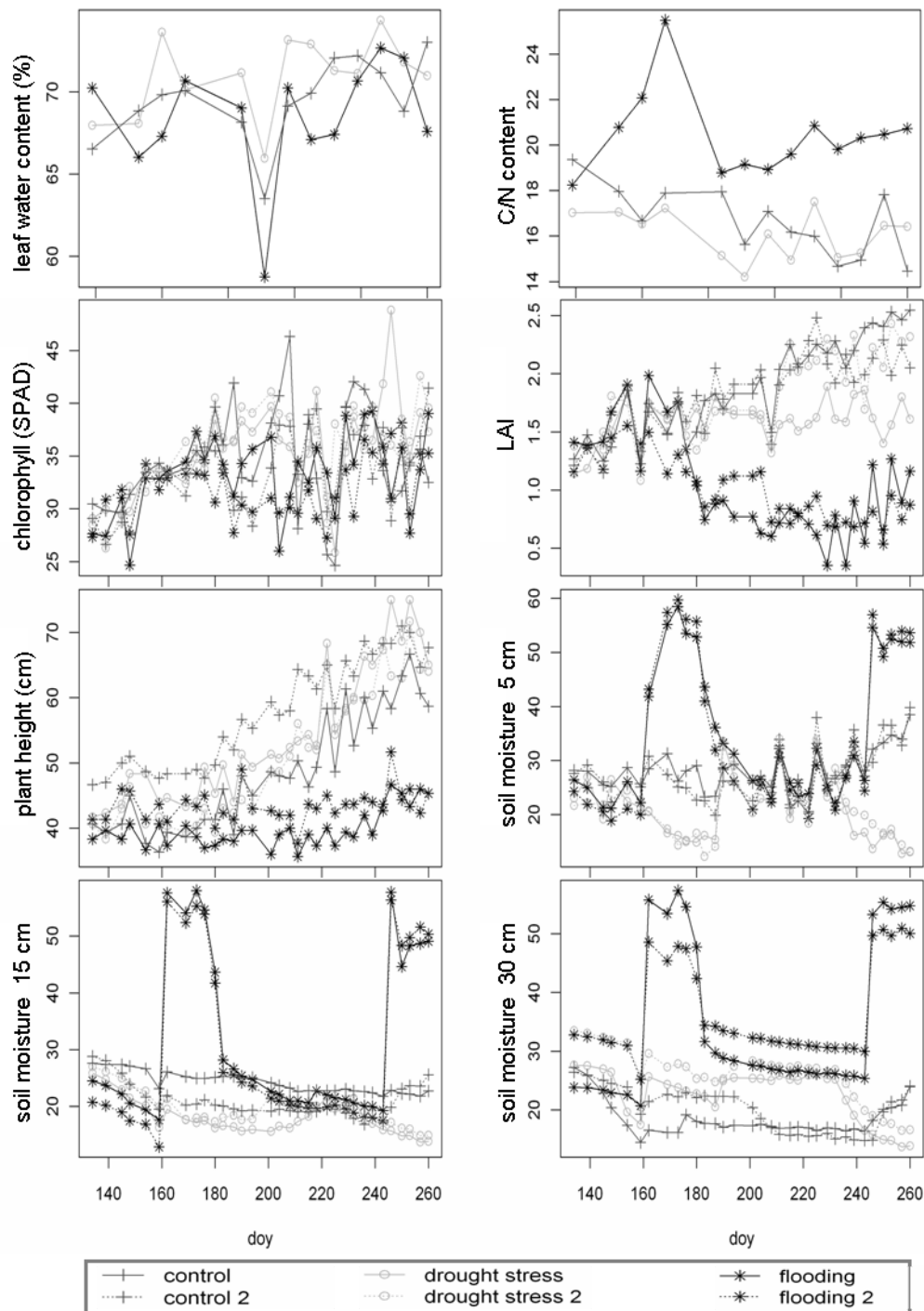
Chlorophyll values show great variation for all containers which is most probably due to the small number of chlorophyll values that the average was calculated from. General trends to be observed from Figure 5.2 are a tendency of chlorophyll values to increase over the experimental period as well as slightly lower values for chlorophyll content in flooded plants.

While LAI increases continuously in both control containers and in one drought stress container in the course of the experiment, it decreases in both flooded containers as a result of the leaf loss until approximately day 230, when the plants started to recover and LAI increases slightly.

The effects of flooding on plant height growth are similar: while plants in control and drought stress containers prosper, plants exposed to flooding only start to show limited height growth to the end of the experiment. Overall, differences in height observed at planting remain until the end of experiment.

Since biomass was sampled only once a week from special sampling containers there is a very limited number of values available for analysis. In addition, the 13 samples per treatment were taken over a period of three month, increasing the influence of external factors such as temperature, humidity and irradiance as possible sources of error. C / N-values derived from the biomass samples show a clear distinction from flooded contain-

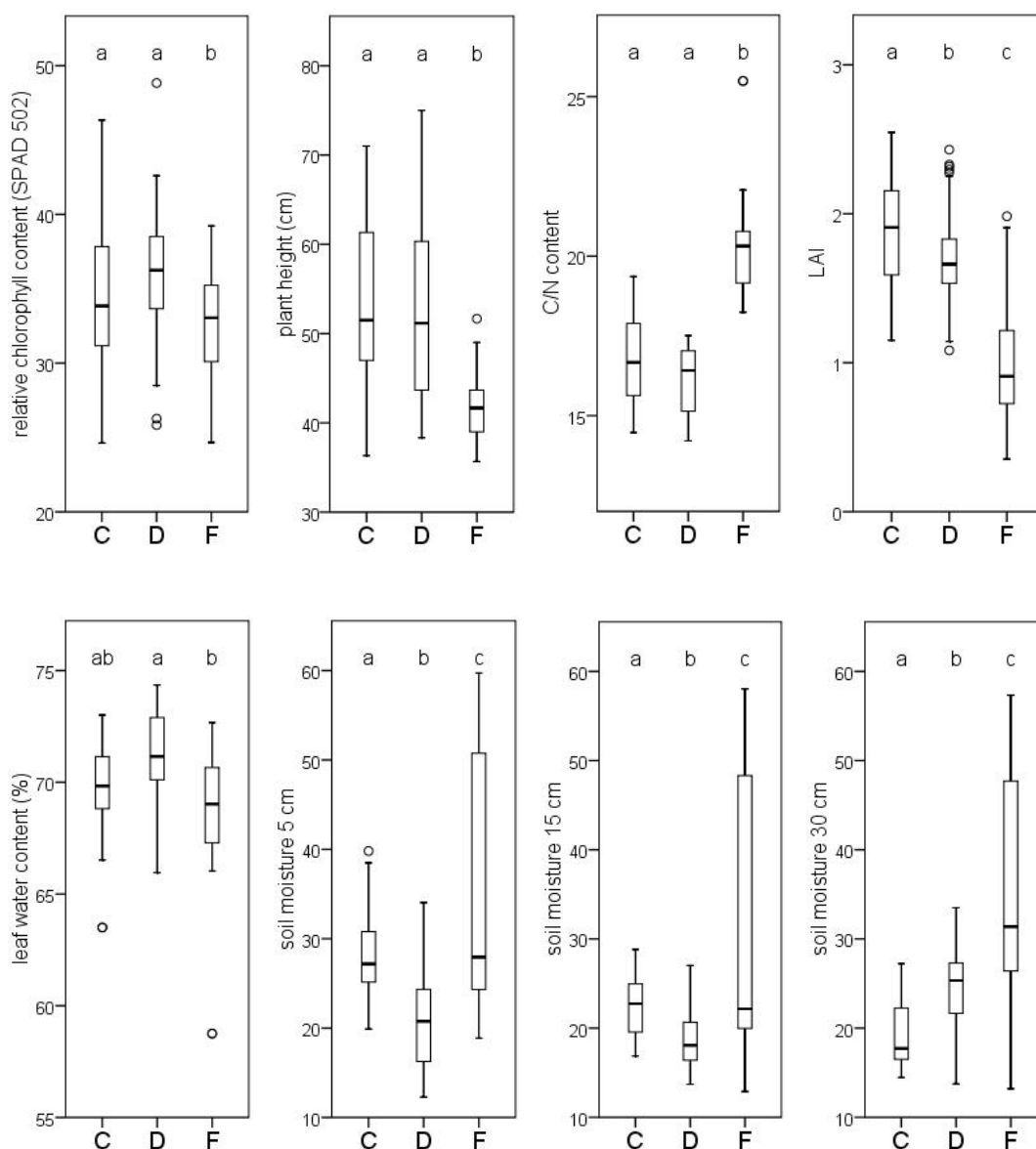
ers and control and drought stress treatments, which, again, are very similar. Leaf water content tends to be slightly lower in flooded containers compared to controls, whereas in drought stress it tends to be slightly higher.



**Figure 5.2:** Plant physiological and Soil moisture data

### 5.1.4 Box Plots and Analysis of Variance

Box plots and results of ANOVA are in general accordance with observations from time series diagrams (see Figure 5.3). There are no significant differences between control and drought stress treatment regarding chlorophyll, height growth and C / N values whereas chlorophyll and height growth are significantly lower and C / N values are significantly higher in water stressed containers.



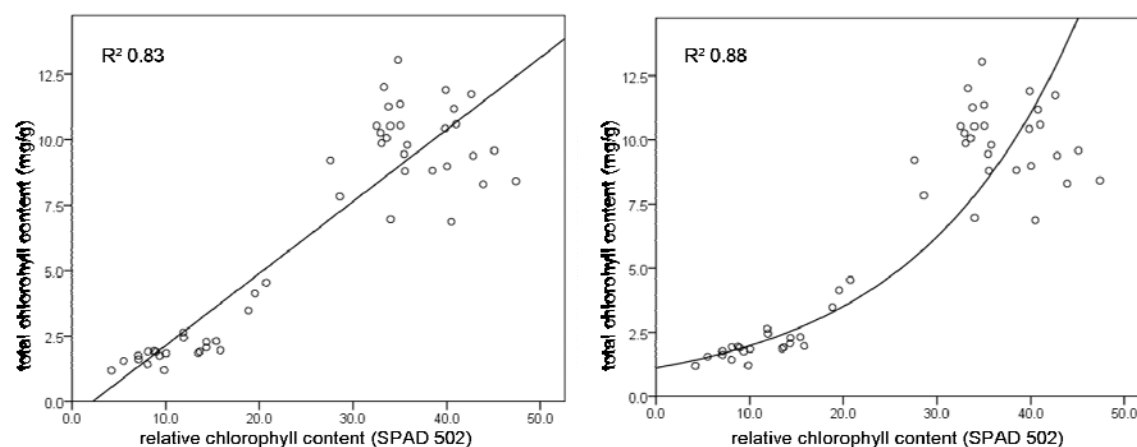
**Figure 5.3:** Differences of plant physiological and soil moisture parameters between treatment levels. C=control treatment, D=drought stress treatment and F=flooding treatment. Box plots show the median (central line), interquartile range (box), maximum and minimum (lines above and below the box), and outliers (circles). Characters over the box plots (a, b, c) indicate classification into groups according to analysis of variance.

LAI values are significantly different in all treatment levels whereas the difference between control and drought stress is strongly influenced by the one drought stress container with stagnating canopy development. Though differences in leaf water content among treatments seemed marginal, leaf water content was found to be significantly higher in drought stressed leaves than in water stressed ones, both being not significantly different from controls.

When it comes to observation of single containers instead of treatment levels, most of this clear distinction vanishes. This is considered a consequence of ANOVA being an inappropriate tool for analysis in this case, thus analysis should not go too much into detail.

### 5.1.5 SPAD Calibration

SPAD 502 relative chlorophyll values were calibrated to total chlorophyll content (mg/g) in order to verify the exact functioning of the SPAD 502 instrument. As shown in Figure 5.4, significant correlations were found between relative SPAD chlorophyll values and extracted total chlorophyll content. This correlation was even stronger when curve fitting was performed using an exponential function as proposed by Markwell et al. (1995). These results endorse the fast and non-destructive use of SPAD 502 in order to accurately assess chlorophyll content.

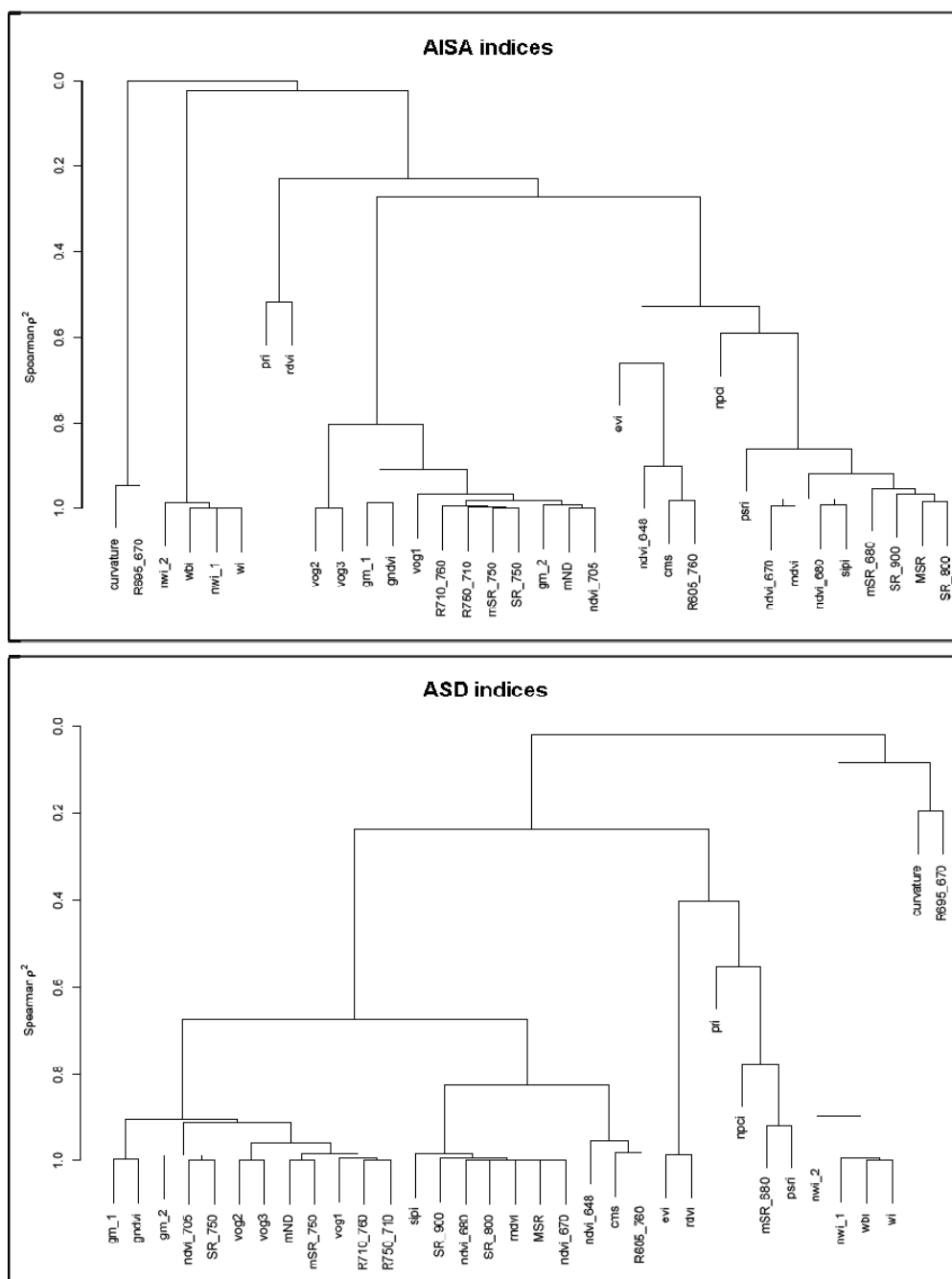


**Figure 5.4:** Correlation of relative chlorophyll content measured with SPAD 502 and total chlorophyll content (mg / g).

## 5.2 Hyperspectral Data

### 5.2.1 Cluster Analysis

The dendrogram shown in Figure 5.5 depicts the results of the cluster analysis performed on the data. For both, ASD and AISA data, vegetation indices are clustered into three larger groups consisting of mainly the same indices.



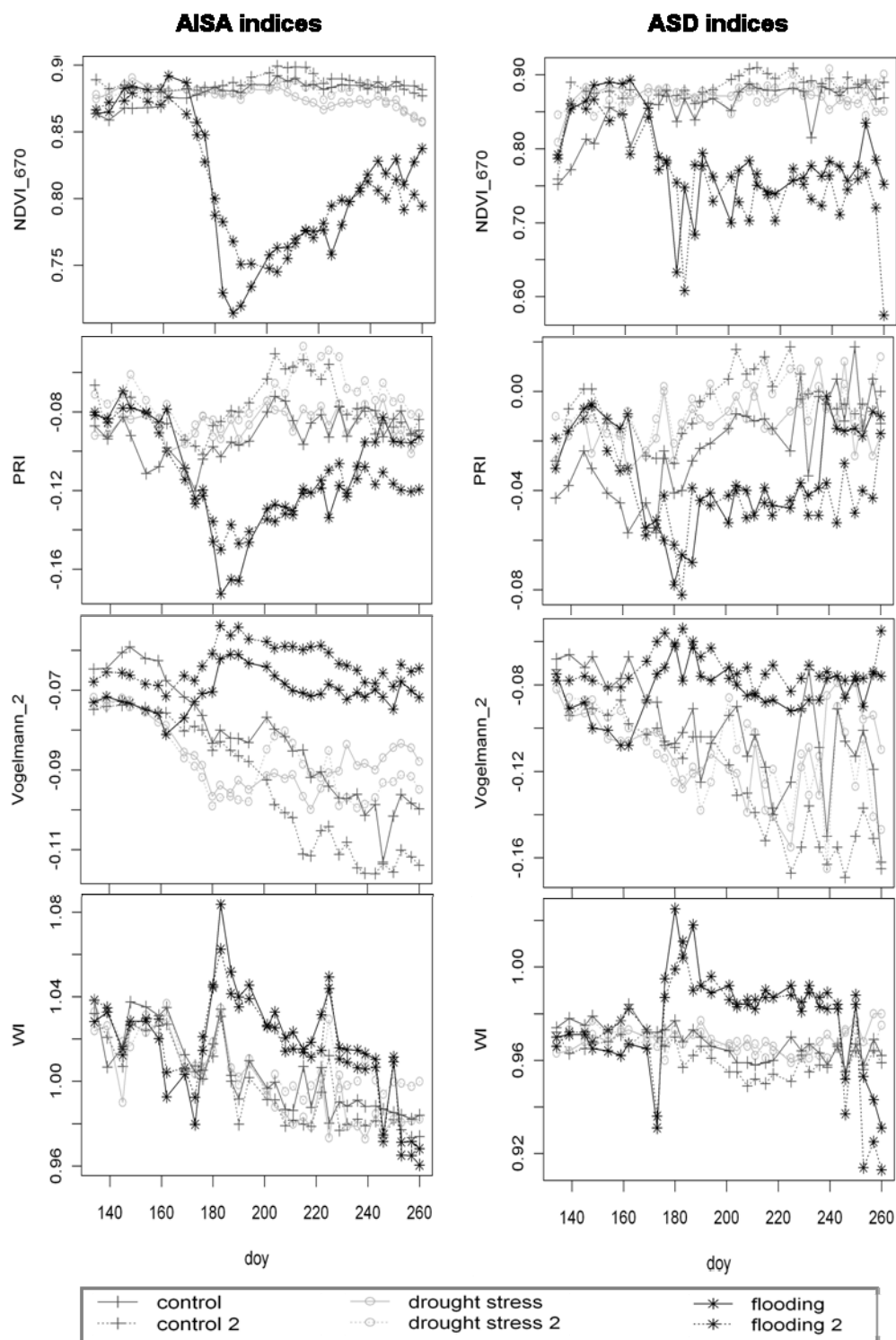
**Figure 5.5:** Dendrogram depicting the results of hierarchical cluster analysis for AISA and ASD data. Grouping results from similarity measures (squared Spearman correlation coefficient)

A small number of indices seem to be very dissimilar and were not allocated to a cluster – these are mainly the same indices for ASD and AISA. By comparing the clusters it becomes evident that they are, apparently, grouped rather by the wavelengths they were calculated from than by their designated use. Despite all differences between data collected by AISA and ASD, indices calculated from the individual instruments seem to be very similar in their structures.

### **5.2.2 Vegetation Indices**

Four vegetation indices selected based on results of the hierarchical cluster analysis are presented in Figure 5.6. The indices calculated from AISA and ASD data show similar trends over the course of the experiment. Whereas drought stress and control treatments are rather similar in all indices, water stress treatments are readily distinguishable. NDVI 670, PRI and Vogelmann 2 seem to react strongly to reduced biomass, while WI corresponds to increased water levels in the containers during flooding periods.

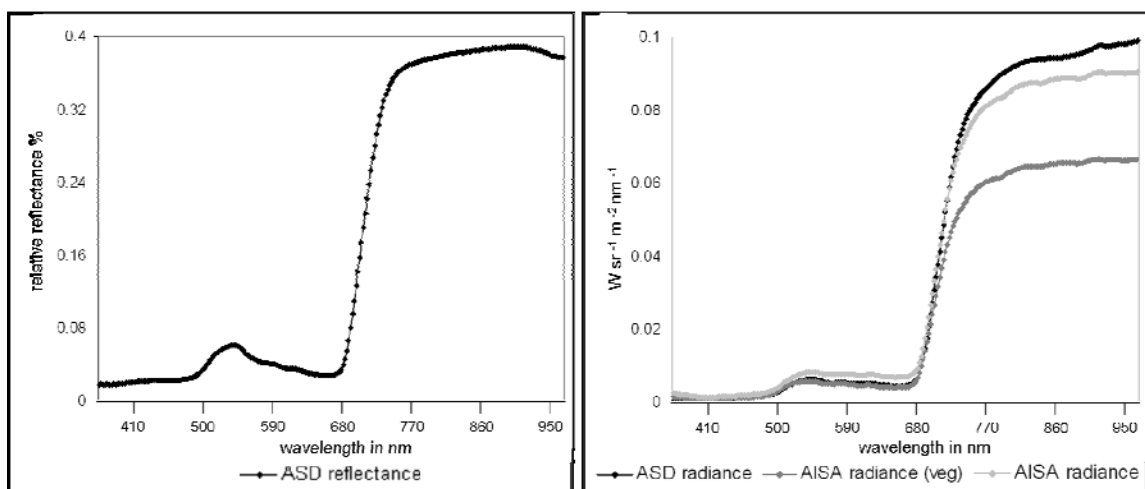
The higher degree of fluctuation of ASD data is mainly due to data acquisition without standardized setup, thus reflectance was measured manually from somewhat differing positions. In addition, the different size of sampled sections (ASD ca. 160 cm<sup>2</sup>; AISA ca. 2500 cm<sup>2</sup>) should be considered when comparing indices calculated from AISA and ASD data. While the ASD spectrometer was aimed at vegetation covered parts only, the AISA sensor simply recorded a 50 x 50 cm subset consisting of vegetation and soil. Though a classification was performed on AISA data to obtain subsets consisting of vegetation pixels only, the reduction in green biomass as well as recovery of flooded containers are well visible. This is most likely due to boundary effects of the classification.



**Figure 5.6:** Selection of vegetation indices and their developing over the course of experiment

### 5.2.3 Comparison of AISA and ASD Radiance and Reflectance Curves

A visual comparison of radiance measured with AISA Eagle and radiance and reflectance measured with the ASD spectrometer shows a generally good comparability of AISA and ASD data regarding the radiance curve (see Figure 5.7). Radiance in the near-infrared portion of the spectrum is lower for vegetation pixels only (AISA radiance (veg)) than for pixels influenced by background and shadow effects (AISA radiance and ASD radiance). Though describing the same general trends, the classic green peak feature seems more pronounced in the reflectance curve (ASD reflectance).



*Figure 5.7: ASD reflectance curve (left) and radiance curves from both instruments (right).*

### 5.2.4 Effects of Canopy Architecture and Shadows on AISA Data – Test

The results of this test, displayed in Figure 5.8, show that the effects of exposure and shadows are minor compared to between-container effects. Only a displacement of 40 cm and tilting of 20 cm appear to have a real impact on some of the containers, especially the second drought stress container, which was observed to have a very heterogeneous canopy structure. Thereby, the indices are all affected differently, with NDVI being most stable and WI the only index reacting to changes in orientation of flooding containers.



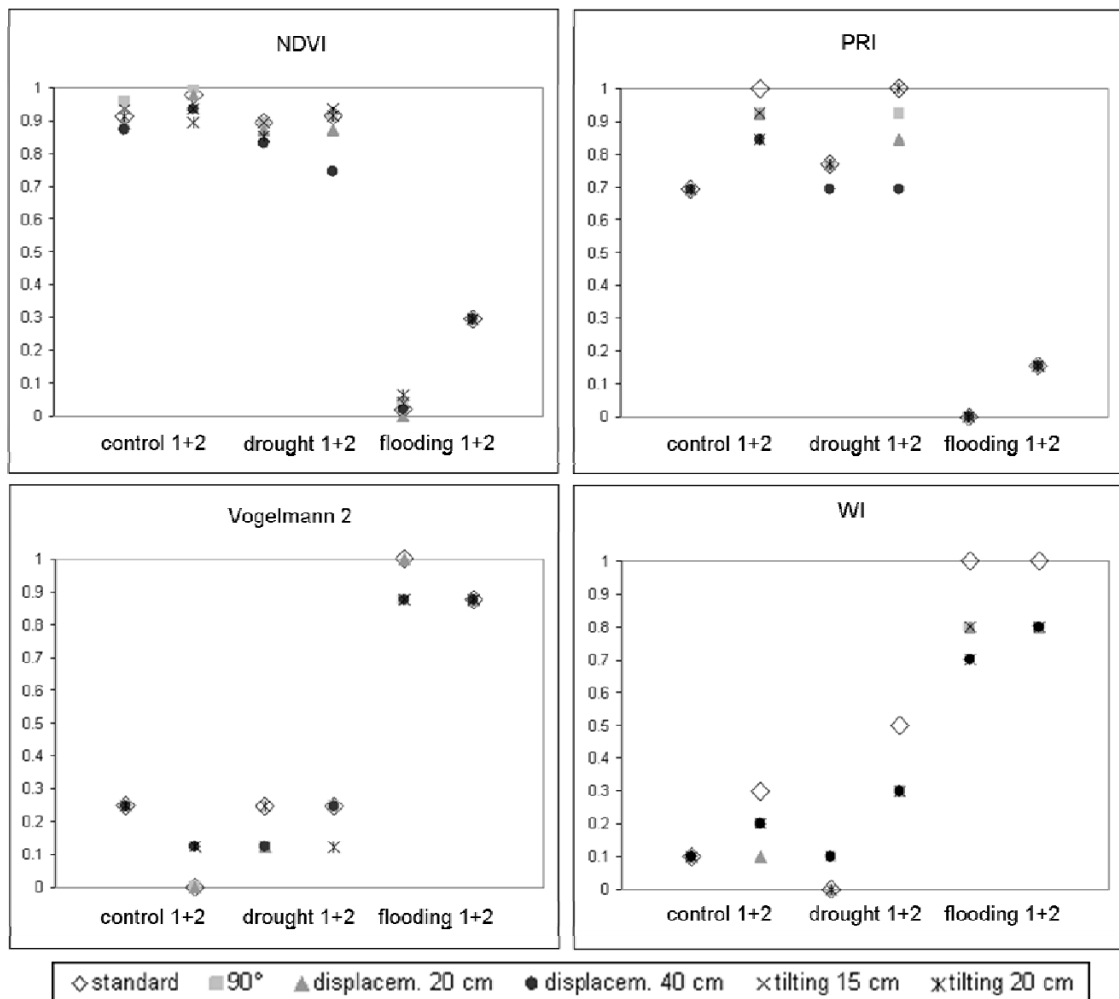


Figure 5.8: Results of test on effects of canopy architecture and shadows on AISA data

## 5.3 Relationships between Plant Physiological Parameters and Vegetation Indices

### 5.3.1 Generalized Additive Model

The results derived from the Generalized Additive Model suggest a medium degree of correlation between most of the 4 vegetation indices selected and the plant physiological parameter C / N, and a slightly lower degree of correlation between indices and LAI (see Table 5.1). Correlation between indices and chlorophyll and plant water content is minor and partly non-existent. These findings are similar for ASD and AISA data. NDVI performs very well in both datasets whereas WI seems to perform somewhat better on ASD data and Vogelmann 2 and PRI on AISA.

These results are in general accordance with findings of Spearman's rank correlation (results not shown).

Table 5.1 shows GAM results with “time”, “treatment” and “container” as co variables. Regarding AISA data, “time” seems to have a strong effect on the relationships in focus, and dramatically improves  $R^2$ . Especially for WI, inclusion of “treatment” as another co variable brings significant improvement to all models. The positive effect of “treatment” on model performance is still evident concerning PRI and LAI and C/N, and Vogelmann 2 and LAI, while NDVI seems hardly affected by treatment levels. “Container” adds little contribution to the model with the only significant – but marginal – improvement being of the relationship between indices and LAI.

These findings are generally very similar to the results derived for ASD data. While “time” seems to add a lot of explanation to the Model, the effect of “treatment” is limited and results are indifferent, and “container” does not contribute much at all. Unlike for AISA data, where WI was the index influenced most strongly by “treatment”, the ASD index most affected by this co variable is Vogelmann 2.

The high contribution of “time” to the model fit is consistent even when it is added as the last co variable, after “treatment” and “container”. Only for LAI, the contributions of “time” and “treatment” seem rather equal and “container” seems to have an effect.

**Table 5.1:** Relationships of AISA and ASD data with plant physiological parameters. The first column shows  $R^2$  for the relationship of hyperspectral data and physiological parameter only, the second column shows  $R^2$  with contribution of “time” and third and fourth columns with extra contribution of “time” and “treatment” and “time”, “treatment” and “container”, respectively. Significant improvement of one model type over the former was tested using ANOVA. Significance levels are indicated as follows: ‘\*\*\*’ = 0.001, ‘\*\*’ = 0.01, ‘\*’ = 0.05, ‘.’ = 0.1.

<b>AISA</b>				
	phys. parameter only	phys. parameter +time	phys. parameter +time +treatment	phys. parameter +time +treatment +container
<b>leaf water content</b>				
Vogelmann 2	0.12	0.47***	0.51*	-
NDVI	0.18	0.46***	0.51*	-
WI	0.06	0.41***	0.50**	-
PRI	0.17	0.49***	0.51.	-
<b>chlorophyll</b>				
Vogelmann 2	0.18	0.36***	0.38*	-
NDVI	0.04	0.37***	-	-
WI	0.16	0.30***	0.36**	-
PRI	0.02	0.37***	0.39*	-
<b>C/N content</b>				
Vogelmann 2	0.67	0.80***	-	-
NDVI	0.67	0.85***	-	-
WI	0.42	-	0.79***	-
PRI	0.61	0.66**	0.82***	-
<b>LAI</b>				
Vogelmann 2	0.61	0.69***	0.74***	0.79***
NDVI	0.59	0.77***	-	0.78**
WI	0.42	0.50***	0.67***	0.68**
PRI	0.47	0.58***	0.71***	0.73**
<b>ASD</b>				
	phys. parameter only	phys. parameter +time	phys. parameter +time +treatment	phys. parameter +time +treatment +container
<b>leaf water content</b>				
Vogelmann 2	0.10	0.47***	0.51**	-
NDVI	0.05	0.52***	0.56*	-
WI	0.06	0.50***	0.53.	-
PRI	0.20	0.52***	0.56*	-
<b>chlorophyll</b>				
Vogelmann 2	0.11	0.34***	0.40***	-
NDVI	0.04	0.31***	0.35***	-
WI	0.01	0.35***	0.37*	-
PRI	0.06	0.32***	-	-
<b>C/N content</b>				
Vogelmann 2	0.63	0.67***	0.84***	-
NDVI	0.66	0.79***	-	-
WI	0.68	0.79***	-	-
PRI	0.47	0.49.	0.79***	-
<b>LAI</b>				
Vogelmann 2	0.46	0.50***	0.63***	0.66***
NDVI	0.60	0.66***	0.70***	0.73**
WI	0.56	0.65***	0.70***	0.72**
PRI	0.44	0.46***	0.66***	0.68**

## 6 Discussion

The presentation of results already reveals a number of difficulties concerning sampling techniques, data analysis and overall success of the experiment. First of all, implementation of drought stress evidently failed, and flooding failed in terms of foliar biomass losses, thus impeding reflectance analysis. Then, appropriate (statistical) tools for data analysis are lacking, a challenge that could only partly be overcome. Much work is still required here. The partly little data available, e.g. chlorophyll data to be averaged, and the length of experimental period added some extra confounding factors complicating interpretation of different effects. However, though a direct comparison of AISA and ASD derived data was little successful, similar relationships with physiological parameters for both, AISA and ASD hyperspectral indices, suggest that the general experimental setup was correct.

### 6.1 Performance of Individual Indices

Since the Vogelmann 2 index did hardly perform better than the other indices examined, the conclusion that red edge indices are more suitable for bioindicator prediction and mapping with hyperspectral remote sensing data (Zarco-Tejada et al. 1999) had to be rejected. Also, the water index (WI) designed by Peñuelas (1993b; 1997b), and reported to accurately estimate leaf water content performed poorly in this study. Whereas WI did not correlate well with leaf water content, which can be attributed to inappropriate sampling design, it apparently did correspond to some extent to raised water levels in flooding containers during inundation. The traditional NDVI index, introduced by Rouse (1974) and intensely used for multitemporal mapping of vegetation dynamics on a global scale (e.g. Townshend 1986, Gutman 1989, Viovy 1992, Goward 1994, Teillet 1997, Lyon et al. 1998, Fung & Siu 2000, Young & Wang 2001, Masek 2001), appeared to be most stable and clearly corresponded to the amount of green biomass. Although PRI was originally designed to assess photosynthetic-radiation-use-efficiency (Gamon et al. 1992, Peñuelas et al. 1997a) and later used for detection of drought stress (Suárez et al. 2008), it mainly corresponded to vegetation cover as well. Apparently, vegetation cover, in part represented by LAI, is the factor dominating spectral response in this study. This is in accordance with findings of Asner (1998), Zarco-Tejada et al. (1999; 2001) and Sampson et al. (2003).

## 6.2 Relationships between Plant Physiological Parameters and Vegetation Indices

The results derived from the Generalized Additive Model suggest mainly correlations between all optical indices and leaf C / N content and LAI.

Asner (1998) found that LAI and leaf angle distribution (LAD) are the dominant controls on canopy reflectance data with the exception of soil reflectance and vegetation cover in sparse canopies. Sensitivity studies revealed that low LAI values ( $< 2$ ) are very critical to the accuracy of predicted leaf chemistry through spectral indices since background and shadow effects can mask the condition of foliage (Asner 1998, Zarco-Tejada et al. 1999; 2001, Sampson et al. 2003).

Due to experimental setup, C / N content was sampled on only 13 occasions within a period of 3 month (June 6 to September 17, 2009), thus a total number of 39 C / N values was included in analysis. Though C / N content was increased in leaves of plants exposed to continued flooding, the contribution of external factors other than stress influencing carbon and nitrogen contents of leaves is expected to be confounding. In addition, the spectral indices used to detect plant stress were not calculated from wavelength reported to be sensitive to nitrogen or carbon. Niemann & Goodenough (2003) report significant correlations between reflectance at 667 nm and nitrogen, whereas e.g. Curran (1989) found relationships between carbon and nitrogen and reflectance only for wavelength beyond 970 nm, which were not covered by AISA Eagle. Owing to the limited data available for analysis, the confounding effects resulting from the length of sampling period, and the lack of reported correlation of carbon and nitrogen with wavelengths studied, it is suspected that the high correlation detected may be coincidental.

Although they represent the parameters most intensely studied in relation to stress detection by means of imaging spectrometry (e.g. Curran et al. 1990, Peñuelas et al. 1993b; 1997b, Gitelson & Merzlyak 1996, Treitz & Howarth 1999, Zarco-Tejada et al. 2000b; 2001; 2002, Sims & Gamon 2002, Sampson 2003), little to no correlation was found between vegetation indices and chlorophyll and leaf water content in this study. Despite the well known fact that leaf chlorophyll and water content may be affected by a range of intrinsic and extrinsic factors and thus undergo natural, non-stress related variation as well (Treitz & Howarth 1999), there is a whole range of possible explanations to these findings.

Just as C / N content, leaf water content was determined on only 13 occasions within a period of 3 month. Whereas this lead to high but possibly coincidental correlations between vegetation indices and C / N content, correlations between spectral indices and

leaf water content are low. Due to the similar sampling design and the number of additional confounding factors - e.g., temperature, humidity, irradiance – these results may be just as coincidental as those derived for C / N content. However, as an indicator of plant stress, leaf water content is less sensitive than chlorophyll content anyway, appearing only at advanced stages of leaf dehydration (Carter 1993b, Sampson 2003).

As mentioned above, chlorophyll values were averaged from only 3 single values taken per container per sampling day. Since chlorophyll content is highly variable between and even within leaves (Markwell et al. 1995), such limited data evidently results in high variation, masking potential stress induced effects.

The length of experimental period may be referred to as another confounding factor. For example, Miller et al. (1991) showed that for leaves of 10 deciduous tree species investigated, the red edge position, attributed to changes in chlorophyll content, varied considerably over the period from bud break to senescence. Thus for the experiment described here, lasting from mid-May to mid-September, phenological effects are expected to contribute to reflectance changes. In addition to seasonal patterns, Sampson et al. (2000) observed diurnally based changes in the ratio of blue to red reflectance in sugar maple (*Acer saccharum*), which were not accompanied by changes in chlorophyll concentration. These changes in red reflectance are attributed, at least in part, to quenching of chlorophyll fluorescence at midday (Sampson et al. 2000, Zarco-Tejada et al. 2000b)

Another factor affecting spectral results is the relative proportion of young and old foliage in a canopy, which was constantly changing during the experiment. Sampson et al. (2000) found higher reflectance in certain regions of the visible spectrum when studying young leaves of sugar maple (*Acer saccharum* M.), white pine (*Pinus strobus* L.) and eastern hemlock (*Tsuga canadensis* (L.) Carr.).

Finally, most studies regarding vegetation indices and foliar chemistry are based on leaf level measurements rather than measurements made at the canopy scale, where correlations of chlorophyll content and spectral reflectance can be readily observed (e.g. Horler et al. 1983, Vogelmann et al. 1993, Carter 1994b, Gitelson & Merzlyak 1996, Peñuelas et al. 1997a, Gitelson et al. 1999, Zarco-Tejada 2000a; 2001, Sims & Gamon 2002, Maire et al. 2004, Liew et al. 2008). The extent to which leaf chlorophyll concentration can be estimated from reflectance measurements at canopy level by the use of vegetation indices remains uncertain. Sims and Gamon (2002) found only weak correlations between previously published spectral indices and leaf chlorophyll at the canopy scale

when indices were applied across a wide range of species, whereas e.g. Blackburn (1998) and Zarco-Tejada et al. (2000b) demonstrated good correlations for uniform canopies. However, most vegetation indices are sensitive to both leaf characteristics and canopy structure, making it difficult to detect changes in chlorophyll content of leaves when canopy structure is variable (Sims & Gamon 2002). Leaf surface reflectance was found to be another important factor in this variation. Asner (1998) found that leaf optical properties are generally under-represented at canopy scales.

Besides these many potential confounding factors that have to be taken into account, results of the GAM emphasize the high contribution of the length of experimental period since “time” added a lot to the explanation of the model fit whereas “treatment” had very limited effect.

The results derived from the container orientation test during the experiment suggest that structural effects within containers were minor compared to between-container differences. However, this may result from the excellent illumination of the scene, masking the effect of view angle while structural differences between containers are still remarkable. Thus, this test only reveals that accidental displacement of the container would have little impact, while structural canopy effects and changes in canopy architecture caused by rapid plant growth may still mask leaf reflectance characteristics.

Rock et al. (1988) conclude that the results of their study suggest “that the red edge parameters detected by the instrument may be related to percent mortality and foliar loss [...], rather than pigment and cellular changes associated with forest decline damage as detected by in situ measurements”. Similarly, Brunn (2006) states that although hyperspectral remote sensing techniques have shown a fundamental potential to provide a fast and easy to use method for monitoring of forest condition on a regional scale, the proclaimed identification of very early signs of stress, invisible by other means, could not be validated in his studies. He could only detect damage symptoms well visible in the field.

### **6.3 Imaging versus Non-Imaging Data**

Although a direct comparison of spectral indices derived from AISA and ASD data via regression functions was impossible due to the number of instrumental and computational differences, examination of radiance and reflectance spectra from both instruments revealed a high degree of similarity. One potential reason for the indices to differ

is the analysis of radiance (AISA) and reflectance (ASD). This is due to the fact that for imaging spectrometer data such as AISA, reflectance is commonly calculated by using atmospheric models which are not applicable to data taken from a height of 2.4 m in the laboratory. By comparing the spectral characteristics of reflectance and radiance curves it was concluded that in laboratory conditions (no atmospheric influence, no changes in illumination) radiance and reflectance are sufficiently similar, and thus radiance was used as reference parameter for AISA data.

A number of further reasons for the indices to differ, such as the classification of AISA data, plot size and standardization of setup, were already given in the results chapter. Nevertheless, relationships between plant physiological parameters and vegetation indices from both, AISA and ASD data, showed analogue trends. These results are in agreement with findings of Pontius et al. (2005), who found that both instruments demonstrated similar relationships between key wavelength and bioindicators when comparing AISA Eagle airborne imagery and ASD spectra. These results are encouraging, not only because the ASD field spectrometer is widely used in ground truthing for airborne campaigns (e.g. Coops et al. 2004, Pontius et al. 2005) but also because it has long been established as a reliable tool in detection of plant stress and foliar chemistry in the lab (e.g. Asner 1998, Zarco-Tejada et al. 2003, Dobrowski et al. 2005, Stellmes et al. 2007). Similar relationships with physiological parameters for both, AISA and ASD hyperspectral indices, suggest that the first-time experimental setup with AISA Eagle in the laboratory was correct. However, these results raise the question whether the complex setup with AISA in the lab is needed or whether non-imaging ASD spectrometer data is sufficient for future laboratory studies.



## 7 Conclusion

During this experiment, which was unique in many respects, a great amount of unique, especially technical difficulties (not all mentioned in this report) had to be overcome. Whereas a number of challenges, such as implementation of stress, appropriate sampling and analysing techniques and confirmation of relationships reported in literature were only achieved in parts, the similar functioning of well-established ASD and AISA Eagle in laboratory conditions indicates the general correctness of experimental setup in the dark room. Relationships between hyperspectral reflectance data and vegetation physiological parameters are still subject to discussion - results at leaf and canopy scales are inconsistent, and derived equations are often not reliable predictors for other remotely sensed data (Kumar et al. 2006). Despite the high complexity of interacting factors and all inconsistencies observed, the overall potential of hyperspectral remote sensing data for monitoring of vegetation condition is considered to be high (e.g. Zarco-Tejada 2000b, Haboudane et al. 2002; 2004, Sims & Gamon 2002, Pontius et al. 2005b; 2008, Brunn 2006, Liew et al. 2008)

Future research trying to separate the impact of individual stress factors should focus on a stronger implementation of stress conditions, a clearer distinction between the treatment levels and possibly different stress agents, not resulting in loss of foliage. When trying to implement drought stress, a less clayey soil material should be used. Besides, a different test organism should be used – in addition to foliar losses, high growth rates (changes of canopy geometry) of common ash hampered spectral analysis. For statistical analysis a greater number of sampling containers exposed to the same treatment conditions would be beneficial. Furthermore, the stress scenarios need to be simplified, since the complexity of treatment levels and length of experimental period apparently resulted in an excess of interfering factors. The development of appropriate analysis and statistical tools, also with respect to future hyperspectral satellite missions such as EnMAP, is in progress.

Many questions, but especially whether the complex setup with AISA in the laboratory is needed, remain unanswered. Thus, analysis of data sampled in the course of this experiment is worth being continued, for example by using different pre-processing and processing methods (e.g. derivatives of reflectance spectra, continuum removal). Some authors report the derivation of good correlations by the use of (inverse) radiative transfer modelling approaches (e.g. Jacquemoud & Baret 1990, Zarco-Tejada et al. 2000b,

Malenovský 2006). Physical models such as PROSPECT or SAIL are available but their application was beyond scope of this study.

## 8 Zusammenfassung

Die vorliegende Arbeit beschäftigt sich mit den kausalen Zusammenhängen zwischen Hyperspektraldaten und physiologischen Vegetationsparametern auf Kronendachebene. Um den Einfluss von stressinduzierten Veränderungen auf diese Zusammenhänge systematisch zu untersuchen, wurde im UFZ Forschungslabor in Bad Lauchstädt eine viermonatige Dauerversuchsreihe durchgeführt.

Neun Messcontainer mit jeweils neun Eschensetzlingen (*Fraxinus excelsior* L.) wurden im Kalthaus platziert und kontrollierten Trockenstress- und Überflutungsszenarien ausgesetzt. Der physiologische Zustand der Pflanzen wurde zwei Mal wöchentlich durch Messungen des Blattchlorophyllgehaltes, des Leaf area index (LAI) und der Wuchshöhe und wöchentlich durch die Messung des Blattwassergehaltes und des C- und N-Gehaltes bestimmt. Zusätzlich wurde die Bodenfeuchte in drei verschiedenen Bodentiefen ermittelt. Parallel dazu wurden in einer Dunkelkammer unter Kunstlichtbedingungen Hyperspektralmessungen mit dem abbildenden AISA-Eagle Sensor und dem nicht abbildenden ASD Feldspektrometer durchgeführt.

Von den AISA und ASD Daten wurden 34 stressensitive Vegetationsindizes berechnet. Da die meisten dieser Indizes ein sehr ähnliches Verhalten aufwiesen wurde bei nachfolgenden Analysen ein Schwerpunkt auf eine Auswahl von vier nachweislich verschiedenen Indizes gelegt, und zwar NDVI, PRI, Vogelmann 2 und WI.

Die Ergebnisse legen nahe, dass die Umsetzung von Trockenstress fehlgeschlagen ist, was vermutlich auf das lehmig-tonige Bodenmaterial zurückzuführen ist. Überflutung hingegen führte zu frühzeitigem und teilweise fast vollständigem Blattabwurf. Eine Kombination von wenigen Messwerten und verhältnismäßig langer Versuchsdauer führte außerdem zu einem Übermaß an Störeinflüssen und teilweise widersprüchlichen Korrelationen zwischen Hyperspektraldaten und Blattchlorophyllgehalt, Blattwassergehalt und C/N-Werten. Der Anteil grüner Biomasse, teilweise repräsentiert durch den Parameter Leaf area index (LAI) stellte sich als einflussreichste Variable heraus. Zwar konnten die in der Fachliteratur beschriebenen Zusammenhänge nur teilweise bestätigt werden, dabei stimmen die Ergebnisse für AISA und ASD Daten jedoch überein. Da das ASD Feldspektrometer ein etabliertes Instrument in diesem Anwendungsbereich ist ist dies als Hinweis zu werten, dass der generelle Versuchsaufbau in der Dunkelkammer korrekt war.

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