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LINKING DIGITAL IMAGE SIGNALS TO THE SEASONAL VARIATIONS IN PHOTOSYNTHETIC PIGMENTS OF DIFFERENT PLANT TYPES

MAIRE Juliette

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Lisa, Wingate chargée de recherche

Alice, Michelot-Antalik, Pchargée de recherche

ECOFUN, UMR 1391 ISPA, INRA Centre de Bordeaux Aquitaine
71 avenue Edouard Bourlau 33882 Villenave d'Ornon cedex

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Résumé

La phénologie est un indicateur sensible pour appréhender les conséquences du changement climatique et des interactions sol-plante-atmosphère de l'échelle locale à globale. Depuis quelques années, l'observation de la phénologie basée sur l'analyse d'images au fil de la saison est devenue un outil répandu. Cependant, les relations entre la phénologie enregistrée avec les cameras et les caractéristiques biochimiques et spectrales des feuilles ne sont pas encore compris. Pendant cette étude, des mesures de propriétés biochimiques des feuilles comme la composition en pigments photosynthétiques et en azote ont été effectuées toutes les semaines durant le printemps et l'été 2015 sur des espèces typiques des Landes de Gascognes (*Pinus pinaster*, *Eucalyptus gundal,* diverses variétés de vigne et des espèces prairiales) autour de Bordeaux en France. La surface des feuilles et les propriétés spectrales des feuilles ont été estimées grâce à des indices de couleurs (Vert, Rouge, Bleu and Excès de Vert) extraits avec un scanner (Canon LIDE 110, CANON, France). En parallèle, des caméras (Stardot SC5 IR, Stardot, California, USA) ont été installées sur le terrain pour automatiser l'acquisition quotidienne d'images des écosystèmes étudiés et extraire les mêmes indices de couleurs que pour le scanner. La technique habituelle d'extraction de chlorophylle en laboratoire est laborieuse donc une pince à feuille portable a été utilisée pour suivre finement la composition foliaire en chlorophylle ((Dualex Scientific +, Force A, Orsay). Le but de cette étude est de comparer les trajectoires saisonnières entre la composition biochimique des feuilles et les indices de couleurs des feuilles et des canopées. Nos résultats montrent que l'évolution phénologique saisonnière est correctement enregistrée par l'information extraite des images répétées de la caméra et du scanner. Ces images ont une importante utilité pour la recherche en phénologie et pour évaluer la réponse de la végétation à des perturbations du milieu (changement climatique, gel, sécheresse)

Mots-clés : Phénologie, télédétection, caméra de surveillance, chlorophylle, trajectoires saisonnières

Abstract

Plant phenology is a sensitive indicator of climate change and influences soil-plant-atmosphere interactions at local and global scales. In recent years, phenology monitoring based on camera images taken over the growing season has become a popular tool. However, relationships between digital camera phenological records and leaf biochemical and spectral properties are poorly understood. In this study, measurements of key leaf biochemical properties such as photosynthetic pigment and nitrogen contents were made on a weekly basis over the spring and summer of 2015 on various Landes of Gascogne species (*Pinus pinaster*, *Eucalyptus gundal,* various vine varieties and grassland species) in Bordeaux, France. The size and spectral leaf properties were measured using a flatbed scanner (Canon LIDE 110, CANON, France) to extract colour indices (Green, Red, Blue and Green Excess indices). At the same time, digital cameras (Stardot SC5 IR, Stardot, California, USA) were installed in the field to automatically record daily pictures of the studied ecosystems and extract the same colour indices. The technique typically used for chlorophyll extraction in laboratory is laborious thus a portable chlorophyll meter was used to collect a larger dataset (Dualex Scientific +, Force A, Orsay). The aim of the study was to compare seasonal trajectories between leaf biochemical content and colour indices of the canopies. Our results show that phenological seasonal evolutions are well captured by information extracted from digital repeat photography. The digital images from camera and scanner have important implications for phonological research application and for assessing responses of vegetation to disturbances (climate change, frost, drought).

Keywords: phenology, remote sensing, digital camera monitoring, chlorophyll, seasonal trajectories

INTRODUCTION

Phenology is the study of periodic life cycle events and how these are influenced by season and by the changes in weather patterns. Vegetation phenology is highly sensitive to climate change (Richardson et al., 2013). Phenology has been an area of active interest and have been recorded for long periods (observations of cherry flowering at the Royal Court in Kyoto date back to 705 AD)(Wingate et al., 2008). Changes in phenological events are robust indicators to monitor vegetation evolution and potential impacts of climate change. The IPCC working Group II report highlights evidence that these changes have occurred over the past decades (IPCC, 2014). The use of phenological metrics is also evolving within different research areas. For example, the agroecology community are now ‘phenotyping’ plant varieties for various properties including budburst and flowering patterns in order to select species that are adapted to future changes in climate. In addition, many studies are also trying to link ecosystem energy, water and CO_2 exchange to variations in phenology that will likely impact the length of the growing season (refs). In turn variations in canopy phenology will also have an impact on climate change by influencing the seasonality of canopy properties such as albedo, surface roughness length, canopy conductance, and fluxes of water, energy, CO_2 and biogenic volatile organic compounds (Richardson et al., 2013).

Typically phenological measurements involve visiting plants on a regular basis and following the different stages of development. This traditional approach used by farmers for many years is typically labour intensive but can provide important information on how particular wine varieties vary with temperature and water and how this relationship has evolved over centuries (Chuine et al., 2004 ; Chuine, 2010). However, more recently the use of digital repeat photography to follow plant phenology has become more popular (Keenan et al., 2014 ; Petach et al., 2014 ; Toomey et al., 2015). In recent decades, ground-based techniques have been developed to support satellite remote sensing estimates of phenological changes on a global scale but also large-scale temporal changes in vegetation (Heinsch et al., 2006 ; Nemani et al., 2007). The ground validation data are based on site-specific observations made at discrete time intervals or continuous time series with camera monitoring (Keenan et al., 2014). Automated near-surface remote sensing techniques have been established as a direct connection between ground-based manual observation and satellite remote sensing products (Richardson et al., 2012, 2009, 2013). The digital repeat photography technique has now been used on several plant canopies including, temperate deciduous and evergreen tree species as well as grass- and croplands (Toomey et al., 2015 ; Toshie et al., 2014 ; Wingate et al., 2011, 2015). These studies have demonstrated that by analysing the seasonal variations in Red, Green and Blue (RGB) fractions in a defined region of interest it is possible to estimate the dates of leaf flushing, flowering, senescence and leaf fall. Time-series of RGB signals above canopies can also provide information on the length of the growing season and estimate the impact of frost or disease on ecosystem productivity (Cannell, Smith, 1986 ; Mizunuma et al., 2013 ; Hufkens et al., 2012). Whilst a large number of studies have identified widespread patterns of change, the impacts of changes in phenology on ecosystem function and feedbacks to the climate system remain poorly understood and quantified (Richardson et al., 2013).

The first objective of this study will consist in establishing the degree of agreement between the dates estimated for key phenological stages estimated from visual observations and digital image archives. This will be facilitated by observing different plant species (*Pinus pinaster* Ait., *Eucalyptus gundal*, Vine hybrids, *Molinia caerulea* L.) known to have different timings for their different phenophases.

The second objective will focus on how image analysis can be used to provide a deeper understanding of plant function. For example, a recent study on an Oak forest growing in the UK has demonstrated a mechanistic link between the seasonal variation in photosynthetic pigments such as chlorophyll and carotenoids and the evolution of RGB signals over the growing season recorded by digital cameras (Wingate et al., 2015). Leaf chlorophyll content is a key ecological variable, both directly through its role in photosynthesis and in the conversion of solar radiation into stored chemical energy. It is also a useful bio-indicator of plant physiological condition e.g. highlighting areas of plant disturbance and environmental pressure (Croft et al., 2015). There are three types of pigments that determine the leaf colour as the results of selective light absorption, reflectance and transmittance: chlorophylls (a and b), carotenoids (including β-catotene) and anthocyanins (Féret et al., 2011). For example a leaf is green due to a diffusion of their reflectance is maximal in the visual spectra around 550nm. Moreover changes in foliage characteristics such as leaf colour and pigmentation, nutrient content, photosynthesis capacity, etc.) over the course of the season can be species specific.(Ma et al., 2011 ; Sims, Gamon, 2002) Thus in my study I will investigate the link between photosynthetic pigments and digital image colour signals over a growing season in the different Landes plant species described above growing on the same soils and in the same climate. This will be achieved by calibrating a leaf chlorophyll index measured with a handheld chlorophyll meter (DUALEX SCIENTIFIC +, Force A, France), against leaf chlorophyll concentration measurements obtained from leaf extracts analysed by a spectrophotometer (UV/Visible Libra 22, Biochrom, France). Once calibrated the chlorophyll meter is used frequently in the field over the growing season to track variations in canopy chlorophyll content and other components including flavonoids and Nitrogen content. These measurements will then be used to interpret:

1/ daily variations in canopy Red, Green and Blue colour signals measured by a digital camera (Stardot SC5 IR, Stardot, California, USA) mounted at each study site and;

2/ discrete variations in leaf Red, Green and Blue colour signals measured with a digital flatbed scanner (Canon LIDE 110, CANON, France)

In particular, the goal of the study is to identify when and under what conditions information can be extracted from digital repeat photography in order to interpret seasonal changes in leaf and canopy level photosynthetic function.

In this project, specific Landes species were selected for the purpose of comparing different plant types (broadleaf forest, needleleaf forests, grasslands and crop plants) and focusing on important economically valuable ecosystems. The Landes is a department in southern France. The Gascogny Landes, near the littoral line are entirely located on Podzolic, acidic, hydromorphic soil and mainly dominated by Maritime Pine forestry.(IGN, 2013) This forest provides on average 48% of the wood industries in France with a production of 4.3 million m^3. In 2009 for instance, after the storm Klaus, the production increased? to 10.5 millions of m^3.(ANON., 2012) The wood production in the French Landes department equals the revenue for wine production estimated in the area ~2.5 billion Euros. These activities provided close to 34,000 jobs in France in 2006.(Héléna CABADIE et al., 2006)

The study was completed within the French National Research Institute of Agronomy, INRA Bordeaux-Aquitaine Centre that conducts innovative and targeted plant science, ecological and environmental research in response to current challenges in the agricultural, forestry and aquaculture sectors. INRA is Europe’s top agricultural research institute and the world’s number two centre for the agricultural sciences. In 2014, INRA is the world’s second greasiest producer of publications in agricultural sciences, 186 laboratories and 49 experimental units across 13 scientific divisions and 17 research centres It is also a community of 12,000 people with an annual budget of 880 million Euros.

INRA are also responsible for the co-ordination of a European digital image network at flux sites (http://european-webcam-network.net) (Wingate et al., 2015). Every day as part of this network, digital images are taken at ~60 sites encompassing a diverse range of ecosystems (crop land, deciduous broad leaf, deciduous needle leaf evergreen broad leaf, evergreen needle leaf, grassland, peatland). (Figure 1) Phenological transition dates recently derived from this network have been used to describe temporal changes in surface-atmosphere gas exchanges and how they vary spatially. More data is now needed to link the digital archives mechanistically to plant function and this motivation underpins the current study.

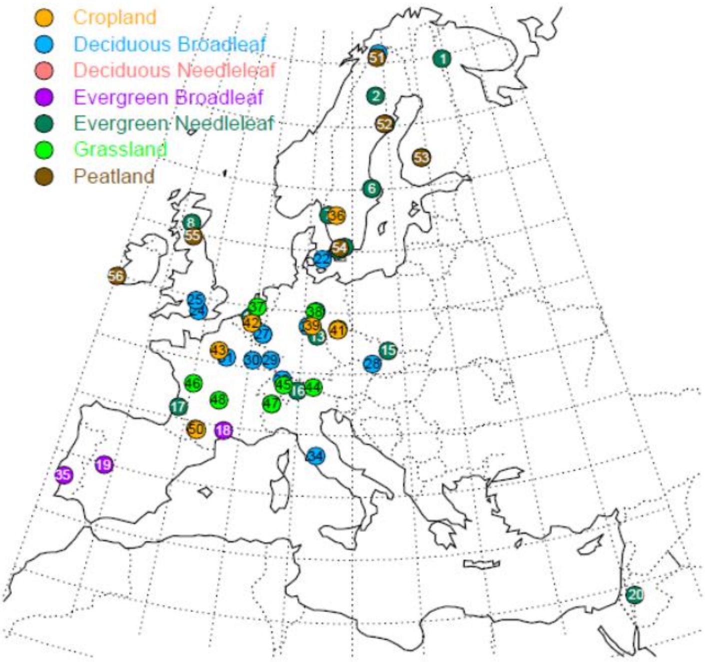


Figure 1: European network of webcam monitoring the ecosystems phenology (Wingate et al., 2015)

MATERIAL AND METHODS

SITES DETAILS

This study was conducted at four sites located around Bordeaux, France. (Figure 2 and Figure 3) All sites are experimental sites used by INRA from two to nine years. The sites are a specific management and monitoring protocol for the purpose to see the climate change adaptations of these plantations with an annual average precipitation of 977 mm and an annual mean temperature of 13°C. (MOREAUX, 2012) These sites are temperate forest plantations dominated by Eucalyptus (E. gundal), evergreen forest dominated by maritime pine (Pinus pinaster) and vineyard (various varieties).

Sites	*Species*	*longitude*	*latitude*	*altitude (m)*
FERRADE	*Vine*	*-0.57771667*	*44.78952000*	*22.1*
PIERROTON	*Eucalyptus-Grass*	*-0.77418333*	*44.74129000*	*60.2*
BILOS	*Maritimes Pines*	*-0.95587000*	*44.49436833*	*41.6*
RESDUR	*Vine*	*-0.58025333*	*44.78863333*	*21.3*

Figure 2: Sites details (data from Dualex Scientific + GPS)

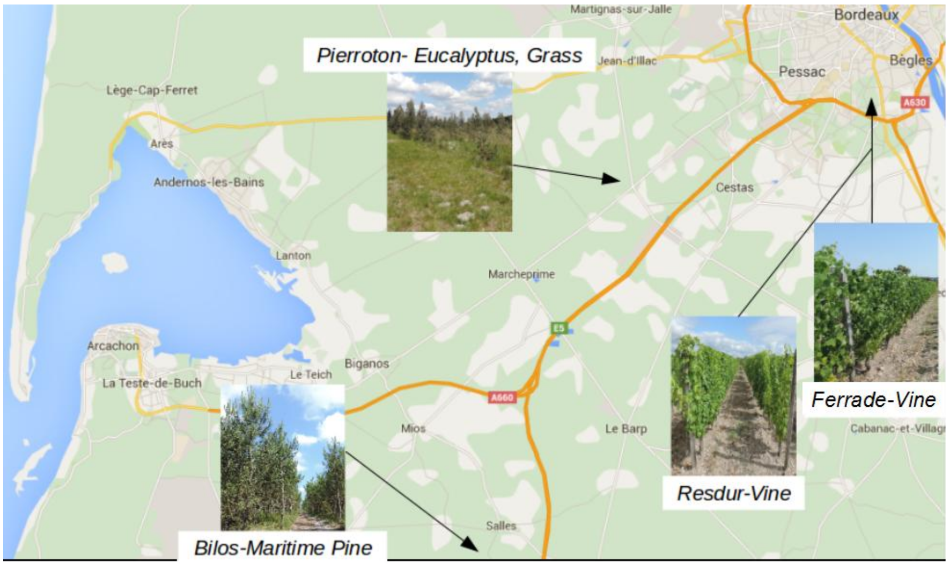
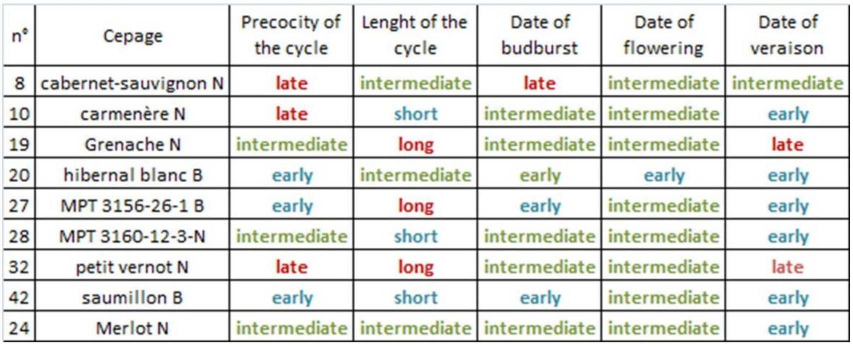


Figure 3: Location of sites

RESDUR and FERRADE sites - Vineyards

The first site containing vine is hereafter called La Ferrade. In this experimental site, 4 blocks were installed to assess the capacity of different vine varieties to adapt to climate change. In each bloc 52 varieties of vine were planted in total randomisation. Each unit of the same variety contains 10 vines (2*5) and covers an area of 1.80m per 5.30m (Table 4). For this study nine varieties of vine were followed in order to have 9 very different varieties in terms of phenology and development cycle as shown below.



n°	Cepage	Precocity of the cycle	Lenght of the cycle	Date of budburst	Date of flowering	Date of veraison
8	cabernet-sauvignon N	late	intermediate	late	intermediate	intermediate
10	carmenère N	late	short	intermediate	intermediate	early
19	Grenache N	intermediate	long	intermediate	intermediate	late
20	hibernal blanc B	early	intermediate	early	early	early
27	MPT 3156-26-1 B	early	long	early	intermediate	early
28	MPT 3160-12-3-N	intermediate	short	intermediate	intermediate	early
32	petit vernot N	late	long	intermediate	intermediate	late
42	saumillon B	early	short	early	intermediate	early
24	Merlot N	intermediate	intermediate	intermediate	intermediate	early

Table 4: Studied vine varieties details on their phenophases

The second site studied during this project is RESDUR. (Figure 5) In this experimental hybrid varieties are being grown to test their capacity to resist disease and pathogens without excessive pesticide treatments with a view of finding new varieties that might be better adapted to the climate of Bordeaux in the future.

At this site, a camera Stardot Netcam SC5-IR (Stardot, California, USA), coupled to a “cheap-raspberry pie” computer, is set-up at 8-8.2m high. Pictures are taken 5 times during the course of each day with a fixed colour balance with the colour settings fixed manually to (R: 256, G: 180, B: 256). In addition this particular camera has the possibility to activate an infra-red filter that can provide information on near-infrared (NIR) wavelengths and calculate the NDVI (Petach et al., 2014). Thus the infrared images were also used to estimate seasonal variations in NDVI at the RESDUR vine site.

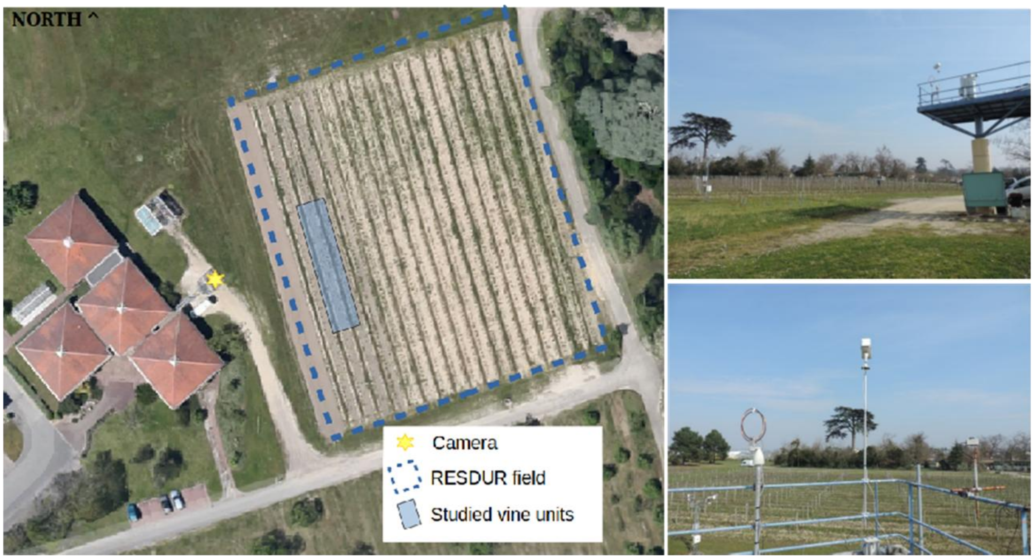


Figure 5: RESDUR field Google maps view and camera installation

BILOS site – Maritime Pine

This experimental site is located at Salles (44°29’38.08″ N; 0°57’22″ O, altitude: 40 m) in the Landes forest, 50km South-West of Bordeaux. The forest, composed of Maritime pine (Pinus pinaster) was planted in July 2004 over an area of 30 hectares. (MOREAUX, 2012)

Figure 6: On the left, picture taken on the 16_03_2015 at 12_01_38 GMT and on the right, camera installation.

Bilos is an important experimental site forming part of the project ICOS (http://www.europe-fluxdata.eu/icos/home) and the European webcam network, EUROPHEN. ICOS (Integrated Carbon Observation System) is a European Research Infrastructure for quantifying and understanding the greenhouse gas balance of the European continent and of adjacent regions. In Bilos, numerous variables have been measured continuously since 2000 (Appendix 2). A website containing treated meteorological data and camera images collected at the site were made available for this project (http://suivibilos.free.fr/Salles2.html). The digital camera was also a Stardot Netcam SC5 (without IR capabilities) set-up to look North across the pine forest at a height of 12m (Figure 6 and Figure 13).

PIERROTON site – Eucalyptus and grassland

The site called Pierroton is an experimental plantation of Eucalyptus E.Gundal (E.gunnii x E. dalrympleana) planted 5-6 of March 2013 with a pattern of 2.4mx4m on a humic Podzol soil. This plantation is part of the project Xyloforest (http://www.xyloforest.org/). Xyloforest is a shared platform for research and innovation systems for forest cultivation – timber products & materials. Its scope covers the adaptation of forest resources, climate change, engineering wood construction, fiber and forest biomass energy and chemical enhancement. In particular, the field followed is part of the experimental system ECO-XYLOSYLVE-2 to compare 24 plots divided in 4 blocs randomly disposed for a long term recording (2014-2034) of production performances and the consequences on the environment of a Eucalyptus plantation in this area. These plots are managed with four different fertilisation and irrigation treatments. The plot that was target by the camera was a non-treated plot in the bloc 3. At this site a camera (Stardot Netcam SC5-IR, California, USA) is set-up looking North at a height of 3,5m. During the project a battery back-up power supply was connected to the "raspberry pie" and the camera system to limit data losses caused by electricity cuts and voltage fluctuations. In addition, at this site it was also possible to investigate the seasonal changes of the understory (grassland). The understory layer in Pierroton is dominated by Ajonc (*Ulex minor Roth*), Molinie (*Kengia serotina (L.) Packer*) and *Erica scoparia L* (Appendix 3)

Figure 7: a) View of the ECO-XYLOSYLVE-2 field taken from a pin monitoring tower. b) Zoom on the plot with the camera. c) Zoom on the "cabane "where the camera was installed

DATA COLLECTION

In the field, leaf and needle collections were made between March 2015 and August 2015, approximately every week in the four sites. Pictures of the sites are presented in Figure 3 showing the different sampling locations. All sampling points were randomly chosen within a specific area or level of the tree/field to follow the evolution of early growth and mature leaf/needle properties.

For each individual leaf or group of leaves/needles three measurement steps were completed.

1/ Leaves were always placed on a flatbed scanner at the same place and in the same direction alongside a 4cm^2 black square (for scaling purposes) and scanned. The image was saved at a resolution of 2400x4800 pixels for subsequent leaf area and RGB analysis. Between leaf samples the scanner was cleaned with ethanol.

2/ The Dualex Scientific + was then used to measure the chlorophyll, flavonol, anthocyanin and nitrogen index of the same leaves/needles that were scanned. The Dualex sensor was cleaned with ethanol before calibration of the instrument. Then group of leaves or needles were placed in a way to always have the same region on the sensor. Each measurement was repeated 3 or 4 times and the measurement were repeated in the same particular area.(Figure 8) For some plant types the Dualex measurement was made on both sides of the leaf, in this case the Dualex was clipped exactly at the same place but on the other leaf side.

3/ The same leaf samples were then labelled in tubes and frozen at -195°C in the field and transported to the lab where they were conserved at -80°C until chlorophyll analysis by spectrophotometry could be completed.

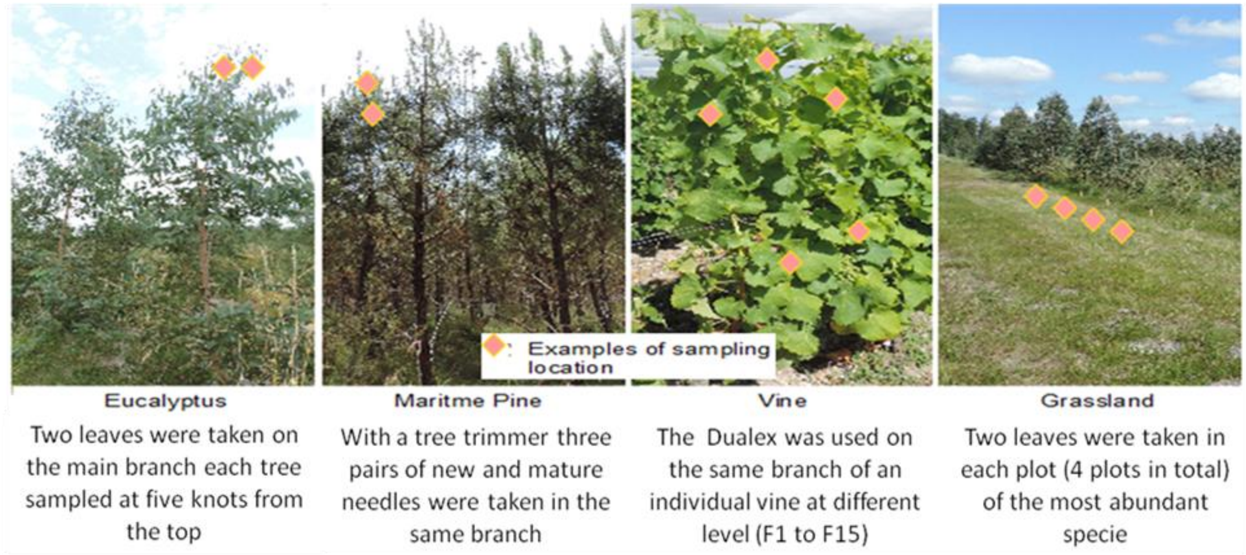


Figure 8: Sampling at the different sites (Right to left, Eucalyptus in Pierroton, Maritime Pine in Bilos, Vine in Ferrade and Grass in Pierroton)

FERRADE

Nine vine varieties were measured and 8 to 9 leaves were taken per vine to calibrate the Dualex Scientific +. The aim was to sample leaves displaying very different colours to obtain a large range of chlorophyll index. The Dualex was used as shown in Figure 9.

Figure 9: Sampling with the Dualex Scientific + and a picture of four very different colour leaves from the same vine type (F1, F3,, F11, F15) of Hibernal Blanc.

RESDUR

For each unit, at every sampling occasion, two individual vines were chosen. For each individual vine (INRA1 to INRA8) only the Dualex Scientific + was used on this experiment because of restrictions on leaf sampling. Only non destructive methods were obtained at this site.

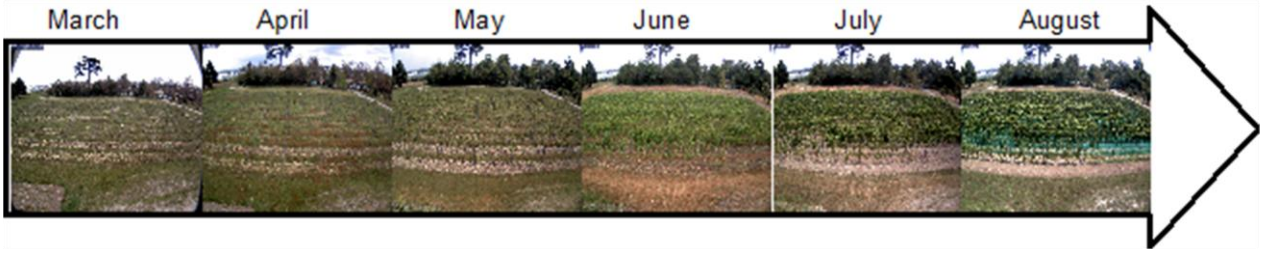


Figure 10: Evolution of the vegetation at Resdur from March 2015 to August 2015 (pictures taken once a month at 12:00:00)

BILOS

At every sampling occasion three individual Maritime pine trees were chosen from each row (1, 2 and 3). For each individual tree the Dualex Scientific + was used on three pairs of needles collected from the upper whorls (6 to 9). Maritime pine typically holds up to 4 needle cohorts on branches. As the needles age metabolic activities tend to decrease slowly until the third year or until a drought is experienced, thereafter the pine needles fall from the tree. In this study measurements were completed on new needles that grew during 2015 and 1 yr-old mature needles from 2014.

Figure 11: Evolution of the vegetation at Bilos from January 2015 to August 2015 (pictures taken on the 11th of each month at 12:00:00)

PIERROTON

At every sampling occasion four individual eucalyptus trees were chosen from each row (D, G, J and M). For each individual eucalyptus tree the Dualex Scientific + was used on two leaves from a main branch of the tree (F6).

Figure 12: Evolution of the vegetation at Pierroton from March 2015 to August 2015 (pictures taken on the 11th or 12th of each month at 12:00:00)

DIGITAL CAMERA, SCANNER AND INSTRUMENTS SETTING, IMAGE ACQUISITION

Dualex Scientific +

The DUALEX SCIENTIFIC+™ is a sensor that measures flavonol, anthocyanin and chlorophyll index. This leaf-clip allows facilitates real-time and non-destructive measurements. This meter measures the transmission of radiation at 710 and 850nm and converts the measurement into a value of chlorophyll in µg.cm^{-2}. http://www.force-a.eu/fr/dualex-plus.php Figure 8Appendix 4)

The calculated indices are:

- NBI (Nitrogen Balance Index), the marker of nitrogen deficiency
- Chl, the chlorophyll index (between 0 and 150)
- Flav, the flavonol index
- Anth, the anthocyanin index (between 0 and 999)

Dualex scientific + has already been tested on vine leaves (Zoran G Cerovic et al., 2012) and for a range of different plants. (Demotes-Mainard S etal., 2008)

Digital camera setting and image acquisition

Canopy images were collected using an automated and networked digital camera (Stardot SC5 IR, Stardot, California, USA) mounted on secure towers, with an oblique viewing angle. Jpeg images from the camera were taken five times per day (every hour between 11:00 and 15:00 local time) and transferred manually or via file transfer protocol (FTP). Camera automatic white and colour balancing was switched off to avoid grey photos (Mizunuma et al., 2013). In contrast, the exposure time was automatically adjusted in response to changing light levels.

For the digital image analysis, the work was completed with the participation of Dr. Gianluca

Filippa, ARPA. Dr Filippa has created a R package called "Phenopix" (Filippa et al., 2015) (http://www.r-forge.r-project.org/R/?group_id=1963). This package was designed to process digital images of vegetation cover in order to compute vegetation indexes and the seasonal development of the vegetation. The analysis can be run on one or more portions of the image (so called regions of interest, ROIs). (Figure 13) Regions of interest can be of any polygonal shape. For data processing, two approaches are available: ROI-averaged analysis or pixel based analysis. ROI-averaged analysis is based on the computation of vegetation indexes as the average of the entire ROI, whereas pixel based analysis allows treating separately each pixel of the image. On each picture, with the Phenopix package the canopy green, red and blue digital numbers (DN) are quantified. These DN are then used to calculate the ratio of green, red and blue indices (Gi, Ri, Bi).

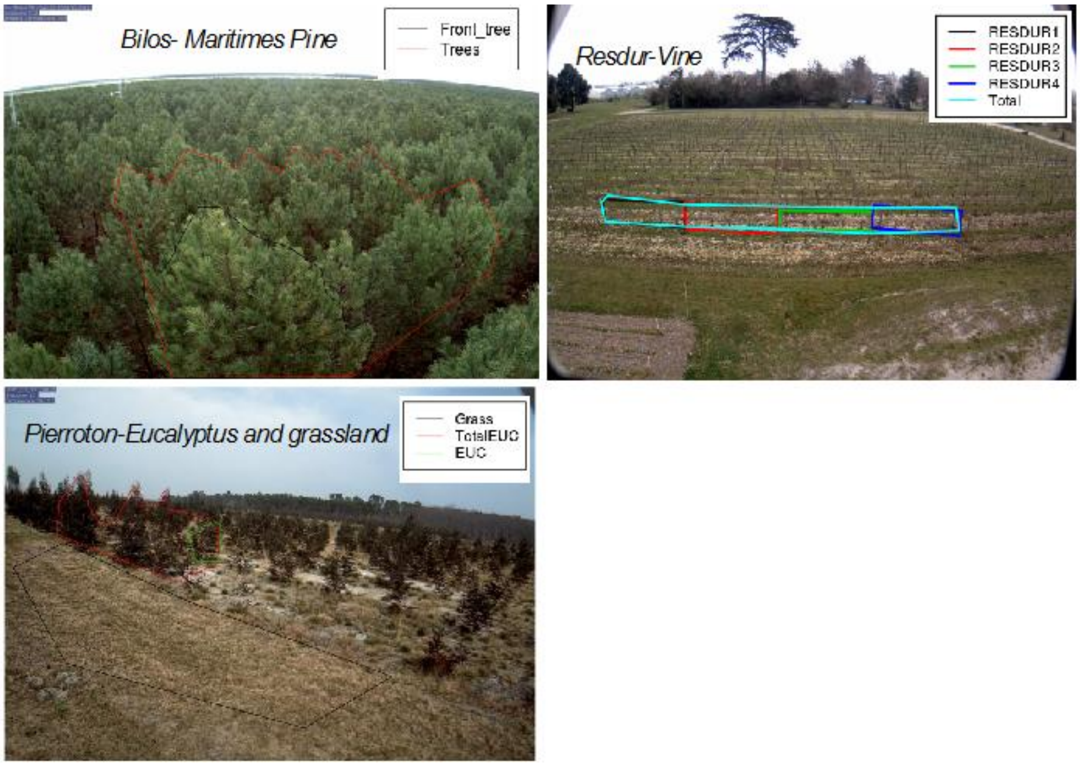


Figure 13: Region of Interest (R.Phenopix packages) used for the sites of this study. The reference pictures that are used as a base for drawing the ROI, were taken on winter pictures (Pierroton: 2015_03_19, Bilos: 2014_11_27, Resdur: 2015_03_20)

Digital scanner setting and image acquisition

Leaf colour and morphological traits were collected using a digital flatbed scanner (Canon LIDE 110, CANON, France). Each leaf was scanned alongside a 4 cm^2 black square. The scanned images were analysed to extract leaf area and leaf colour (red, green and blue digital numbers) using a new R package developed for this purpose during the internship with the participation of Dr. Gianluca Filippa and Dr. Tim Brown (Borevitz lab, Australian National University). The code uses the concept of grayscale threshold to "find" on the scanned image the pixels corresponding to the leaves/needles or to the scale. Using the ratio between the amount of black pixels and the amount of leaf sample pixels the algorithm calculates the leaf/needle area. Thereafter, the colour fractions are calculated using a simple mean of the colour fraction by pixel. The scanner automatic white and colour balancing was switched off to optimise the measurements.

For the scanner and digital camera images colour indices where calculated as show below:

$$\text{Colour index} = \frac{n_{\text{colour}}}{(n_{\text{green}} + n_{\text{red}} + n_{\text{blue}})}$$

Where Colour index is the colour signal of red, green or blue (0-1) and n is the average digital numbers of each particular colour.

$$\text{GEI} = 2 * n_{\text{green}} - (n_{\text{red}} + n_{\text{blue}})$$

Where GEI is the green excess index (Richardson et al., 2013).

Photosynthetic pigment extraction in the laboratory

Frozen leaf samples were then prepared for pigment concentration analysis following the method of Yang *et al*., (2014). A few methods were considered, and two were chosen. The first method uses pure acetone as the solvent to extract the photosynthetic pigments, whilst the second uses pure dimethyl-sulfoxide (DMSO). (Figure 14)

In brief, in the first method, two leaf discs of 1.13 cm² were prepared from each leaf and then ground in a mortar with the 100% acetone solution. The solution was then transferred to a glass vial and centrifuged for 8 min at 4500 rpm. The resultant supernatant was then transferred to a cuvette and the absorbance measured using a spectrophotometer (UV/Visible Libra 22, Biochrom, France).

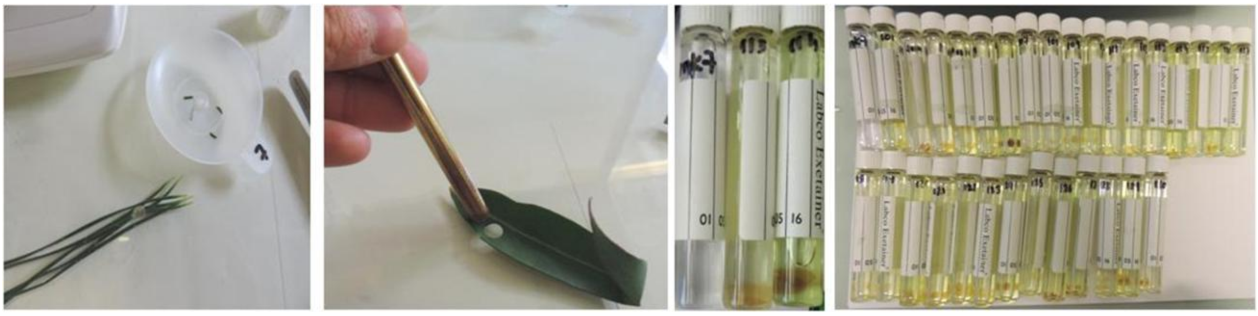


Figure 14: Laboratory analysis, leaf disks sampling and DMSO tubes after 3hours incubations à 65°C

Chlorophyll a and b concentrations were then calculated using the readings from 470, 520, 645, 662, and 710 nm. (Lichtenthaler, Buschmann, 2001) Chlorophyll a and b absorb with narrow bands in the blue (near 428 and 453nm) and red (near 661 and 642nm) spectral range.(Xue, Yang, 2009)

Acetone (pure) equations from (Lichtenthaler, Buschmann, 2001):

$$\text{Chl a } (\mu g.\, mL^{-1}) = 11.24 * A_{661.6} - 2.04 * A_{644.8}$$

$$\text{Chl b } (\mu g.\, mL^{-1}) = 20.13 * A_{644.8} - 4.19 * A_{661.}$$

Absorbance maxima of extracted pigment depends strongly on the type of solvent and spectrophotometer used thus it is necessary to correct with a calibration obtained from the literature (Lichtenthaler, Buschmann, 2001).

This technique is time-consuming and has the added difficulty as one has to calibrate for the equations used and the settling duration. The second method using DMSO follows that for acetone (Barnes et al., 1992 ; Alan.R Wellburn, 1994). One or two discs of 0,65cm diameter are weighed then two discs are transferred to 10mL of pure DMSO in 12mL glass tubes. The tubes were then closed and put in an incubator at 65°C for 3 hours. After incubation, the tubes were cooled down under the laboratory fume hood. Using butyl gloves and protective glasses, 2mL of supernatant from each tube was transferred to plastic cuvettes for spectrophotometer analysis (UV/Visible Libra 22, Biochrom, France). Absorbances were then measured at 665nm, 649nm, 648nm, 750nm and 480nm and equations for pure DMSO were used to calculate pigment concentrations (A R Wellburn, 1994 ;

Parry et al., 2014):

$$\mathbf{Chl\ a}\ (\mu g.mL^{-1}) = (12.47 * A_{665} - 3.62 * A_{649}) * \mathbf{dilution}$$

$$\mathbf{Chl\ b}\ (\mu g.mL^{-1}) = (25.06 * A_{649} - 6.50 * A_{665}) * \mathbf{dilution}$$

$$\beta carotene\ (\mu g.mL^{-1}) = \frac{(1000 * A_{480}) - 1.29 * (\frac{Chl\ a}{dilution}) - 53.78 * (\frac{Chl\ b}{dilution})}{220 * dilution}$$

The results of the chlorophyll extraction were checked to ensure the Chl a/ Chl b ratio was within the range expected (Barry et al., 2009 ; Curran et al., 2001 ; Datt, 1998)

STATISTICS ANALYSIS

The dataset collected was stored on a database and treated with R in Rstudio. The point sample ROI data for each method was averaged to obtain a daily mean with standard error for each day of year and by individual statistic sample. In this study, the individual statistic sample is considered as an average of all the pseudo-repetition done on a specific day on a specific tree/individual vine/plant. After this step, descriptive data were made to identify and check outliers in the dataset. A total of five data points were removed from the analysis because of obvious difference with the average value of the dataset. These outliers may have been caused by inadequate sample storage or an error by the spectrophotometer during the absorbance reading or laboratory manipulation. Only a sub-sample of the entire Dualex and scanner field dataset were compared to Chlorophyll and Nitrogen concentrations determined in laboratory. From this reduced dataset a calibration was made using a parametric linear regression followed by a bootstrap test to evaluate the error of the regression. The rest of the dataset were then used to indirectly follow the seasonal variability of the photosynthetic pigments. Then leaf/ needle biochemical data were average with a standard derivation and compared using an ANOVA test.

RESULTS

CALIBRATION RESULTS

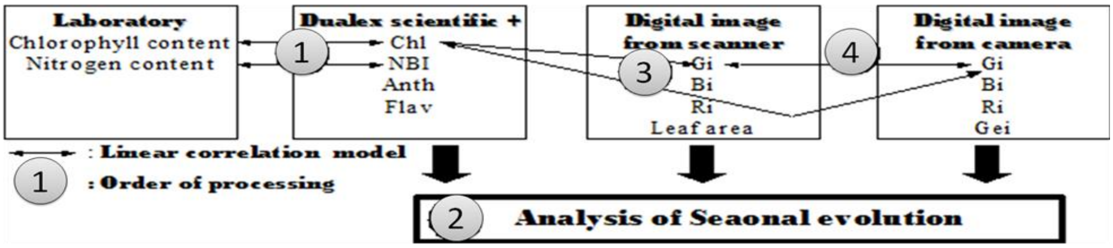


Figure 15: Flow diagram of data processing

The Dualex Scientific + was used on all sites and was calibrated against data measured in the laboratory (chlorophyll and nitrogen content) (Figure 20). Appropriate equations to calculate the concentrations of total chlorophyll were used. The extraction of nitrogen where done in laboratory by Kjeldahl method with sulphuric acid. A linear correlation was found between chlorophyll contents (Chla, Chlb and Chla+b) and the Chlorophyll Index from the Dualex Scientific +. A significant correlation was found for the eucalyptus (n=62) and the vineyard (n=60) with an r^2 of 0.80 and 0.69 respectively ($P<0.05$). The basic statistics and the linear correlations are presented in Appendix 1. For the maritime pine (n= 41) the correlation was statistically significant because it was above the critical Bravais-Pearson value of R ($p<0.05$) with an r^2 of 0.37($P<0.05$). Within the pine dataset there were two distinct groups of data found, one corresponding to the newly grown needles (Chl between 0 and 60 $\mu g.cm^{-2}$ and NBI between 0 and 200 $\mu g.g^{-1}$) and the one year-old needles (Chl between 60 and 145 µg.cm^{-2} and NBI between 200 and 490 µg.g^{-1}).

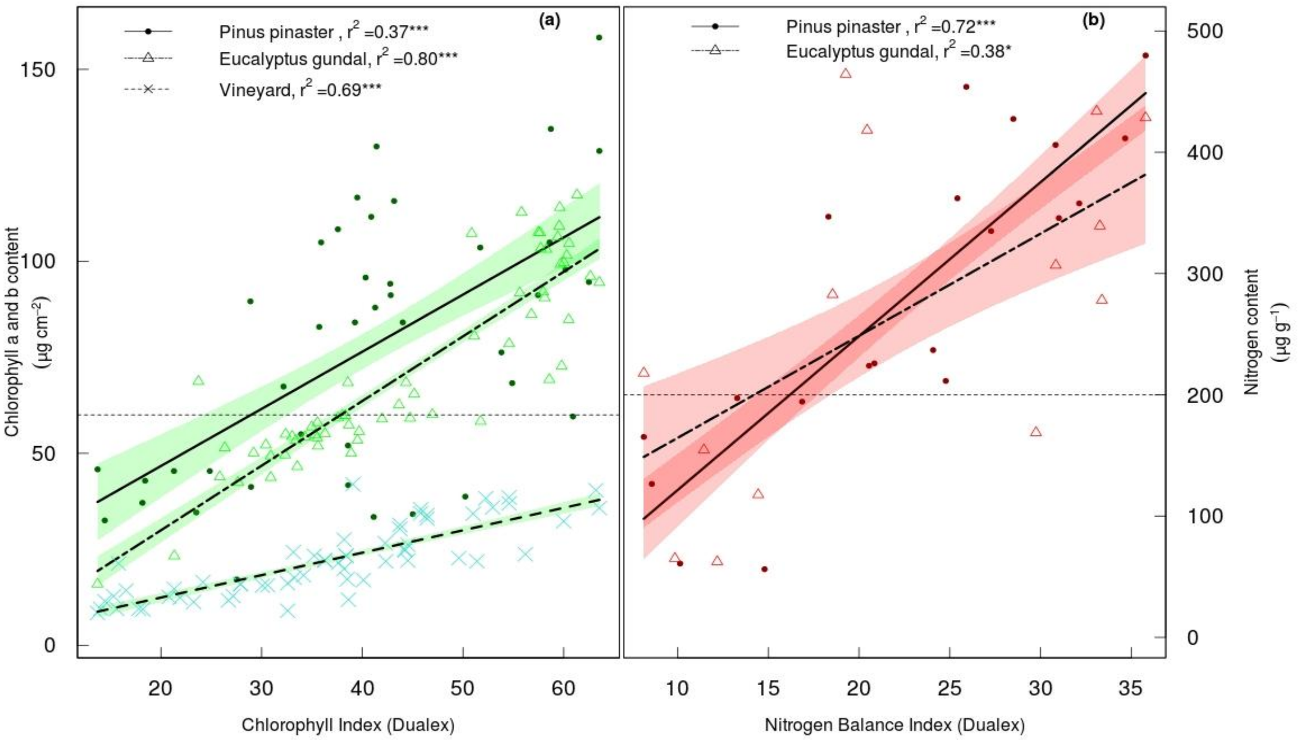


Figure 16: Relationship between leaf total chlorophyll and nitrogen content and Dualex Scientific + indices (Chlorophyll index and nitrogen balance index) for eucalyptus, maritime pine and vineyard samples. Data were pooled for linear regression analysis. Coloured bands indicate confidence intervals

The relationship for nitrogen in maritime pines needles (n=20) was also significant (Fig 20 and Appendix 1). For the eucalyptus (n=14) the correlation was significant with P<0.05. The significant positive correlations found between the chlorophyll and nitrogen concentrations measured in the lab and the measurements obtained from the Dualex Scientific indicate that the Dualex can be reliably used to extend the seasonal dataset and predict absolute variations in total chlorophyll and Nitrogen contents over the growing season. The results found in this study are also consistent with the results of Cerovic et al. (Zoran G. Cerovic et al., 2012) who found a similar relationship for vines (Cabernet, Merlot), maize, kiwi and wheat. In a different set of studies on *Red imperator*, *Profusion*, *Macrocarpa* and muskmelon a good correlation between lab pigment content and the chlorophyll index was also found (Demotes-Mainard et al., 2008 ; Padilla et al., 2014).

SEASONAL TRAJECTORIES OF CANOPY-LEVEL INDICES

Seasonal trajectories of RGB colour indices obtained from the field cameras are presented in Figure 18. Datasets were available from January 2015 to August 2015 for the Maritime pine site. The other site cameras were installed later between March and April 2015. The seasonal pattern for the pine green index consisted of a slow but noisy increase of the green index during winter and spring (DOY 1-125) whereafter the signal became stable and less noisy during early summer (DOY 125-212). From day 127, the pine trees began to flower but did not impact the green fraction strongly. From around day 152 needle growth began and continued for several weeks leading to a slow increase in the green fraction over time. However from around day of year 186, the Red index increased strongly whilst the green index and the blue index to a lesser extent decreased. This strong change in the colour signals also corresponded to a dry and hot period of weather across the Landes forest. Rainfall in the region was scarce over the period (DOY 163-209) and only returned at the very end of July (day 212) and beginning of August. As the frequency of rainfall increased during August the red signal was observed to drop whilst the green began to increase. During the field visits of July, one to three year old needles were observed turning brown as they dried out. The brown needles where still attached to the branch and visible from the camera pictures and visual observation.

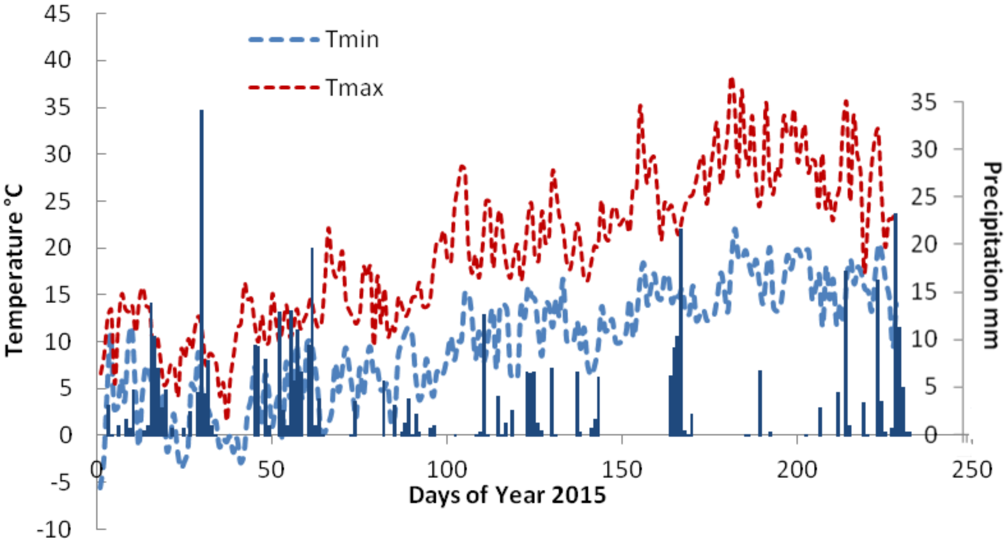


Figure 17: Seasonal course of air maximal and minimal temperature (°C) and precipitation (mm) for the studied year (2015)

The pattern on eucalyptus trees and grassland data is difficult to interpret due to a multiple gaps in the dataset. It is hard to judge what exactly was happening at Pierroton site from the camera data alone.

The day-to-day variability in the RGB indices was much smaller than that observed at the pine site. The vine green index displayed a gradual increase in the red, blue and green indices over several weeks (DOY 84-196). However, the GEI indicated there were clearly different phases to the signals. The GEI index increased between DOY 96 to 118 and DOY 150 to165 could be due to a strong influence of new green leaves. On the DOY 118 the vineyard inter-row widths was mown that has for consequences a reduction of the GEI index. On the DOY 157 a short session of irrigation was done on the vineyard and during July (DOY 184 to 212). After day 188 the green index increased rapidly. The DOY 188 corresponds of the date where they cleaned the grown under the vine and in the inter-rows. Shortly, after on day 204, a green fence was installed to protect the developing grapes from birds. This period was associated with a strong increase in the green index.

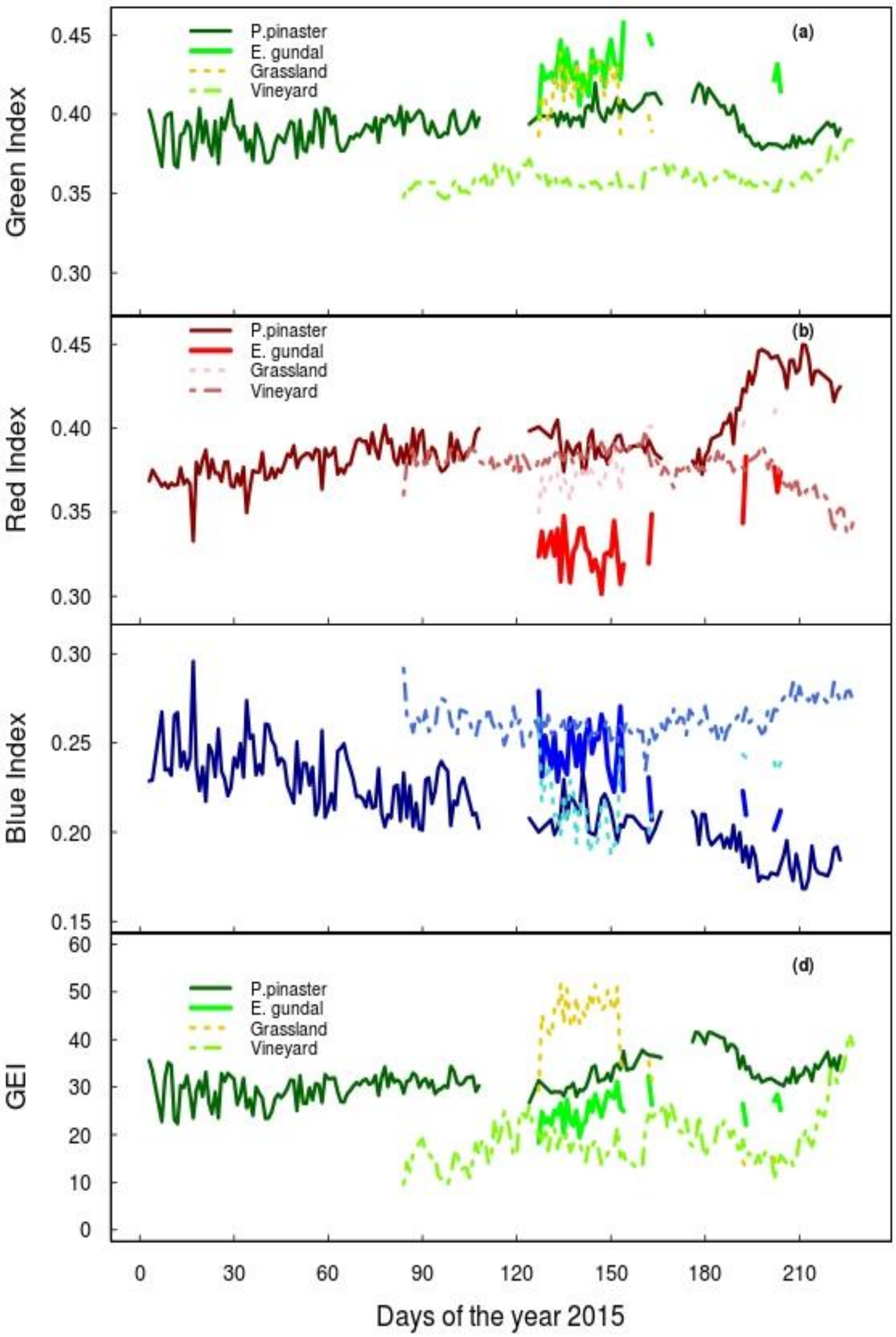


Figure 18: Colour indices (Green index, Red index, blue index and Green Excess Index measured from digital camera images for the four sites during the year of 2015.

SEASONAL TRAJECTORIES OF LEAF BIOCHEMICAL AND SPECTRAL PROPERTIES

Seasonal trajectories for the pine needle characteristics obtained from the Dualex Scientific + and the flatbed scanner are presented in **Erreur ! Source du renvoi introuvable.** for new (light symbols) and mature needles (dark symbols). A difference in chlorophyll, nitrogen and area (per six needles) was found between needles of a different age (ANOVA test: $P<0.05$, diff = 20.01 µg.cm^{-2} of chlorophyll, ANOVA test: $P<0.05$, diff = 11.78 µg.g^{-1} of nitrogen). Several leaf biochemical indices (chlorophyll and NBI) decreased gradually during the growing season reaching a minimum in the mean values around DOY 160, where after the values began increasing again until a maximum was reached around DOY 212. Overall, the mature needles presented stable biochemical properties over time compared to the new needles that presented an increase of chlorophyll, nitrogen content and area over time. The processed scanner images tracked the growth of the new needle area and indicated that the red and green indices increased slightly over time. In contrast, the blue index decreased over time. For the scanned signals the errors bars overlap for most of the season (new and mature needles mixed signals). The other features observed by the camera are not strongly visible on the leaf data.

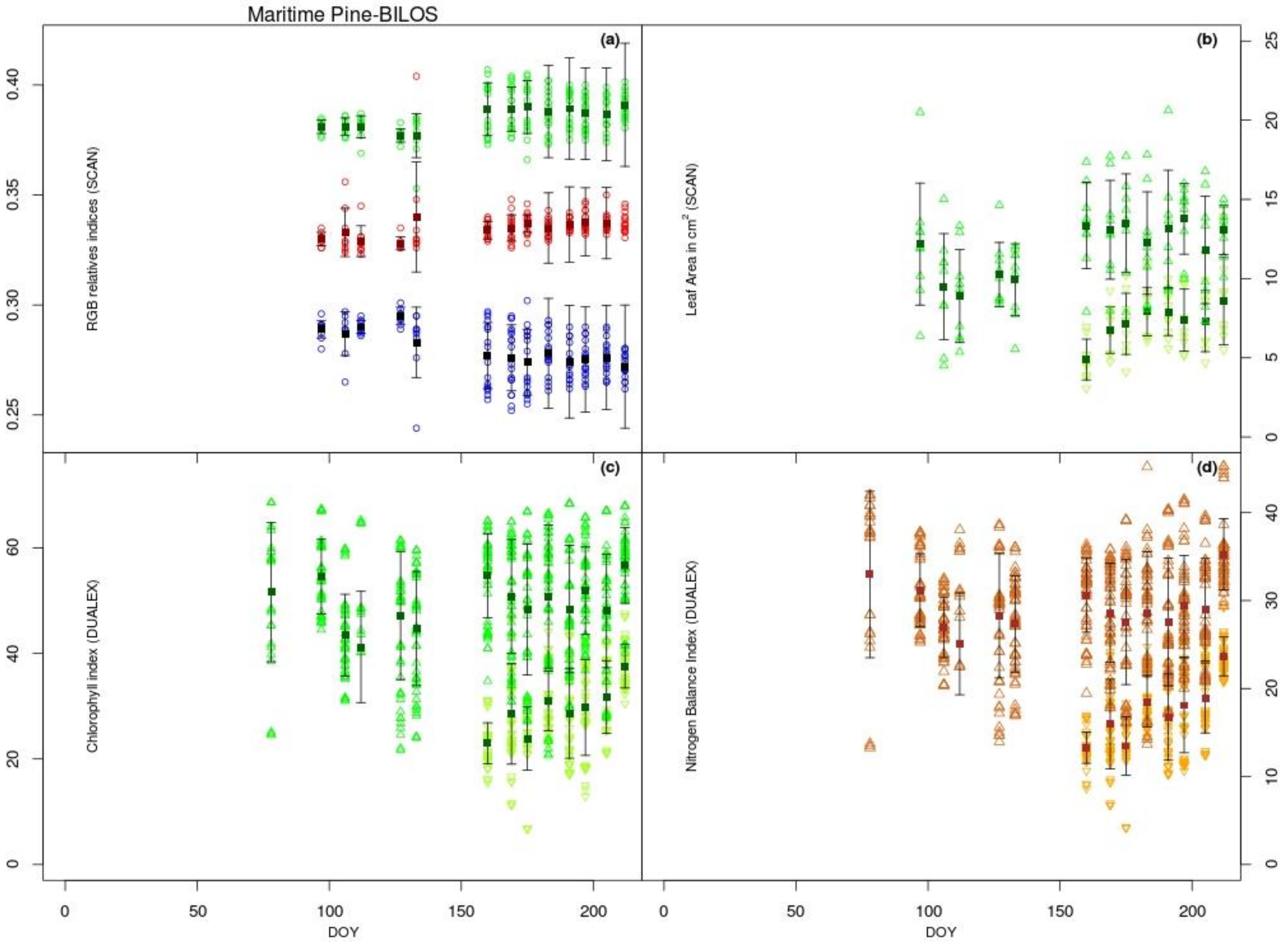


Figure 19: Biochemical and morphological properties of maritime pine needles over time from digital images analysis taken with a flatbed scanner and from the measurements with Dualex Scientific +. Light coloured triangle facing down symbolise the new needles and dark coloured triangle symbolise the mature needles. The mean value of the measurements per day has been calculated and has been shown on the graphs with the standard error bars.

At the Pierroton site, the results clearly show that the leaf area for eucalyptus trees increased during the course of the growing season. (**Erreur ! Source du renvoi introuvable.**) The biochemical indices presented a clear step change around DOY 125 that coincided with the growth of new leaves. Both the upper and lower sides of the eucalypt leaves were analysed. The lower side of the leaf is shown using light coloured symbols on the graphs. Statistical analysis of each of the indices demonstrated there was no significant difference between leaf sides measured is not significant (ANOVA test. Not different. P =0.93 for chlorophyll, P=0.87 for nitrogen). As shown for maritime pine, there was a minimum mean value for the chlorophyll, nitrogen and leaf area around DOY 120.

In contrast the anthocyanin content peaked around DOY 120 and again around DOY 145. After day 120 the chlorophyll and nitrogen indices began to increase again slowly but never reached the same values observed prior to the growth of the new leaves during the timeframe of the present study. Unlike the canopy RGB values it was possible to follow the seasonal trajectories of the leaves. The scanned leaf green fractions indicated a peak on DOY 160 and thereafter a gradual reduction in strength to date. On the other hand, the red and blue fractions tended to decrease in value until DOY 160 but started to rise again thereafter.

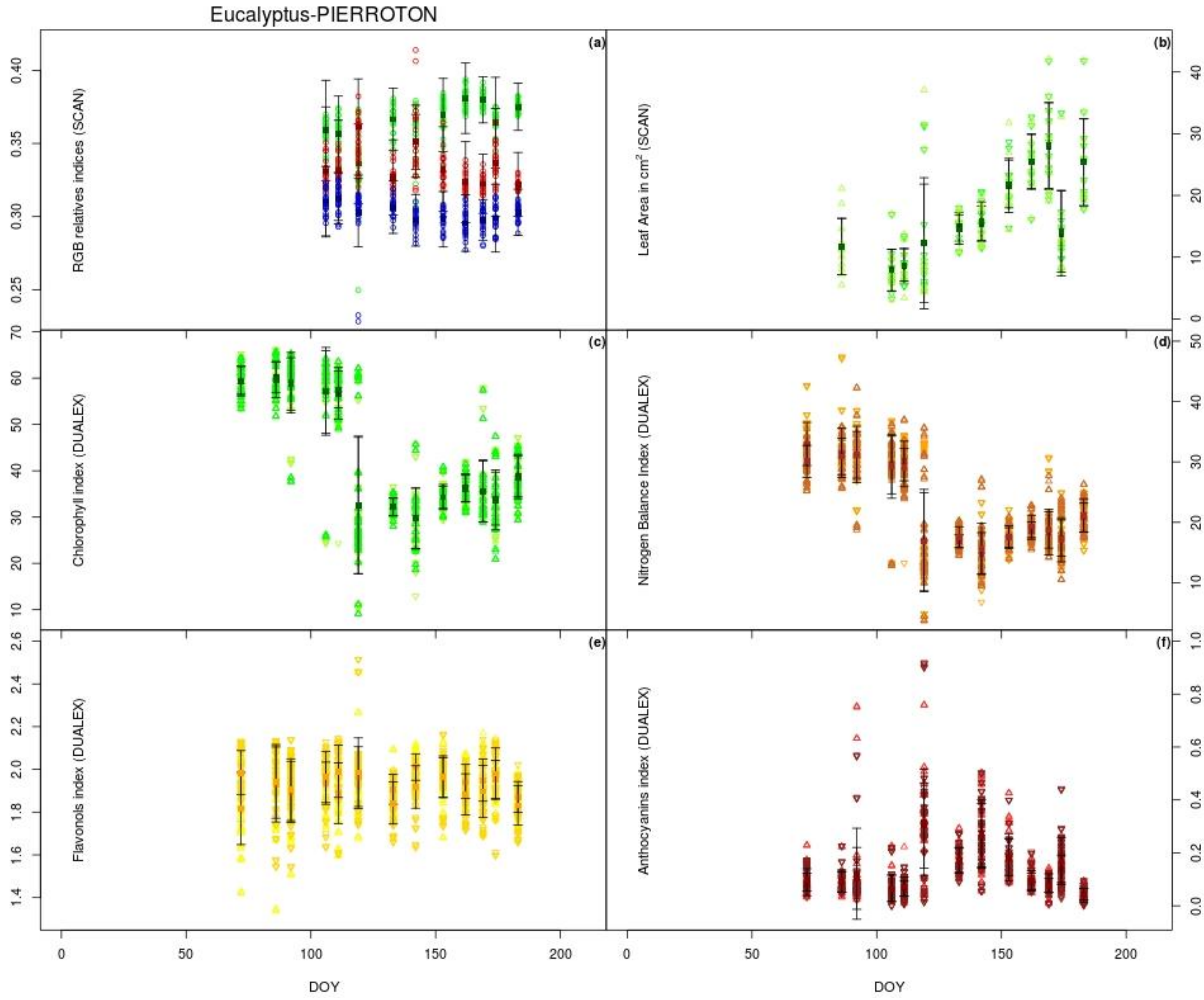


Figure 20: Biochemical and morphological properties of eucalyptus leaves over time from scanned digital images and the Dualex Scientific +. Light coloured triangle facing down symbols indicate data from the lower side of the leaf, dark coloured triangle symbols indicate data collected from the upper side of the leaf. The mean value per day has been calculated and is also shown on the graphs with the standard error bars.

On the RESDUR site, weekly Dualex Scientific + measurements were made on the vines. Unfortunately it was not possible to destructively harvest samples from this site. For most of the season at this site chlorophyll, nitrogen and flavonols increased steadily over time. On one occasion DOY 175 the data were collected for the first time by a new operator, thus it is uncertain whether this anomaly is a feature of the seasonal trajectory or an artefact introduced by the sampling protocol. In addition there was also a strong decrease in the anthocyanin index of the vine leaves over the season. The strong increase in the green fraction observed by the camera at this site may be linked to the increase observed in the chlorophyll index for the same period.

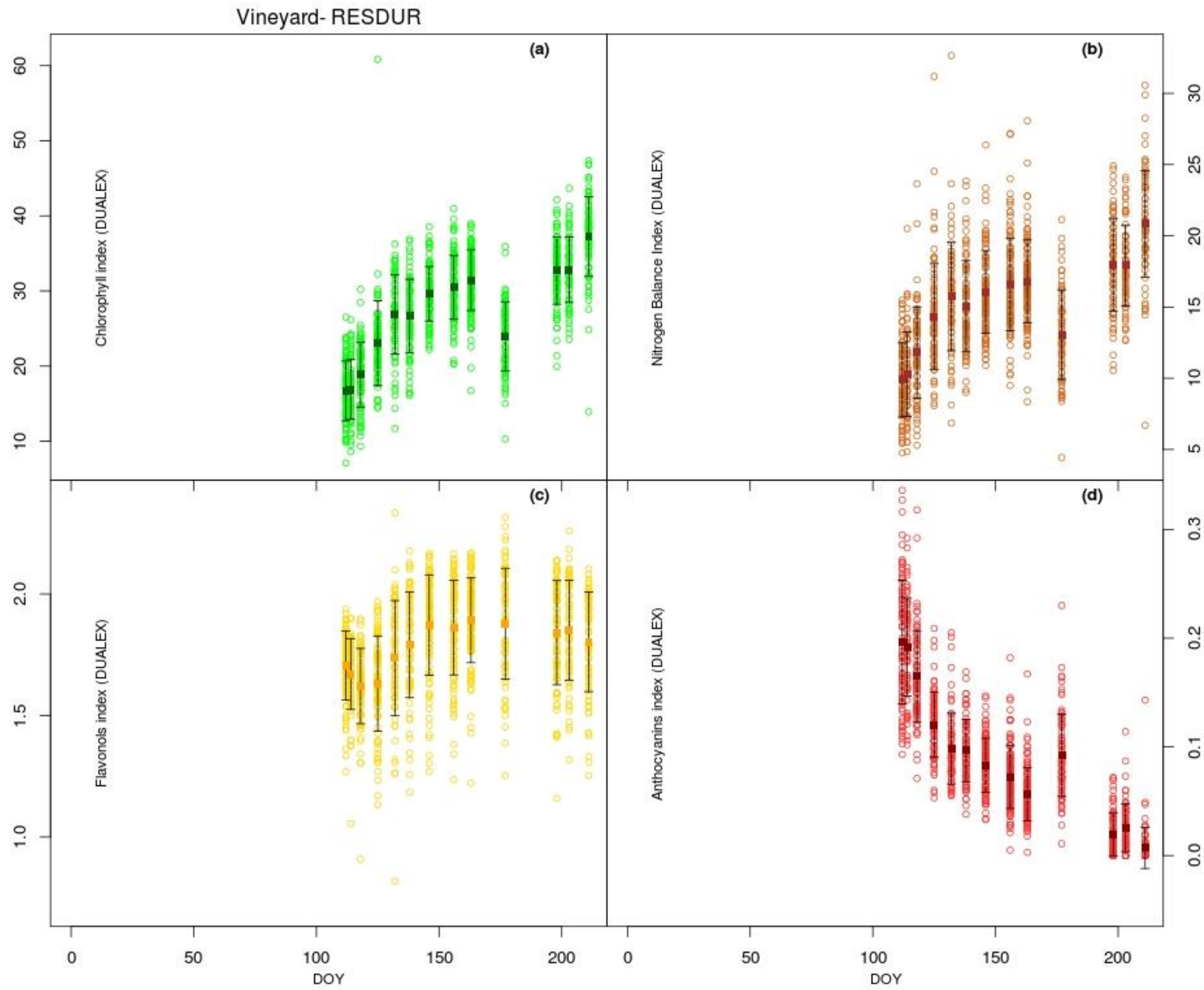


Figure 21: Biochemical and morphological properties of vine leaves of numerous varieties over time from measurements with Dualex Scientific +. The mean value of the measurements per day has been calculated and has been shown on the graphs with the standard error bars.

COMPARISONS BETWEEN LEAF SPRECTRAL PROPERTIES AND LEAF BIOCHEMICAL PROPERTIES

The technique of comparing chlorophyll content measurements to the scanned colour properties of the leaf is fairly novel. Results for this analysis are presented in Appendix 1 alongside an extended analysis between scanned leaves and the Dualex which could be performed on a much larger dataset. The strongest correlation with total chlorophyll content of leaves was found for the scanned blue index for all species (r^2 between 0.48 and 0.59 with $P<0.05$). For the scan estimated green fraction the strongest correlation with total chlorophyll was found for the pine needles ($r^2= 0.48$, $p<0.05$). This indicated that as the chlorophyll content decreased the green index increased. For the other species no significant relationship was found for the scanned green index and total chlorophyll concentrations. In contrast, for these two species there were stronger correlations between the red index and the total chlorophyll content. For the pine needles there was only a weak relationship found.

When comparing the results between the scanned colour indices and the Dualex for the pine needles no significant relationship could be found. Whereas, for the red and blue signals significant correlations were only found for the pine and eucalypt leaves, but not for the vines.

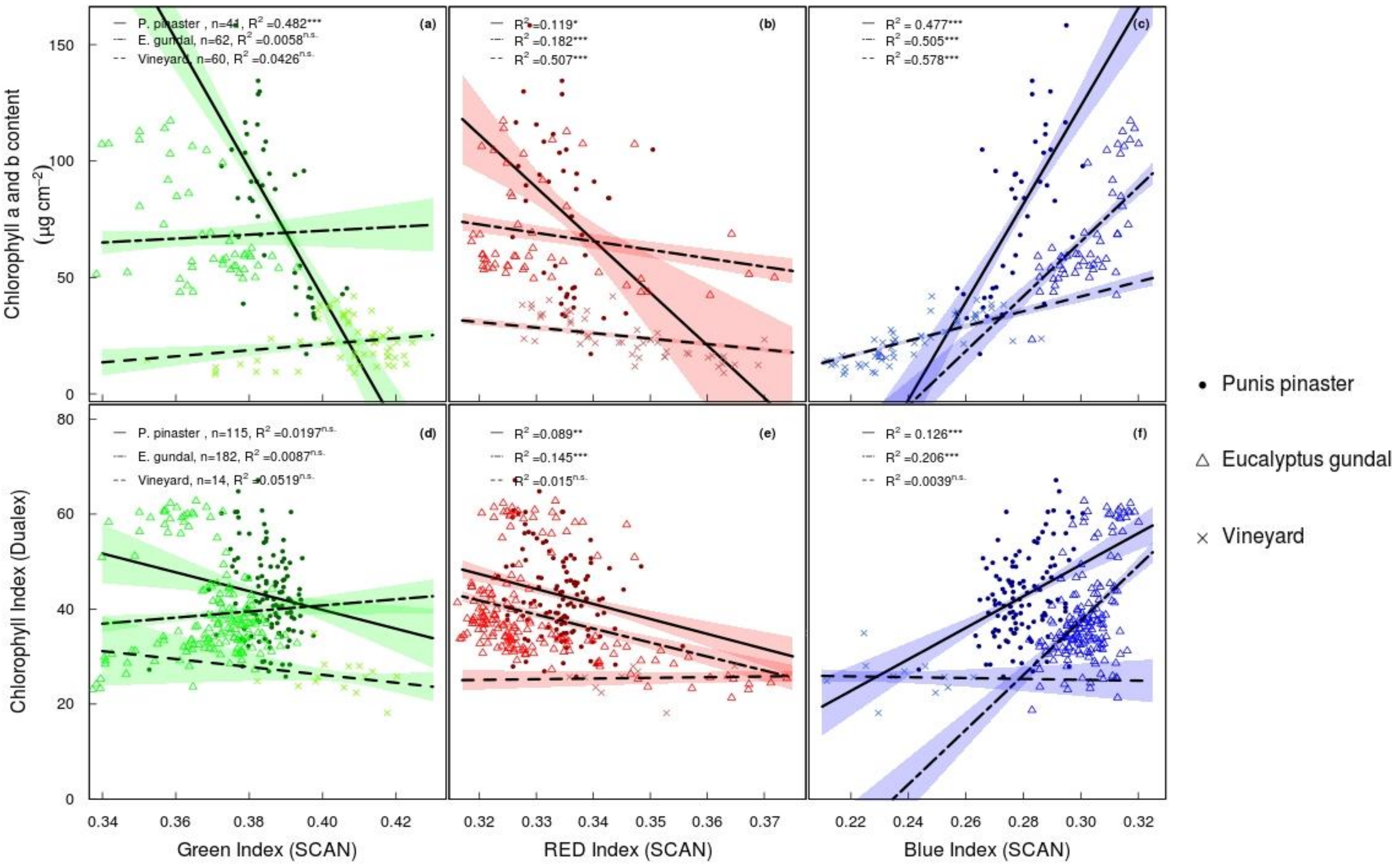


Figure 22: Linear correlation between Chlorophyll content/ index and Colour indices (Gi, Ri, Bi) from digital images taken with a flatbed scanner of the different sites samples (maritime pine, eucalyptus and vine varieties). The coloured bands indicate confidence intervals.

COMPARISON BETWEEN CANOPY COLOUR AND LEAF SPECTRAL PROPERTIES

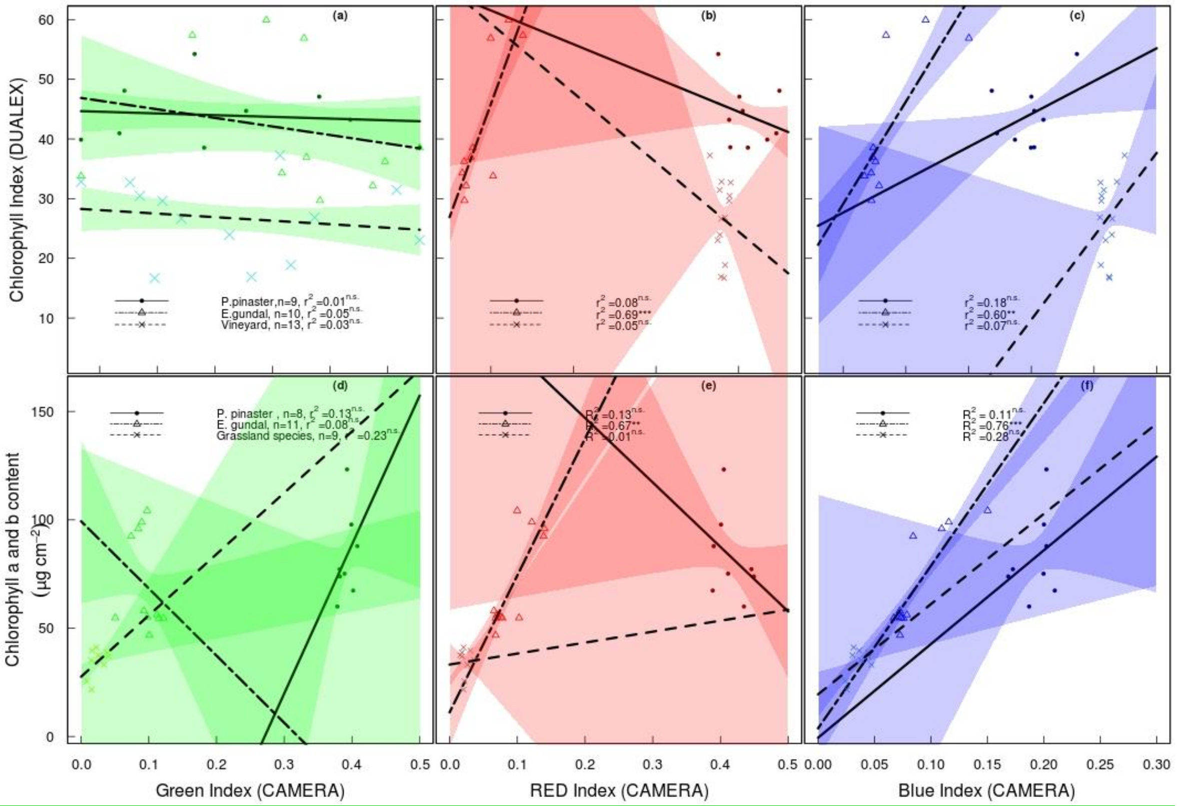


Figure 23: Linear correlation between Chlorophyll content/ index and Colour indices (Gi, Ri, Bi) from digital camera images of the different sites samples (maritime pine, eucalyptus and vine varieties). The coloured bands indicate confidence intervals.

Finally, a comparison of the RGB Figure 27 reports a scatter plot between RGB indices average by from digital camera images and chlorophyll contents and chlorophyll index derived from Dualex scientific + measurements. The data set were very small (n=8 to 13). The Blue index is again the more correlated with the chlorophyll but it is only significant on eucalyptus trees. The correlation is significant also between red index and chlorophyll for eucalyptus trees measurements.

DISCUSSIONS

Several previous papers have shown that canopy greenness and redness derived from digital images can effectively aid the identification of different phonological dates between species. (Wingate et al., 2011 ; Filippa et al., 2015 ; Toomey et al., 2015) However, the linkage between the leaf biochemical changes over the year and the greenness, redness and blueness is still elusive. Only a few of these studies compared species phenology and even fewer projects that have attempted to use digital images, studied leaf biochemical properties of co-existing plant species. (Richardson et al., 2009 ; Yang et al., 2014)

This study started by a calibration phase of the Dualex Scientific +. The results indicated that chlorophyll and nitrogen content could be estimated with the Chlorophyll index and Nitrogen Balance index (Figure 16). The hypotheses were that the chlorophyll content depends on the specie, sample age (mature or young) and the leaf side (upper or lower). There are a few papers using Dualex and calibrated based on laboratory extraction. The previous comparisons between vine leaf chlorophyll content and chlorophyll Index from Dualex measurements report regression coefficients similar to those found in this study. (Zoran G Cerovic et al., 2012)The researchers in this paper showed a better R-squared due to a less important noise in their data and a larger range and quantity of data (Chl Index: 10 to 60).

Further analyses were done to see the influences of factors on this correlation. Indeed the model is influenced by the sample age and the tree but is independent of the day of sampling (LME test). The chlorophyll extraction was a difficult task and the data was compared with the results numerous papers and acetone extraction methods on 50 samples. The results were consistent with the DMSO extraction.

The seasonal evolution of the Green, Red, Blue and Green Excess indices from digital camera images was studied. The results showed clearly specific trajectories depending on the specie. As a consequence of the species-specific difference in chlorophyll content we observed a contrast in the Green index and Green Excess index values over the growing season (Vineyard vs Maritime pine). Specifically, for the maritime pine the analysis of the digital images provided an opportunity to investigate the direct and indirect consequences of the heat and dry wave in South of France in summer 2015. The consequences could be evaluated not only on the canopy growing season length but also its physiological capacity (photosynthetic, water efficiencies etc.). The vineyard has shown a steep increase of the green index during the summer. But the installation of a green fence disturbed our records. We were expected a big step on the green index records. In contrary the green index increase rapidly and this tendency is also shown on the chlorophyll content measured over time. A hypothesis could be emitted that the fence amplified the actual green index augmentation. The results concerning the eucalyptus tree and their understorey are difficult to interpret due to important gaps in the dataset. The camera installation was improved on August and hopefully the data collection will be better for August to December.

Field measurements are often substituted by automated monitoring systems to follow phenology evolution on various ecosystems (Gonsamo et al., 2013). Indeed, more attention is devoted to understanding the impact of phenological changes or annual variability can have on ecosystems component as photosynthesis and carbon sequestration that is directly link to photosynthetic pigments concentration. (Richardson et al., 2012, 2010). In this study, the pigments photosynthetic leaf contents were used to characterize phenological evolution. The maritime pine field showed a pattern through the season of chlorophyll and nitrogen contents in early growth needles. This evolution is correlated at the Blue index records by the digital camera and by the flatbed scanner (Figure 22 and Figure 23). This could be predicted by the fact that chlorophyll pigments absorb blue wavelengths. For the eucalyptus trees, the measurements of biochemical leaf properties were able to be compared between upper and lower side of the leaf. Indeed, the differences between eucalyptus leaf side's measurements were not significant. Therefore, there is clearly a step in the results between sampling on new growth leaves and mature leaves. The difference is currently measure in greenhouses on *Pinus pinaster, Quercus robur, Molinia Caerulea, Pteridium aquilinum, Eucalyptus guni, Zea mays, Betula spp* in collaboration with Dr Teresa Gimeno and Noelia Saavedra. The vine leaf biochemical properties have shown a very clear pattern over the season. The evolution of the chlorophyll index measured with the Dualex Scientific + presented similar trajectories than the colour indices recorded with the digital camera. Because of this pattern, we can conclude that the Green index from the digital camera imagery increased on the last period (DOY 188-227) is not only due to the green fence.

The link between the leaf spectral index and the leaf biochemical was explored by Xi Yang and is team (Yang et al., 2014). Similar tendencies were found for deciduous forests with more than a strong correlation. The correlation between Red, Green and Blue index from the camera and the biochemical properties of the leaves were done (Figure 23) The correlations founded were statistically significant for the Blue index. A model will be applied on scan imagery data to use the full light visible spectra recorded by the scanner to predict the photosynthetic pigments leaf content. This model is based on the model PROSPECT calibrated on the reflectance and transmittance sensitivity of the scanner (Barry et al., 2009).

Finally, the link between chlorophyll content of leaf samples where compared to the R, B, and G indices extracted from the digital camera images. The correlation was significant for blue and red index on eucalyptus leaves. The eucalyptus records from digital camera were fragmented. More data will be necessary to conclude on this relationship. Data collection will continue over autumn season 2015.

CONCLUSIONS

The use of automated digital cameras for monitoring vegetation phenology is becoming widespread. Digital repeat photography has been used to characterize the phonological evolution of canopies, correlated to C02 fluxes and photosynthetic capacities (Wingate et al., 2008 ; Migliavacca et al., 2011 ; Richardson et al., 2013). Despite that this approach become central to phenological records, there has yet to be a critical assessment of the relationship between colour indices extracted from digital camera and canopy or leaf physiology. We used four different experimental sites to measure the biochemical leaf properties in laboratory or using a Dualex Scientific + directly on a field in combination with colour indices extracted from camera and scanner digital photography analyses. The results showed that digital camera RBG indices are not only useful for highlighting phenological transitions as bud burst, flowering but also characterizing consequences of drought or irrigation on the plant physiology. Therefore the digital camera is useful for identifying differences over season in photosynthetic pigment leaf concentration. This will provide an opportunity to investigate climate changes impacts plant phenology and ecosystems interactions. However, the digital camera monitoring is strongly dependent of the light conditions and canopy structure. Use a flatbed scanner to extract leaves light reflectance could increase our ability to predict the synthetic pigments content. This study showed a clear link between blue index and chlorophyll content. Developing a model based on PRESPECT model to calibrate the scanner reflectance and transmittance will be valuable focus of future work.

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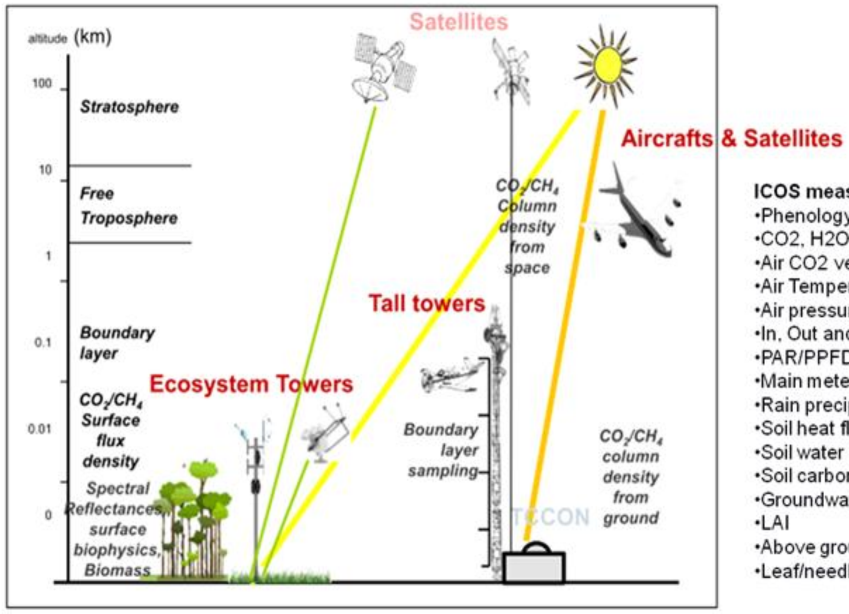
APPENDIX

1- LINEAR REGRESSION STATISTICS

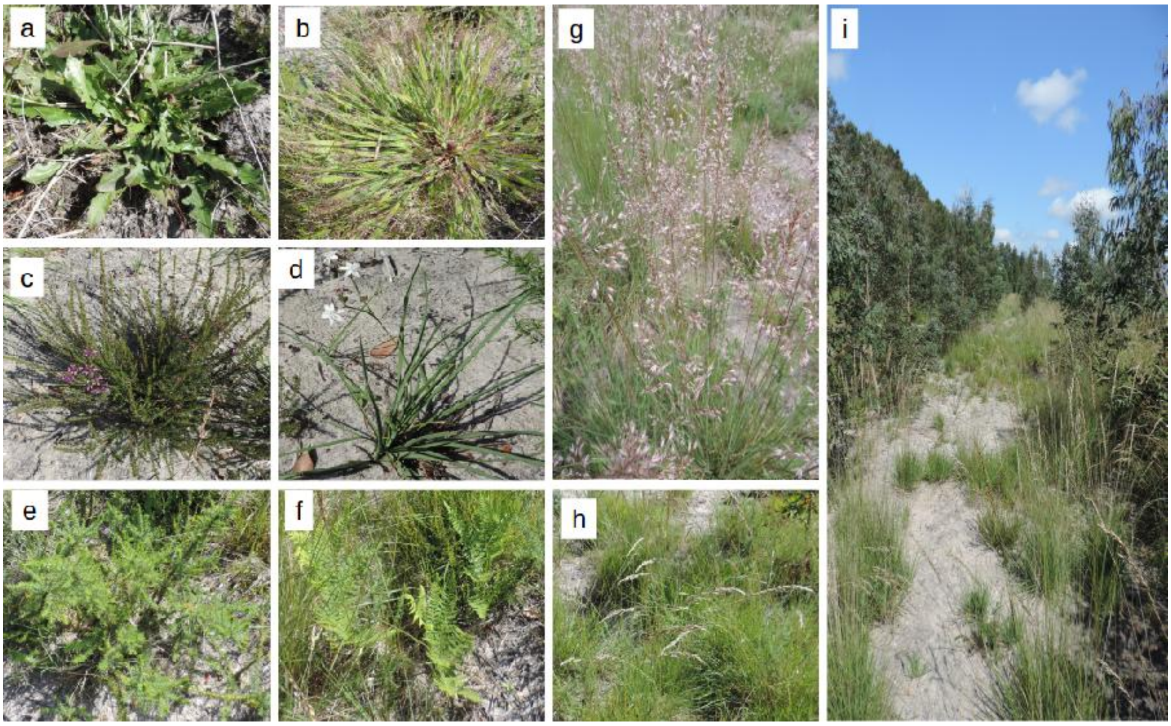
X	y	Specie	Model parameters y=ax+b		Model statistics		Descriptive statistics (x unit)			Descriptive statistics (y unit)			N	Critical Bravais-Pearson Value of R (p<0.05)
			a	b	r^2	RMSE (x unit)	Mean x	Min x	Max x	Mean y	Min y	Max y		
Chlorophyll a μg.cm^{-2} (laboratory)	Chlorophyll Index (Dualex Scientific +)	Eucalptus	0,76***	-0,96^{ns}	0,85***	4,42	32,32	5,94	54,36	43,95	10,39	64,74	62	0,2502
		Maritimes pine	0,73***	8,50^{ns}	0,37***	13,36	38,27	10,31	78,93	40,73	13,71	63,55	41	0,3045
		Vine	0,48***	-1,58^{ns}	0,58***	3,05	10,975	3,68	23,91	25,16	12,43	40,33	60	0,2502
		Total	0,90***	-6,68***	0,67***	9,36	24,38	3,68	78,93	36,22	10,39	64,74	163	0,1582
Chlorophyll b μg.cm^{-2} (laboratory)	Chlorophyll Index (Dualex Scientific +)	Eucalptus	0,79***	4,28^{ns}	0,72***	6,9	38,84	10,00	64,23	43,95	10,39	64,74	62	0,2502
		Maritimes pine	0,76***	8,43^{ns}	0,36***	14,15	39,27	6,85	79,38	40,73	13,71	63,55	41	0,3045
		Vine	0,56***	-3,18*	0,70***	2,74	10,95	2,50	21,84	25,16	12,43	40,33	60	0,2502
		Total	1,02***	-8,33***	0,68***	10,4	26,61	2,50	79,38	36,22	10,39	64,74	163	0,1582
Chlorophyll ab μg.cm^{-2} (laboratory)	Chlorophyll Index (Dualex Scientific +)	Eucalptus	1,54***	3,32^{ns}	0,80***	10,97	71,16	15,95	117,29	43,95	10,39	64,74	62	0,2502
		Maritimes pine	1,49***	16,93^{ns}	0,37***	27,44	77,54	17,16	158,31	40,73	13,71	63,55	41	0,3045
		Vine	1,04***	-4,22^{ns}	0,69***	5,25	22,04	8,42	41,94	25,16	12,43	40,33	60	0,2502
		Total	1,92***	-15,01*	0,68***	19,41	54,68	8,42	158,31	36,22	10,39	64,74	163	0,1582
Nitrogen μg.g^{-1} (laboratory)	Nitrogen balance Index (Dualex Scientific +)	Eucalptus	9,23*	65,44^{ns}	0,37*	110,2	267,01	62,4	464,33	21,85	9,04	34,28	14	0,4975
		Maritimes pine	12,69***	-5,48^{ns}	0,72***	67,3	281,27	56,24	479,83	22,59	8,14	35,79	20	0,4229
		Total	11,17***	26,42^{ns}	0,56***	87,33	275,4	56,24	479,83	22,29	8,14	35,79	34	0,3293
Chlorophyll ab μg.cm^{-2} (laboratory)	Green Index (Scanner, Canon, LIDE 110)	Eucalptus	84,03^{ns}	36,48^{ns}	0,006^{ns}	23,69^{ns}	71,16	15,95	117,29	0,363	0,274	0,393	53	0,2734
		Maritimes pine	-2767***	1148,3***	0,48***	24,81	77,54	17,16	158,31	0,387	0,373	0,407	41	0,3045
		Vine	130,13^{ns}	-30,68^{ns}	0,04^{ns}	9,16	22,04	8,42	41,94	0,405	0,352	0,424	60	0,2502
		Total	-617,61***	290,44***	0,20***	30,21	54,68	8,42	158,31	0,386	0,274	0,424	154	0,1579
Chlorophyll ab μg.cm^{-2} (laboratory)	Red Index (Scanner, Canon, LIDE 110)	Eucalptus	-363,26**	189,05***	0,18***	21,49	71,16	15,95	117,29	0,336	0,319	0,495	53	0,2734
		Maritimes pine	-2258,7*	834*	0,12*	32,35	77,54	17,16	158,31	0,335	0,326	0,350	41	0,3045
		Vine	-2355,76***	106,29***	0,51***	6,57	22,04	8,42	41,94	0,357	0,328	0,440	60	0,2502
		Total	-641,58***	273,02***	0,25***	29,24	54,68	8,42	158,31	0,344	0,319	0,495	154	0,1579
Chlorophyll ab μg.cm^{-2} (laboratory)	Blue Index (Scanner, Canon, LIDE 110)	Eucalptus	1173,9***	-286,8***	0,51***	16,71	71,16	15,95	117,29	0,301	0,230	0,320	53	0,2734
		Maritimes pine	2102,4***	-507,1***	0,48***	24,93	77,54	17,16	158,31	0,278	0,257	0,301	41	0,3045
		Vine	317,1***	-53,3***	0,59***	6,08	22,04	8,42	41,94	0,238	0,176	0,286	60	0,2502
		Total	760,99***	-153,42***	0,54***	22,83	54,68	8,42	158,31	0,27	0,176	0,32	154	0,1579

2- THE CONCEPT ICOS: OBSERVATION IN LONG TERM OF FLUX AND GHG CONCENTRATIONS



ICOS measurements:
- •Phenology monitoring with repeat digital photography(Stardot SC5 IR)
- •CO2, H2O and sensible heat fluxes (eddy covariance tower)
- •Air CO2 vertical profile
- •Air Temperature and Rh profile
- •Air pressure
- •In, Out and Net SW, LW radiation, surface temperature
- •PAR/PPFD incident/reflected
- •Main meteo vars (Ta, Rh, Swin, precipitation)
- •Rain precipitation
- •Soil heat flux
- •Soil water content profile, Soil temperature profile
- •Soil carbon content
- •Groundwater level
- •LAI
- •Above ground biomass
- •Leaf/needle N content

3- GRASSLAND SPECIES OF THE UNDERCOVER OF THE EUCALYPTUS PLANTATION



a) Hypochaeris radicata (Porcelle enracinée, Cat’s ear)
b) Holcus lanatus (Houlque laineuse, Yorkshire Fog)
c) Calluna vulgaris (Callune, Heather) Éricacées
d) Simethis mattiazzii (= S. planifolia, Phalangium à feuilles planes, Kerry Lily)
e) Ulex minor (= U. nanus, Ajonc nain, Dwarf gorse)
f) Pteridium aquilinum (Fougère-aigle, Bracken) (Dennstaedtiacées)
g) Deschampsia flexuosa (Canche flexueuse, Wavy Hair-grass)

h) Molinia caerulea (Molinie bleue, Purple moor grass)

I) View of under Eucalyptus grass layer

4- DUALEX SCIENTIFIC+

The DUALEX SCIENTIFIC+™ is a sensor that measures flavonol, anthocyanin and chlorophyll indices.

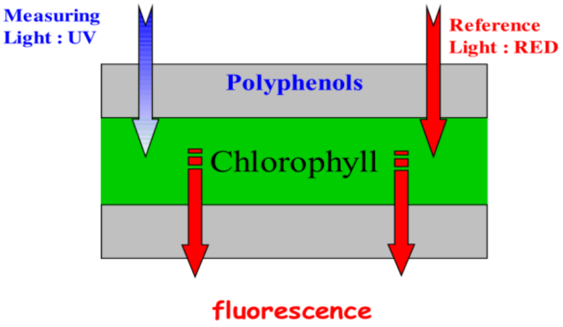
Epidermal polyphenols were estimated using the Dualex portable leaf-clip (Force-A, Orsay, France) according to Cartelat et al. (2005). Dualex measures UV absorbance of the leaf epidermis by double excitation of Chl a fluorescence using UV (375 nm) and red (650 nm) light. Epidermal UV-absorbance is determined from the UV/red Chl fluorescence excitation ratio. Red light is not absorbed by the epidermis and reaches the mesophyll where it excites Chl. Therefore, red-excited Chl fluorescence can serve as a reference signal to which UV-excited Chl fluorescence can be related. Absorbance at 375 nm is due mainly to water-soluble flavonoids stored in epidermal vacuoles. (Louis et al., 2009)

The measurement of the UV optical absorbance of the epidermis is based on the fluorescence emitted by the chlorophyll located in the mesophyll. This principle is described below (Figure 1).

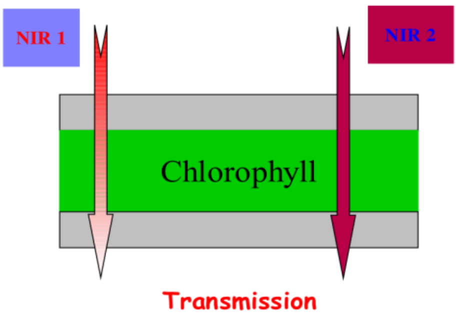
It is well known that chlorophyll emits fluorescence when illuminated. This fluorescence is induced by UV light as well as by red light. In a leaf, the epidermis absorbs the UV while it transmits the red.

Then by comparing the fluorescence induced by UV and induced by red, the absorbance of the epidermis can be determined. For example in the case of flavonols, the radiation is highly absorbed by the epidermis, whereas in the red range the absorption is very low. Thanks to its unique patented technology, DUALEX leaf-clip measures quantitatively the optical absorption of the leaf epidermis in UV.

The epidermal absorption is directly linked to the concentration of the leaf polyphenols:



Description of fluorescence and absorption



Differential transmission for two NIR wavelengths

The optical absorbance of the epidermis in the UV is therefore calculated as the ratio of chlorophyll fluorescence induced by the Measuring Light compared to that induced by Reference Light. It is important to note that the chlorophyll acts, in the case of DUALEX, like an internal detector (sensor) of photons. This method is known as the LogFER method. The measurement of the chlorophyll content of the leaf is based on the measurement of the difference in transmission of two wavelengths, both in the near infra red (NIR).

A chlorophyll-specific absorption index is calculated as a difference of the optical transmission at two different wavelengths in the NIR.