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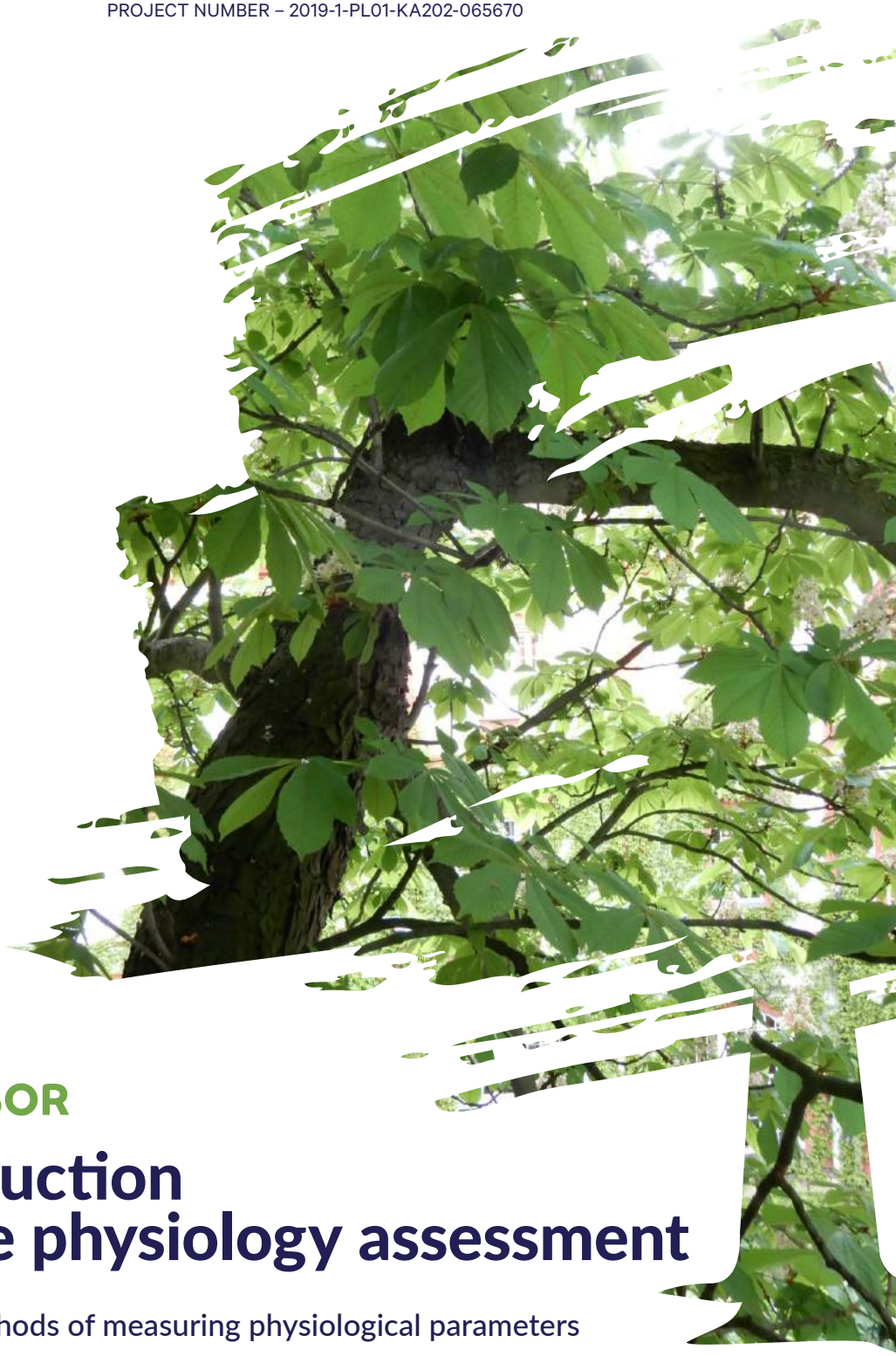

TREE
ASSESSOR

Introduction to tree physiology assessment

Practical methods of measuring physiological parameters

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Practical methods of measuring physiologial parameters

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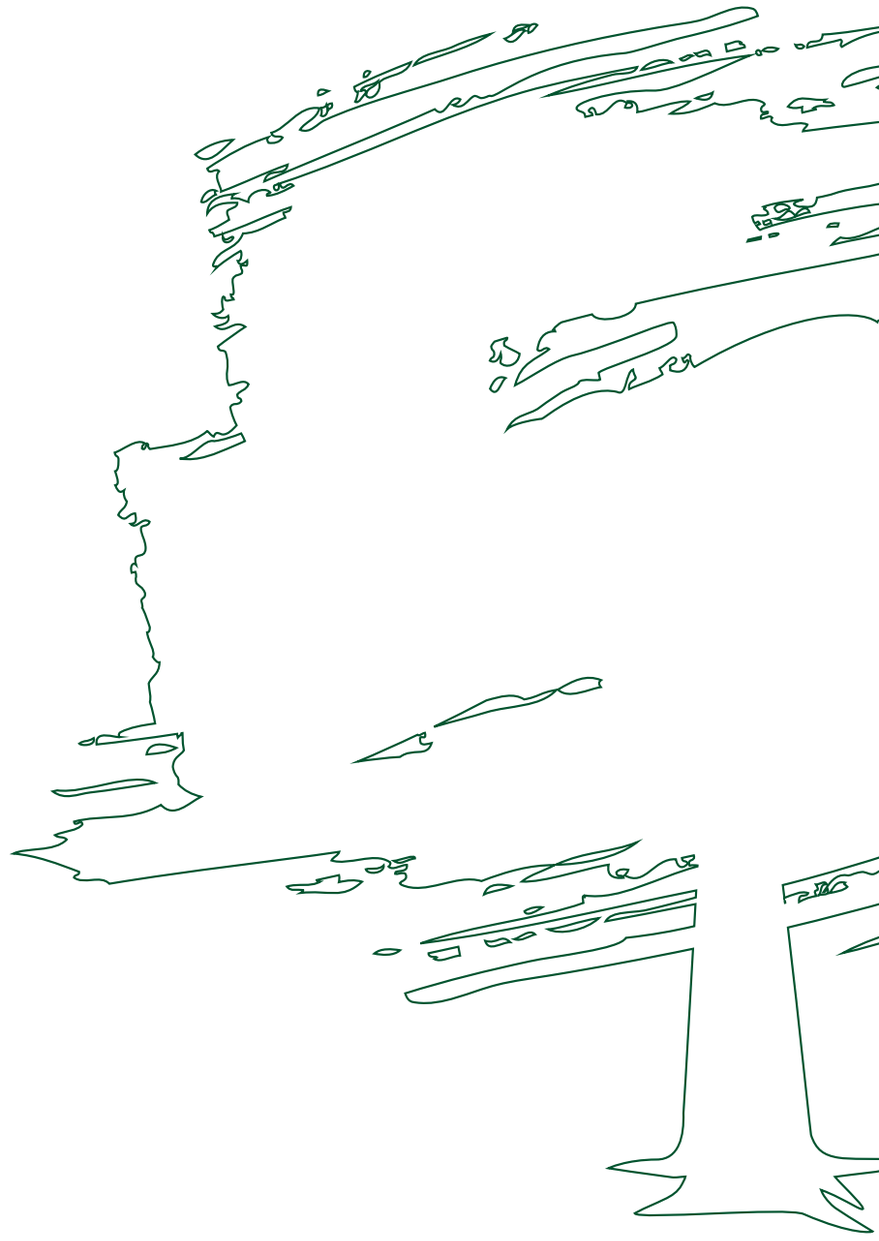

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TREE ASSESSOR

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Introduction

Trees are complex organisms and environmental stress impacts their diverse physiological processes on various levels – from cells and tissues, to whole body processes. The resulting changes are not easy to measure. Most popular research methods focus on gas exchange, chlorophyll content and fluorescence, and water potential of leaves, shoots or a whole stem. It should be noted, however, that values of these parameters do not necessarily reflect the processes on a whole organism level. Countless physiological experiments and measurements were conducted on simple annual plants, driven by the needs of agriculture. However, analogies to trees are not always warranted. Tree parts possess certain degree of autonomy, and their interconnections are not well known. Also, lifespan, as well as time scale of tree's reaction, are different than those of smaller, short living plants.

When faced with stress, trees first try to adapt to it, and then make efforts to compensate for losses. For example, in response to drought, stomata close. If the stress continues, leaves wilt, and are shed to prevent further water loss. Precocious leaf discolouration is observed and if the drought is chronic, impacted trees may die, usually beginning from the top. The decline may be limited to the top part, which is most difficult to be supplied with water. Similar symptoms to those of drought are observed after damage to roots due to their severance or soil compaction. In the longer term, water deficit mobilises the tree to expand or restore its root system. When coupled with reduced photosynthetic production (closed stomata), this leads to a decrease in tree growth, both longitudinal (twigs) and in thickness (stem and branch diameter). New leaves are often smaller and may contain less chlorophyll (being chlorotic). Damage to leaves can be also caused by harmful substances, such as salt and salt aerosols, pesticides, and exhaust fumes.

The processes, described above, usually take few years. This is for several reasons, first,

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most of the processes in trees are slower, in comparison to the human organism. Second, healthy trees possess abundant reserves that help with survival in the face of austerity. Third, an environmental stimulus sometimes elicits a cascade of effects, of which later factors impact adversely tree's health. For example, a minor wound to roots may start fungal decay which in turn could lead to the decline of an entire organism and death or failure of the affected tree later in its life.

Contemporary research methods enable to assess the magnitude of stress reaction when the stress in the plant has been ascertained or to detect stress reactions prior to visible damage. Every method has its own pros and cons. As a tree tends to keep all physiological processes balanced within an entire organism, there is a possibility that early symptoms of tree decline may not be detected by particular physiological parameters. Moreover, each of the methods may illustrate a particular physiological problem. Therefore, it is good to keep in mind not only the advantages but also limitations of every applied method. In fact, the combining of different methods is a better way to get an overall insight into the health of a tree.

The Authors would like to thank Prof. Stefan Pietkiewicz and Tomasz Horaczek Ph.D. for their valuable comments on this chapter. We would also like to express our thanks to Ms Charlotte Aldred for the proofreading of the English version of the text.

Fot 1 (left): Tatiana Swoczyna, Ph D. (fot. Jacek Mojski)
Fot 2 (right): Piotr Tyszko-Chmielowiec, Ph D. (fot. Woong Lee)

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I.

Relative chlorophyll content

Sugar production in plants is possible due to chlorophyll (Chl) molecules providing conversion of light energy into chemical energy. Therefore, chlorophyll content in photosynthesising organs, especially in the leaves, is of high importance. The restricted chlorophyll content can directly limit the photosynthetic potential and, in consequence, primary production. This is important not only for tree growth but also for the potential to recover after stress occurrence.

In higher plants, two types of chlorophyll molecules, a and b, cooperate in light harvesting. The concentration of chlorophylls in leaves (and their ratio a/b) depends on light conditions, shaded leaves have a higher Chl content (Hatamian et al. 2014). Water availability also affects chlorophyll concentration. Leaves of woody plants developed under water deficit may have smaller and more tightly packed cells with a lower fraction of air spaces (Poorter et al. 2009), thus may reveal relatively higher Chl concentrations than under optimal water availability (Borowiak and Korszun 2011). As in leaf tissues some amount of nitrogen (N) is incorporated into chlorophyll, Chl content is highly dependent on N nutrition in a plant (Percival et al. 2008). Hence why, in agriculture the nitrogen fertilization demand is often determined by estimating Chl content. However, in natural habitats N deficit in plants may not only be caused by N scarcity in the soil. The mineral forms of N which are available for plants, i.e. nitrates and ammonium, are released from the soil organic matter by soil microorganisms. Severe drought may restrain microorganism activity and thus N supply. Likewise, high soil acidity (low pH) may diminish N availability for many plant species.

Trees growing in manmade habitats often experience unfavourable soil conditions (Fig. 1). Their growth in paved areas is usually affected by restricted soil volume in planting pits. This often leads to depletion of nutrients which are not complemented by decaying organic matter. The lack of organic matter in planting pits and the absence of destruents, such as invertebrates and microorganisms, contribute to soil compaction. And

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conversely, compacted soil is lacking in oxygen which is necessary for the microorganisms' activity. As a consequence, the process of soil degradation slowly increases. In the case of highly compacted soils chlorophyll content in tree leaves may be restricted.

A direct method for determining chlorophylls' content requires the extraction of Chl using a solvent, followed by spectrophotometric analysis of the chlorophyll solution. As a result, the amount of chlorophyll in a leaf is expressed in terms of concentration, i.e. µg Chl per 1 g of the tissue, or content, µg Chl per 1 cm^2 of the tissue. Relative concentrations of Chl *a* and b can provide information on stress effect. However, the interpretation of the results requires more knowledge. Moreover, for the laboratory procedure leaf sampling in the field is necessary and the chemical analysis is relatively time and cost consuming.

Recently developed optical methods allow for non-destructive and quick determination of chlorophyll content. They are based on the absorbance and/or reflectance of light by an intact leaf. The obtained results do not show an absolute Chl content per unit leaf area neither concentration per gram of leaf tissue. They express a relative chlorophyll content which is usually sufficient enough in field work. The commercially available devices are hand-held and battery-operated with the records displayed immediately. Some devices also allow for data to be stored in the device's memory for a later transfer.

Chlorophyll absorbance meters measure absorbance by the leaf of two different wavelengths of light: 620-660 nm (red light absorbed by

Fig. 1. Margin chlorosis in Ginkgo leaves caused by deicing salt in street trees. (TS)

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Fig. 2 Disturbed colouration of young leaves in field maple due to compacted soil and drought in April, Warsaw 2007. (TS)

chlorophylls) and 930-960 nm as a reference irradiance, which is used to adjust for differences in leaf structure. Two light-emitting diodes (LEDs) in the illuminating system emit red and infrared light. The light which passes through the leaf sample strikes the receptor, which converts the transmitted light to analogue electrical signals (Fig. 4). The leaf chlorophyll absorbs red light but not infrared, the difference in absorption is calculated and expressed as relative chlorophyll content. The so-called chlorophyll content index, proportional to the amount of chlorophyll in the sample, gives an overall estimation of chlorophyll in the leaf (Loh et al. 2002, Chang and Robison 2003, Torres-Netto et al. 2005).

The first commercially available device was the **SPAD-502** (Konica-Minolta, Osaka, Japan), initially developed in Japan to diagnose foliar nitrogen (N) status and determine N fertilizer requirements of rice (Fig. 5). The newer version SPAD-502plus can hold up to 30 records and calculate the average value of the collected records. All data are deleted when the device is switched off. However the newest version

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SPAD-502PDL is equipped with a Data Logger which allows the compilation of readings for statistical analysis and communication with a PC or portable GPS receiver. Calibration should be made whenever the meter is switched on and can easily be done by the operator. It is also possible to input compensation values if necessary.

The **CCM-200 plus Chlorophyll Content Meter** (OptiSciences, Tyngsboro, Massachusetts, USA) operates similarly. Beside instantaneously displaying the measurements, the on-board data logger and included USB cable allow for data transfer to a PC. Here the averaging of multi-point readings, or averaging of 10 to 30 readings, is possible using the standard deviation elimination function, used to eliminate readings that are outside of a two-sigma range. The instrument includes a port for external GPS.

The **CL-01 chlorophyll content meter** (Hansatech Instruments Ltd., Kings Lynn, UK) is also a lightweight instrument, allowing for 60 measurements, with dual-wavelength optical absorbance: 620 and 940 nm. The connection to a PC is not possible.

MultispeQ (PhotosynQ Inc., East Lansing, Michigan, USA) is a new multifunctional instrument which enable measurements of several parameters of photosynthetic activity of leaves. The device houses LEDs of different wavelengths

Fig. 3. Gradual degradation of chlorophyll can be detected using a chlorophyll meter before the visible symptoms. (TS)

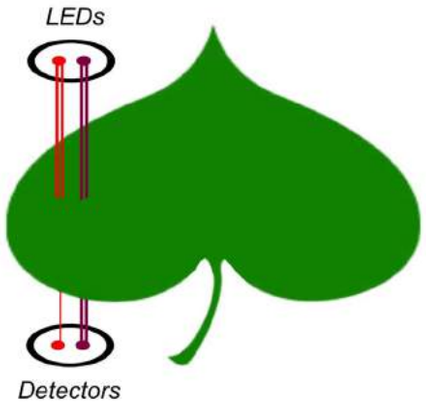

Fig. 4. Chlorophyll absorbance meter working principle. (TS)

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Fig. 5. The measurement of relative chlorophyll content with a chlorophyll meter SPAD-502, Konica-Minolta, Osaka, Japan. (TS)

and sensors allowing for the assessment of both chlorophyll and anthocyanin contents. The measurements are not displayed on the instrument itself. It is, however, intended to be used alongside an Android phone or tablet, or a computer running Windows, Mac OS or Linux, connecting via Bluetooth or micro-USB. The device is dedicated to work in combination with the open science PhotosynQ platform (https://photosynq.org) and to be linked to larger communities of researchers and practitioners who use this instrument (Kuhlgert 2016, MultispeQ 2020). This approach enables the collection and analysis of data obtained by other users working on the same task.

Chlorophyll content may be also assessed indirectly using the reflectance method. Leaf cells re-emit a significant part of solar radiation in the near-infrared spectral region (in the range of 700-1100 nm) while their reflectance of visible light (400-700 nm) is relatively low due to absorption by photosynthetic pigments. The difference in leaf reflectance in the visible and near-infrared wavelengths is used to calculate so called 'normalized difference vegetation index' (NDVI). For direct measurements of NDVI in leaves the PlantPen NDVI device (Photon Systems Instruments, Drasov, Czech Republic) was constructed.

The optical instruments give the information expressed in relative units which may vary between different types of devices. Thus, when sharing results, it is important to state which device was used. There are numerous scientific papers which show and discuss results of measurements performed using the above-mentioned devices. The user can compare his/her results and find out whether the obtained records are of

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the optimal values or not. However, we encourage the owner of the instrument to test it by measuring numerous leaf samples of different tree species growing under varying habitat conditions, in order to realise which values can be identified as optimal. Although the measured chlorophyll content values are universal, the relative Chl content in particular tree species may vary from each other to some extent (Loh et al. 2002). Nevertheless, chlorophyll content is much more dependent on environmental factors than on species specific traits. Thus, it is hard to determine optimum values for individual tree species. Moreover, Chl content depends on the leaf ontogeny stage.

The experienced operator will be able to interpret the results in terms of proper edaphic conditions. Although the results of relative Chl content are related in crop plants to nitrogen nutrition, in case of landscape trees they should be treated rather as an indicator of good or poor overall soil conditions. In landscape habitats the nitrogen availability is highly bound to soil organic matter transformation. The higher the amount of soil organic matter, the more nitrogen is released to soil solution. This could be also related to other nutrients, like potassium, phosphorus, magnesium, etc. Prolonged drought usually inhibits soil organic matter transformation, as it diminishes water availability to soil biota. Likewise, high soil compaction restricts oxygen availability for microorganisms' respiration.

Thus, inadequate (not high enough) levels of relative chlorophyll content suggest the need to improve soil condition in the range of tree root system.

Although the chlorophyll meters cannot indicate an abundance of nitrate, only a possible deficiency, they may be a useful tool for arborists to detect stress in trees, particularly young specimens. The above-mentioned devices are lightweight, portable, handheld, and easy to operate, there are no limits to data collection except device memory, and beside battery charging there are no additional costs of the usage. The obtained measurement results are relatively easy to interpret.

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SUMMARY

1. Chlorophyll content is an indirect measure of the nitrogen status in a plant.

2. Diminished chlorophyll content may indicate not only soil N scarcity but also be a symptom of an improper soil condition.

3. Relative chlorophyll content is a valuable indicator of improper soil conditions in field research due to non-invasiveness and quick processing when using portable devices.

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II.

Chlorophyll fluorescence

In higher plants, two types of chlorophyll molecules, *a* and *b*, are cooperating in light harvesting, most of them are assembled in light-harvesting antennae. The antenna pigments collect the excitation energy coming from the light and channel it to the so-called reaction centres (Strasser et al. 2004). There are two types of photosystems (PS), PSI and PSII, each contain two specific chlorophyll a molecules acting as a reaction centre (a simplified scheme of PSII and PSI connections is shown in the Fig. 6, however, the structures involved in photochemical reactions are more complicated and differentiated).

Light energy is converted to chemical energy when one of chlorophyll a molecules of the reaction centre loses a photochemically excited electron, which is then trapped and used to reduce a primary electron acceptor. In PSII this is a plastoquinone Q_A molecule, forwarding the electron to the subsequent molecules in a so-called electron transport chain up to the PSI reaction centre. In the PSII, a water splitting complex (or oxygen evolving complex), separating electrons, protons and oxygen atoms, acts as a donor of electrons to replace electrons used for plastoquinone Q_A reduction in the PS II reaction centre.

If the energy of excitation is not used for photochemical reactions, it is dissipated mainly as heat and to a lesser extent as emitted radiation, i.e. **fluorescence**. Photochemistry, heat dissipation and fluorescence are competing processes, thus fluorescence measurements can be used to assess the balance between photochemistry and non-photochemical dissipation of absorbed light (Kalaji et al. 2016). This is possible due to the development of specific techniques and protocols. The size of emitted fluorescence is determined by the redox state of the reaction centres, electron donors, and electron acceptors of the photosystems, by the reactions within the electron transport chain,

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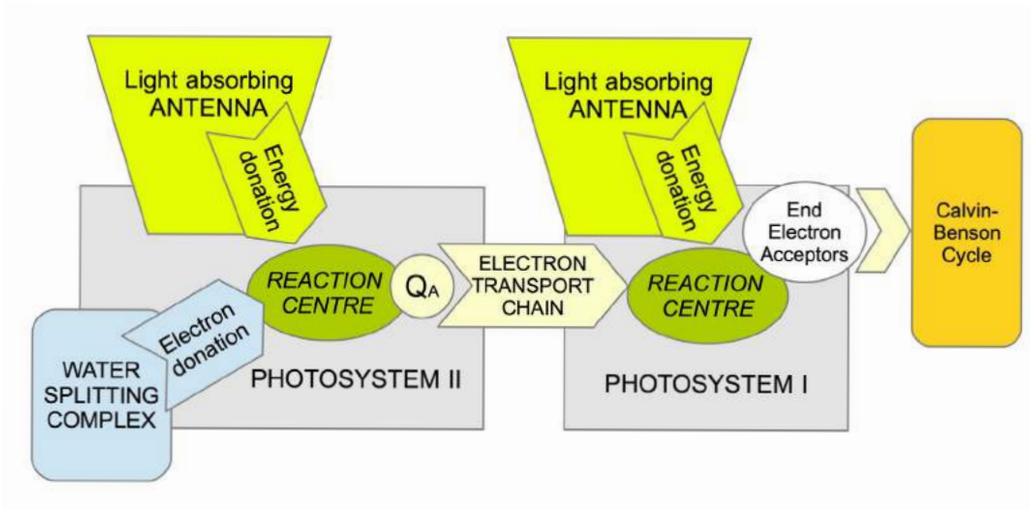

Fig. 6. A simplified scheme of photosynthetic electron transport. (TS)

but also by pigments engaged in photosynthesis (Kalaji and Łoboda 2009).

Chlorophyll *a* fluorescence (ChF) analysis from PSII has recently become a popular research method for many different purposes, including detection of environmental stress in plants. There are two main methods for examining environmental stress performed on living leaves. The first is based on pulse amplitude-modulated (PAM) technique. PAM fluorimeters use actinic light (blue or red), that drives photosynthesis, and additionally emit so-called measuring light, that is used to probe the state of the photosynthetic system (Kalaji et al. 2014). Monitoring the dynamics of chlorophyll fluorescence pulses in response to saturating light pulses allows for fluorescence quenching analysis, in which two basic categories are considered, i.e. so-called photochemical quenching related to photochemical energy utilisation at the reaction centres of PSII and non-photochemical quenching expressing nonradiative energy dissipation into heat (Schreiber 2004).

The second method is based on the application of actinic light as a measuring light and is performed using Plant Efficiency Analysers (PEA). Application of light emitting diodes (LED) of a specific wavelength and a high time resolution detector enables quick and precise measurements after a single exposure of a sample (Kalaji et al. 2014). Therefore, the latter method facilitates non-time-consuming measurements when a high number of records are needed in a relatively short time (at the same light/temperature conditions) in field experiments. In order to detect the PSII performance, it is necessary to quench all photochemical reactions. This is made using light-excluding clips attached to leaf samples for 25-30 minutes. After dark adaptation, samples are illuminated with 660 nm light of minimum 3,000 µmol m^{-2} s^{-1}. High-time resolution measurements (with intervals of 10s) provide a dataset for plotting a fluorescence

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rise which, when shown on a logarithmic time scale, is called a fast (or prompt) fluorescence curve. This curve with the visible points marked as O, J, I, P, enables an insight into the particular phenomena concerning light absorption and its conversion to biochemical energy (Strasser et al. 2004, Fig.7).

Much research is completed using only a few ChF parameters, which are applied as stress markers, these are:

THE MINIMUM CHLOROPHYLL FLUORESCENCE YIELD

in the dark adapted state, F_0,

MAXIMUM CHLOROPHYLL FLUORESCENCE YIELD

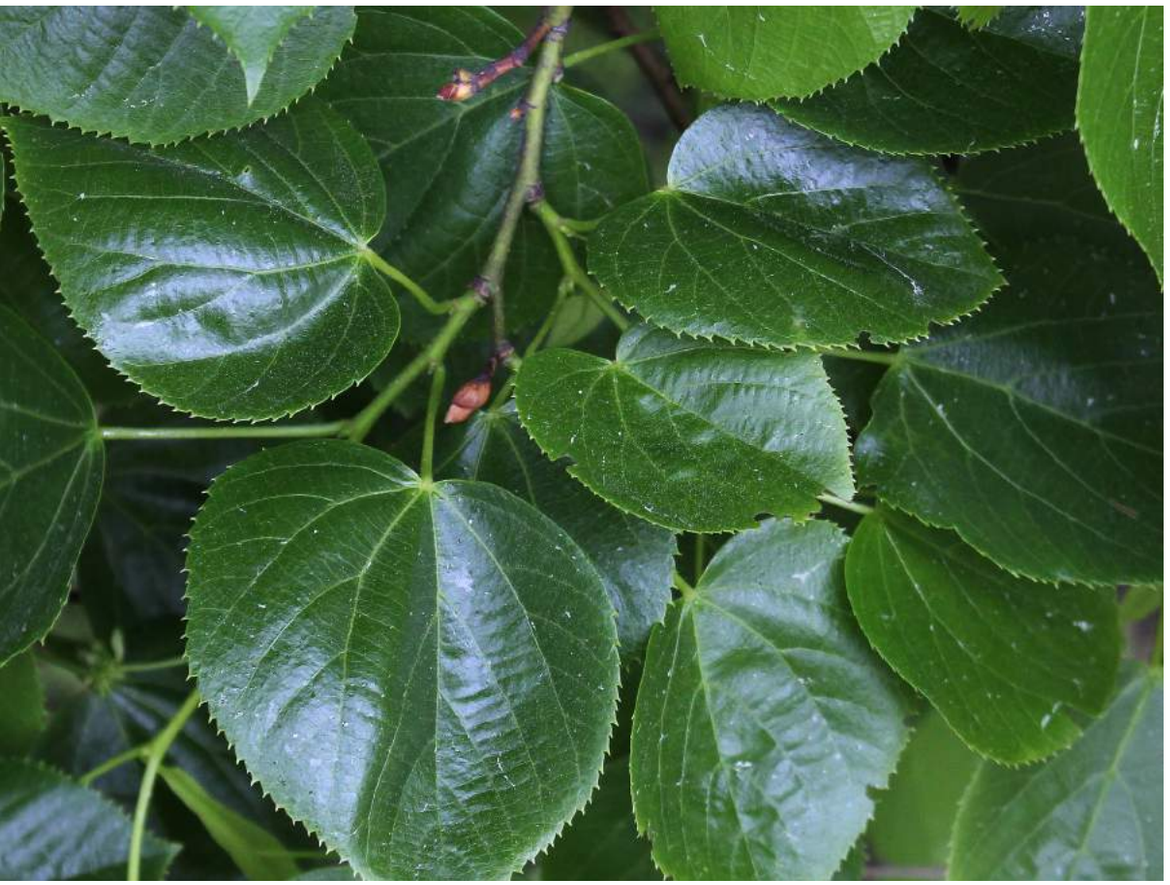
in the dark adapted state, F_m (or F_M),

and

MAXIMUM QUANTUM YIELD OF PS II PHOTOCHEMISTRY,

F_v/F_m, calculated as $(F_m - F_0)/F_m$.

These simple parameters may be obtained using both PEA and PAM fluorimeters (Schreiber 2004, Kalaji et al. 2017).



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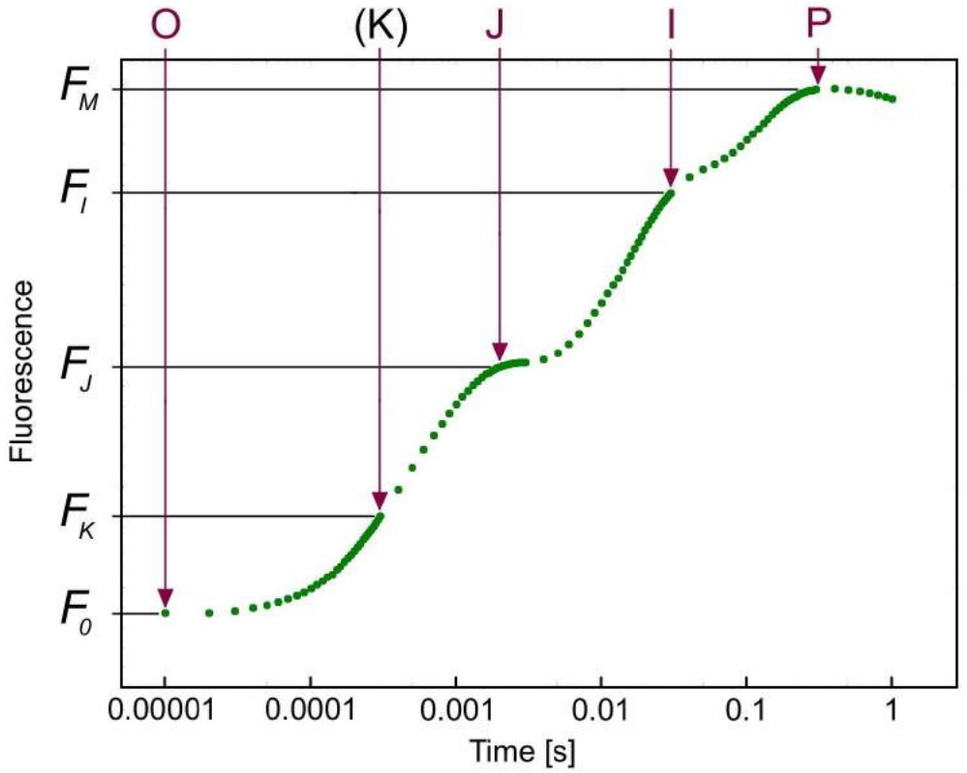

Fig. 7. The fluorescence curve obtained from a Plant Efficiency Analyser after dark adaptation and following illumination of leaf sample with strong actinic light. F_0, minimum chlorophyll a fluorescence yield in the dark adapted state, F_m, maximum chlorophyll a fluorescence yield in the dark adapted state, O(K) JIP, consecutive steps marked artificially at the fluorescence transient, O=F_0, K at 300 μs, J at 2 ms, I at 30 ms, P=F_m. Fluorescence measure is given in arbitrary units depending on a device. (TS)

Based on the points marked as O, J, I, P, so called 'JIP-test' was developed and numerous JIP-test parameters were introduced (Strasser et al. 2004), which describe energy fluxes occurring inside and around reaction centres (RCs) of numerous PSII localised in chloroplasts. In this way, the consecutive energy fluxes of the average photon absorption (ABS), trapping of the excited electrons (TR), energy dissipation as heat (DI), electron transport towards PSI (ET), and reduction of end electron acceptors at the PSI acceptor side (RE), can be evaluated (Fig. 6). Especially, parameters defining quantum yields and efficiencies (or probabilities) are valuable owing to their universal meaning, these are the maximum quantum yield of primary photochemistry (at t_0), TR_0/ABS (φ_{Po}), which equals F_v/F_m, and probability that an electron will be moved into the electron transport chain beyond primary acceptor Q_A, ET_0/TR_0 (ψ_{Eo}) (Strasser et al. 2004).

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Based on numerous laboratory experiments and calculations some other useful parameters have been introduced by prof. Reto Strasser and his co-workers which allow an insight into the physiological condition of leaves and, indirectly, the plants themselves. RC/ABS reflects a relative number of active RCs per average antenna size. RC/CS_0 is a measure of density of active RCs (Q_A reducing RCs) per cross section (at point 0). ABS/CS_0, measured as minimum chlorophyll *a* fluorescence yield in a dark adapted state, F_0, reflects constraints in electron trapping at the initial phase of light conversion. An integrative parameter, so-called performance index, PI_{ABS}, was introduced by Strasser et al. (2004). It combines a pool of active RCs per PSII antenna chlorophyll (RC/ABS), maximum quantum yield of primary photochemistry ($TR_0/ABS=\varphi_{Po}$), and probability that a trapped exciton moves an electron into the ETC beyond the primary acceptor Q_A, ($ET_0/TR_0=\psi_{Eo}$). The mathematical formula of PI_{ABS} is given as follows:

$$PI_{ABS} = RC/ABS \times \varphi_{Po}/(1-\varphi_{Po}) \times \psi_{Eo}/(1-\psi_{Eo})$$

Numerous ChF parameters may be seen as confusing for non-professionals, thus it would be valuable to list and comment on some of the most informative ones.

Fig. 8. Health status of young urban trees may be monitored using chlorophyll fluorescence technique. (TS).

These are:

F_0 – minimum chlorophyll a fluorescence yield in a dark adapted state, higher values indicate prolonged stress caused by, among others, drought or salinity (Percival 2005, Percival et al. 2006, Wang et al. 2012); the obtained values depend, among others, on a device, as fluorescence is measured using arbitrary units, thus, it is recommended to compare the results with any well-performing trees or plants of the same ontogenic state and age;

F_v/F_m – maximum quantum yield of PSII photochemistry (denoted also as TR_0/ABS or φ_{Po}), a measure of efficiency of PSII in light energy conversion, the optimal values are at least 0.8 (Kalaji et al. 2014); in case of weak stress, it may not show any decrease. However, drought, salinity, thermal stress, trunk vessel diseases may be reflected by diminished F_v/F_m;

ET_0/TR_0 (ψ_{Eo}) – probability of electron movement into the ETC beyond the primary electron acceptor Q_A; stress factors like prolonging drought, elevated temperatures, salinity, soil contamination may reduce the values of this parameter, even though F_v/F_m does not show any decrease;

RC/CS_0 – a measure of density of active RCs (Q_A reducing RCs) per cross section of a sample (at point 0); this parameter is affected by N deficiency and prolonging drought (Swoczyna et al. 2019), however, it is strongly dependant on the ontogenic stage of leaves (Lepeduš et al. 2010);

PI_{ABS} – an integrated parameter, very sensitive to different stress factors; it is difficult to interpret its magnitude without any reference values because PI_{ABS} ranges from decimals to values above ten, however, when comparing trees growing in the same habitat, e.g. in the same street, the PI_{ABS} may show the best and the worst performing specimens (Hermans et al. 2003).

The above-mentioned parameters can give some indication as to physiological condition of a plant, however long-living plants, especially trees, tend to keep all physiological processes balanced. Thus, in field work a more comprehensive analysis of ChF parameters should be performed (Ugolini et al. 2012). It should be made clear that, in order to make an appropriate interpretation of the data, it is recommended to compare all the fluorescence records of stressed specimens to reference individuals (Kalaji et al. 2014). In field research most ChF parameters are influenced by numerous environmental factors, thus, it is rather difficult to indicate any reference values.

The time of day when the measurements are taken is of high importance. At midday, when there is high sun, PSII performance is often altered by photoinhibition (Kalaji et al. 2016, Mlinarić et al. 2017), thus recorded values of fluorescence may lead to confusing conclusions. Additionally, cold temperatures associated with high light intensity (e.g. mornings) may cause photoinhibition. Thus, for optimal measurements cold mornings should be avoided. In warmer conditions the process should be completed by about 11:00 a.m. for optimal results. The orientation of a leaf should also be considered. Leaves receiving more light (sun leaves, south-oriented leaves) cannot be compared with shade leaves. The ontogenic stage of a leaf also affects fluorescence records, younger leaves may show higher fluorescence owing to a thinner cuticle and, on the other hand, very young leaves may be more susceptible to photoinhibition. The problem of influence of temporal photoinhibition may be partially solved by collecting leaf samples, putting them into a bag to avoid dehydration and

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leaving them in darkness at room temperature for four-five hours (Pollastrini et al. 2016).

The following models of handheld fluorimeters available today:

HandyPEA and PocketPEA fluorimeters (Hansatech Instruments Ltd., Kings Lynn, UK), the first instruments with high-resolution light measurements enabling the plotting of OJIP transient; the package includes Windows® data transfer and analysis software (Fig. 8).

FluorPen FP 110 (Photon Systems Instruments, Drasov, Czech Republic), a portable, battery-powered PAM fluorimeter, also allowing OJIP transient to be obtained; the device and the package include leaf-clip(s) for dark adaptation, integrated GPS module, USB and Bluetooth communication for data transfer, and comprehensive software for data processing;

MultispeQ (PhotosynQ Inc., East Lansing, Michigan, USA); the instrument combines a pulse-amplitude-modulated fluorimeter, a chlorophyll meter, and a spectrometer in one; the fluorescence parameters are based on PAM protocol; the measurements are not displayed by the instrument, it is intended to be used together with an AndroidTM phone or tablet, or a computer running Windows, Mac OS or Linux, connecting via Bluetooth or micro-USB.

It should be stated here that, in the case of some instruments, the dark adaptation of leaf samples is obtained by using special leaf clips which are sometimes not included with the instrument set and should be purchased separately (Fig. 9). This refers to most devices performing OJIP transient. In field research it is convenient to use a higher amount of leaf clips in order to save time during the measurements.

When choosing a device, it should be considered which parameters are of interest. Several fluorimeters provide only a few parameters which may be difficult to interpret in a field work. Moreover, even when a producer declares the measurements of dark adaptation dependent parameters (like F_v/F_m), the device may not have an option for dark adaptation and therefore samples should be taken to a laboratory equipped with a dark room, meaning fast field measurements cannot be undertaken. Thus, when purchasing a device, it should be established if a product allows for dark adaptation during a field work.

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Fig.. 9 (left): A special clip placed on a leaf for adaptation to the dark, Hansatech Instruments Ltd., Kings Lynn, UK (TS)
Fig. 10 (right): The measurement of chlorophyll a fluorescence with a HandyPEA fluorimeter, Hansatech Instruments Ltd., Kings Lynn, UK. (TS)

Fig. 11. Chlorophyll fluorescence technique may be used in evaluation of urban trees' resistance to harsh street conditions. (TS)

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SUMMARY

1. Chlorophyll *a* fluorescence method enables the monitoring of environmental stress in plants, the stress is detected by examining processes of the light phase of photosynthesis.

2. The two most popular protocols of field measurements are based on: (1) pulse-amplitude-modulated (PAM) fluorescence and (2) fast -fluorescence measurements enabling OJIP analysis.

3. Among numerous ChF parameters, some may be more informative, however, the interpretation of results should encompass a wider set of parameters.

4. The ChF results of stressed plants should be interpreted in relation to the non-stressed individuals.

5. There are handheld devices available, portable and battery-operated, easy to operate in a field work, no chemical reagents are needed to take a measurement.

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III.

Gas exchange (photosynthesis, respiration, transpiration)

Electrons captured in the light phase of photosynthesis are consumed by RubisCO (ribulose-1,5-biphosphate carboxylase/oxygenase), the primary enzyme responsible for CO_2 uptake by plants. This enzyme drives two different catalytic processes: **carboxylation** (fixation of CO_2) and **oxygenation** (fixation of O_2). The latter is called **photorespiration** and ends up in the net release of CO_2 which is then exhausted into the atmosphere. Under normal conditions, carboxylation outperforms oxygenation resulting in CO_2 incorporation in sugar molecules. Simultaneously, respiration is driven in mitochondria which release CO_2 to intercellular spaces in leaf tissues (Taiz et al. 2015, Kopcewicz and Lewak, 2019).

These three processes:

- CO_2 fixation,
- photorespiration and
- mitochondrial respiration,

influence net balance of CO_2 fluxes which are detected using the gas exchange method. It is important to note that the gas exchange method, based on CO_2 flux via stomata, does not inform about plant photosynthesis in total but only about net CO_2 assimilation (A_N).

The leaf gas exchange via stomata also involves a water vapour release process, i.e. transpiration. Transpiration is the final stage of water flow from the roots to stomatal pores via the xylem and the leaf mesophyll tissues. This flow is necessary to support all plant tissues in water and to transport nutrients, biochemical signals (e.g. hormones) and other substances. When the roots signalise water stress, stomata in leaves tend to close. Thus, net CO_2 assimilation is in close relation with water losses by transpiration (E).

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Both gases, CO_2 and H_2O, absorb radiation at specific sub-millimetre infrared wave-bands, each gas having a characteristic absorption spectrum. Infrared gas analysers (IRGA) include an infrared source, gas cell, optical filter, and detector. Gas molecules decrease the radiation reaching the detector, which is calculated to concentrations proportional to the measured transmittance. The IRGA cells are designed to allow a continuous flow of gases. In this way, a continuous and real-time record of CO_2 and H_2O mole fraction is given. In a typical IRGA dedicated for plant CO_2 assimilation measurements two cells are included: the analysis cell and the reference cell. The analysis cell is filled with the air coming from a closed chamber or cuvette where the examined leaf is placed. The reference cell is filled with the same ambient air which is supplied to the leaf cuvette. The amounts of radiation passing through the cells are compared and based on the difference, the rates of absorbed CO_2 and released H_2O are calculated (Fig. 12).

A typical instrument, so-called photosynthesis system, consists of a leaf chamber (where the examined leaf is placed) and a console with the infrared gas analyser, pumps allowing for air flow, flowmeters, keyboard, display, batteries, and memory card.

There are two modes of gas measurements: in a closed system, where the air is recirculated through the leaf chamber, and an open system, where the air passes the chamber only once. Recently, open-system devices were developed because of their advantages and many of them are dedicated also to field work (Long et al. 1996). In open-system instruments the main console supplies the leaf chamber with the air at a known rate and with a known concentration of CO_2 and H_2O. Air is continuously passed through the chamber and afterwards the CO2 and H_2O concentration is determined again. Due to photosynthesis the outgoing air will have a lower CO_2 concentration and due

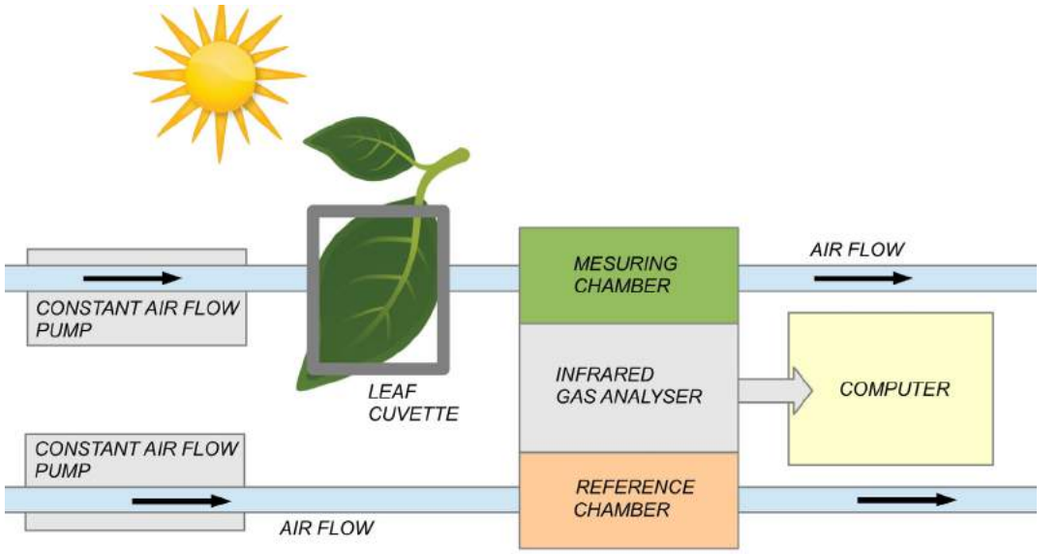

Fig. 12. The scheme of the working principle of an open-system infrared gas analyser. (TS)

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to transpiration, the H_2O concentration will be higher. Besides determining net assimilation/photosynthesis rate (A_N or earlier denoted P_N) and transpiration rate (E) the instruments may calculate stomatal conductance (g_s), intercellular CO_2 concentration (c_i), water use efficiency (WUE = A/E), and intrinsic water use efficiency (WUEi = A/g_s). Modern photosynthesis systems may also enable measurements of photosynthetically active radiation (PAR), chamber air temperature, and leaf temperature.

Photosynthesis measurement systems may be additionally equipped with a unit containing a light source for light control (enabling measurements on days with high cloud cover) and for determining photosynthetic response to irradiance (PRI). The use of an artificial light source with a defined high value of PAR (for example 1,500-2,000 µmol m^{-2} s^{-1}) enables values of light-saturated net assimilation (A_{max}) to be obtained. The systems may be also equipped with the CO_2 supplying module.

Here are some examples of photosynthetic systems dedicated for field work:

LI-6800

(LI-COR Inc., Lincoln, Nebraska, USA) – the console provides the operating system, interface, air conditioning, and data logging but the sample and reference gas analysers are incorporated in the head, a leaf chamber is attached to the head; weight: console 6.1 kg, head 2.15 kg, leaf chambers 0.3-0.35 kg; memory: 512 MB RAM, 8 GB Flash memory; data transfer possible by copying to a USB storage device, or directly to a computer or a computer network using the Ethernet connection; the Producer states that the incorporated fluorimeter measures both modulated and continuous fluorescence signals, and can fully characterise the fluorescence induction transient (also called an "OJIP curve") of a leaf at high resolution.

TARGAS-1

(PP Systems Ltd.) – weight: the console 2.1 kg, the basic leaf cuvette (PLC5) 0.7 kg; data are recorded and stored on USB flash, additional one mini USB for connection to external PC; optional extras include: a light unit, a soil respiration chamber, a temperature/PAR probe and a soil temperature probe (Fig. 13, 14).

GFS-3000

(Heinz Walz GmbH, Effeltrich, Germany) – a portable photosynthesis system consisting of the control unit (console), weight 12.3 kg (including batteries), and the standard measuring head with special plates for different leaf sizes, optional cuvettes for lichens and conifers, weight 1.6 kg; data storage: Solid State Drive 32 GB, PC interface: USB 2.0; the modified system package GFS-3000FL contains the LEDArray/PAM- fluorimeter 3057-FL, measuring PAM fluorescence parameters.

LCI T

(ADC BioScientific Ltd., Hoddesdon, UK) – weight: the console 2.4 kg, the plant leaf chamber 0.6 kg; data storage up to 1000 records on removable SD cards (up to 32 GB supported); equipped with GPS; Broad and Narrow leaf chambers are compatible with the OS5p+ and OS1p portable fluorimeters, supplied by ADC; fluorescence data can be recorded to a fluorimeter at the same time as gas exchange data are recorded to the LCi T.

CI-340

(CID Bio-Science, Inc., Camas, Washington, USA) – a portable, single-handed, light-weight (1.5 kg) main unit; data storage: 4 MB Internal FLASH RAM; the optional accessory modules allow control of CO_2, H_2O, temperature, light intensity, and measurement of chlorophyll fluorescence; 10 different customised chambers accommodating different leaf sizes are available, including conifer needles and cacti; the basic functions

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Fig. 13. The IRGA (Infrared Gas Analyser) console of Targas-1, PP Systems Ltd., Amesbury, Massachusetts, USA. (TS)

Fig. 14. The measurement of photosynthesis intensity with the leaf cuvette of the photosynthesis system Targas-1, PP Systems Ltd., Amesbury, Massachusetts, USA. (TS)

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are incorporated in the main unit thus direct chamber connection to the CO_2/H_2O gas analyser reduces measurement delay.

Before purchasing an IRGA the decision should be made as to what kind of leaf chamber will be necessary. As the rate of gas exchange is related to the sample area, optimal results are obtained when the flat leaf blade fills the chamber entirely. If not, special care should be taken when interpreting the records, for example, in the case of differentiated leaf shape the calculation of leaf area would be necessary. In many instruments a range of chambers are now available: for typical dicot leaf blades, grasses, conifers and small leaves.

The photosynthetic rate depends on various environmental factors. Under natural conditions the most important limiting factor of photosynthesis is light availability, followed by water shortage, but temperature and nutrient status are also meaningful. Other stressors, such as hypoxia within the root system and soil compaction, may also indirectly affect photosynthesis. Moreover, the examination may concern the plant itself or the influence of current environmental features. Thus, the protocol of gas exchange measurements must take into consideration the goal of the examination.

Most gas exchange studies deal with light-saturated rates of CO_2 assimilation (A_{max}). Under field conditions light (irradiance) is the most variable factor affecting photosynthesis. Thus, in order to examine photosynthetic capacity of a given individual, a photosynthetic response to irradiance (PRI) curve should be determined. The measure of irradiance used for photosynthesis is the photosynthetic photon flux density (PPFD), defined as the number of photosynthetically-active photons incident per unit area per unit time (µmol photons m^{-2} s^{-1}). For example, in sunny open sites PPFD may reach 1300 µmol photons m^{-2} s^{-1} while in the shaded understory of forests may achieve about 50 µmol photons m^{-2} s^{-1} (Abrams and Mostoller 1995). The additional unit for light control enables the creation of uniform light conditions. On the other hand, it is important to remember that leaves can accommodate both to long-term and current light conditions. Therefore, when monitoring the effect of environmental conditions other than light, e.g. air pollution, drought stress etc., the gas exchange measurements should be performed on the leaves under the same light exposure. It should be pointed out that in case of insufficient light (or under other serious stress) the A_N may show values below zero. In case of light scarcity, the below-zero records inform that the light intensity have not reached the compensation point at which CO_2 fixation exceeds the sum of photorespiration and mitochondrial respiration (Fig. 15).

Another function of IRGAs may be to assess plant potential for CO_2 fixation in case of saturated CO_2. For that examination the additional CO_2 source may be incorporated or attached to the device.

Every IRGA instrument is equipped with three absorber columns. The first column contains a CO_2 and H_2O scrubbing desiccant, known as Molecular Sieve. It removes CO_2 and H_2O from the air stream in order to check the analyser zero and to ensure long term stability and accuracy of the gas analysers. The second column contains soda lime (CO_2 scrubber) and the third contains a desiccant (H_2O scrubber). These chemicals allow for CO_2 and H_2O control during the gas flow. Each absorber column should be checked periodically, and the absorbers should be replaced when necessary.

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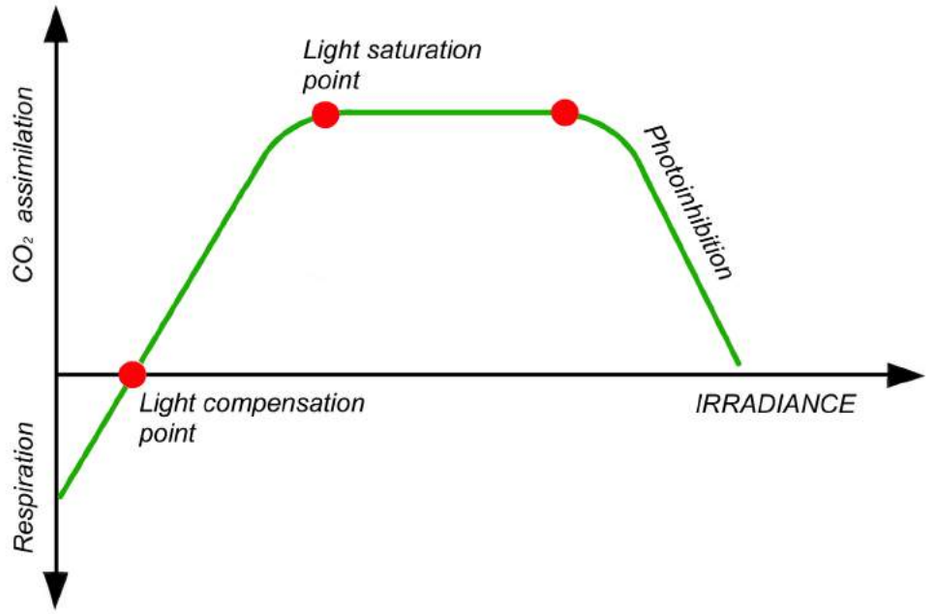

Fig. 15. Photosynthesis-irradiance curve. (TS)

When starting to use with the IRGA, it is necessary to warm-up the instrument. This is because mass flow of gasses is detected at the temperature of ca. 55 °C. In the infrared gas analysers dedicated for field work the reference air source is normally the ambient air. Therefore, the source of reference air should be drawn from a stable source away from any CO_2 influences or disturbances such as people breathing, ventilation systems, carparks or motorways, automobiles, etc. The air supply intake unit is usually incorporated in the photosynthetic system set. After placement of the leaf inside the chamber, monitoring either the CO_2 value or A value should be taken during ca. 60 s in order to stabilise the air flow in the chamber, and then the measured data may be recorded. In fact, CO_2 assimilation measurements take much time, approximately 20 minutes to half an hour for warming-up the device and for measurement collection in one site.

As described above, the assimilation rate depends on light availability. In order to compare photosynthetic performance of different specimens the examined leaves should perform in the same light conditions. If full sunlight is not available for all the specimens, shaded leaves are preferred. The measurements both under high irradiance and extremely low light should be avoided. Likewise, high air humidity may affect the results. Assimilation goes on only in intact leaves, if a leaf has lost its connection to the shoot, contrary to chlorophyll fluorescence, the performance of gas exchange analysis is not possible. Net assimilation values are dependent on species characteristics. Abrams and Mostoller (1995) found that early successional tree species reveal higher A_N than late successional ones. Moreover, middle successional tree species show high plasticity in tolerance to drought. Therefore, a recommendation of reference values is difficult, although

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some ranges can be introduced. According to Abrams and Mostoller (1995) A_N in shade leaves may obtain up to 0.45 µmol m^{-2} s^{-1} in average, while in sun leaves may range between 4 and 7 µmol m^{-2} s^{-1} in average.

Transpiration rate, E, may be analysed itself or in combination with A_N, giving the water use efficiency index (WUE). The WUE is helpful in plant phenotyping. It enables to classify examined species and cultivars according to their potential to conserve water in their tissues. It may also give information on the plant acclimation to difficult environmental conditions.

Stomatal conductivity (g_s) provides information on the current status of stomata opening or closure. In relation to given environmental conditions, g_s informs about the answer of the plant to vapour deficit in the air and water deficit in the soil. Typically, stomatal conductance ranges from 50 to 500 mmol.

Fig. 16. Disturbances in photosynthetic intensity may result from harsh environmental conditions affecting newly planted trees. (TS)

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SUMMARY

1. Gas exchange measurements provide information on how a plant is performing at the moment and on the basis of the results it can be concluded if the plant is stressed or not, assuming that we compare plants in different growth conditions, including the optimal ones. It is also possible to test photosynthetic capacity of a given individual using the additional unit for light control.

2. The measurements in the field should be undertaken before midday because at noon most of stomata may be closed and the gas exchange may be stopped.

3. Each IRGA operation requires several minutes for warming-up the device before starting the measurements and then a couple of minutes for each record, thus, the method is time consuming.

4. The IRGA instruments are rather expensive and require special absorbers which should be purchased periodically.

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IV.

Water potential

Plant cells need to be more or less saturated with water because water is a constituent of plant tissues. It acts as a solvent, takes part in many biochemical reactions, supports transport of nutrients, sugars and hormones, gives shape to non-woody tissues due to turgidity, determines cell divisions and plant growth, and provides thermal stability. Water is taken up by plants from the soil via roots and passes through the vascular system, namely xylem tissue, towards leaves, here it is released into the atmosphere through stomata. Only ca. 1% of water taken up by the roots is estimated to be left in plant tissues. The water transport in plants is explained by Soil-Plant-Atmosphere Continuum model where the difference between water potential (Ψ) values in the soil, the plant, and the atmosphere, is the driving force for the water flow (Table 1). The water potential in soils is almost always negative and held under tension. The more negative this value, the lower the water potential (Taiz et al. 2015, Kopcewicz and Lewak, 2019).

When growing in the natural environment, plants often experience insufficient water availability which causes difficulty in water supply for the whole organism and for the water flow in xylem. On the other hand, vapour pressure deficit (VPD) in the air triggers transpiration from leaves through stomata. The higher VPD in the air, the greater potential gradient between the atmosphere and leaf tissues, and this leads to transpiration increase. A faster rate of water loss causes more negative potentials in the leaf tissues and this in turn diminishes the water potential in the stem.

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Table 1. Water potential in the Soil-Plant-Atmosphere Continuum (Ψ).

Plant tissues/Environment	Water potential [MPa]
Atmosphere	- 30.0 or less
Leaf mesophyll	ca. - 1.2
Xylem	ca. - 0.7
Root Surface	ca. - 0.3
Soil	0 (clear water) to - 0.15

In the 1960s Per Fredrik Scholander and his colleagues developed a pressure chamber technique to measure the water relations in trees and shrubs, particularly water potentials in shoots and leaves. The pressure chamber comprises an aluminium, steel or stainless steel pressure vessel that can withstand pressures up to 10 MPa connected to a pressurised supply of inert gas (usually nitrogen) or compressed air through a pressure reducing valve and a metering valve and connected to pressure gauges. The vessel is closed with a lid having a holder fitted for shoots or leaf petioles of different sizes (Turner 1988). The air/gas pressure is applied to a leaf (or small shoot), where most of the leaf is inside the chamber but a small part of the leaf stem (the petiole) is exposed to the outside of the chamber through a seal (Fig. 17). The increasing pressure causes xylem water to appear at the cut surface of the petiole. The pressure required to force water out of the petiole (or stem) reflects the water potential. The units of pressure most commonly used are megapascals (MPa) or bars: 1 bar = 0.1 MPa.

The time of measurements and the mode of leaf treatment before the measurement determines from which part of the plant the water potential is measured. Predawn leaf water potential Ψ_{PD} measures plant water status at zero plant water flux and provides information on the overnight recovery in plant water potential (Fulton et al. 2001). Leaf water potential Ψ_{LEAF} measured on a single leaf reflects a combination of many factors: local leaf water demand (i.e. VPD, leaf intercepted radiation), soil water availability, internal plant hydraulic conductivity and stomatal regulation. Stem water potential Ψ_{STEM} is the result of whole plant transpiration, and soil and root/soil hydraulic conductivity. It indicates the capacity of the plant to conduct water from the soil to the atmosphere. Ψ_{STEM} has been reported as a good indicator of water deficit affecting the whole plant (tree) and is successfully applied to schedule irrigation in orchards (Fulton et al. 2001, 2018).

In order to determine predawn Ψ, measurements should be made in the dark before sunrise. Alternatively, leaves should be wrapped with light-excluding, hermetic material for the night and measurements taken early in the morning. Leaf Ψ is measured on a fully transpiring leaf in mid-afternoon when photosynthetic activity and water demand is highest, and stem Ψ is measured on a non-transpiring leaf between

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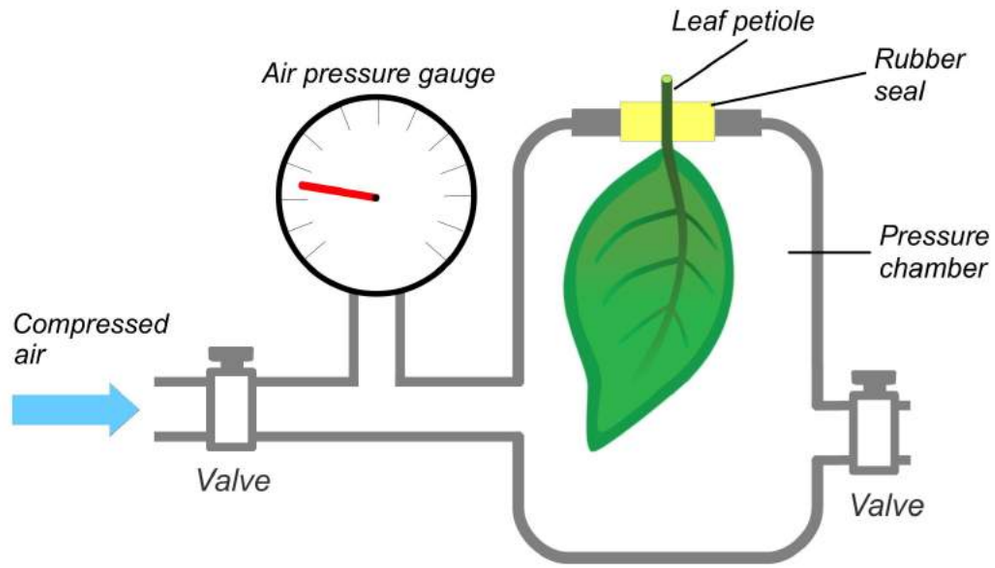

Fig. 17. The scheme of Scholander pressure chamber. (TS)

12:00 to 4:00 p.m. (Fulton et al. 2014). For Ψ_{STEM} 10 minutes up to an hour prior the measurement leaves must be covered with aluminium-wrapped (to avoid solar radiation effect) and hermetic (to prevent transpiration) plastic bags.

Leaves can be pulled from the tree or preferably cut with a knife or a razor blade. The leaf petiole (or the shoot) should be inserted in a holder of the lid. The leaf should be placed inside the vessel, while the petiole should be viewed outside. The lid should be firmly locked and then the vessel is filled with gas under pressure. This process is controlled by the operator. The role of operator is to observe the petiole (here a magnifying glass is useful) and note the pressure value when water from petiole xylem appears at the cut surface. The determination of the end-point, when liquid water is forced to the cut petiole or stem surface, is the essence of the estimation of accurate water potential. The operator's lack of vigilance can result in over-run errors (Model 3115 Portable Plant Water Status Console, Operating Instructions, 2017).

Contemporary instruments are built in a carrying case and are easy to transport (Fig. 18). It is possible to purchase the pressure chamber console with or without a gas tank. In the latter case the gas tank should be purchased separately. Pressure chambers are manufactured commercially by e.g. PMS Instrument Company (Albany, Oregon, USA) and Soil Moisture Equipment Corp. (Santa Barbara, California, USA).

As shown above in Table 1, typical Ψ_{STEM} values are around -0.7 MPa, while in fruit trees, when affected by drought stress drought stress,

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Fig. 18. The pressure chamber instrument Model 615, PMS Instrument Company, as an example of an instrument for the water potential measurement. Source: PMS Instrument Company, Albany, Oregon, USA. Reprinted with permission.

Ψ_{STEM} may achieve -1.4 MPa or even -1.8 MPa (Naor 1998, Williams and Araujo 2002). Variation in daily values of Ψ_{LEAF} are dependent on current weather conditions, thus, may be in the range of -1.19 to -2.3 MPa. In drought-affected fruit trees Ψ_{LEAF} decrease up to -2.3 MPa or -3.4 MPa (Naor 1998, Williams and Araujo 2002). Therefore, in order to assess the size of drought stress it is recommended to relay on Ψ_{STEM} rather than on Ψ_{LEAF}.

When examining trees during field work, some rules should be considered in measurement protocol:

1. It is recommended to choose well-developed internal leaves from the northern side of the crown, especially when Ψ_{PD} or Ψ_{STEM} is to be measured.

2. Recutting of the petiole must be avoided as this can lead to erroneous results;

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3. Identification of the end-point of pressure application should be done carefully;

4. SAFETY NOTE: the stored energy of compressed gas is dangerous and care must be taken in the use of the pressure chamber, the use of safety googles is recommended; the operator should keep his/her face away from the centre of the leaf holder.

The pressure chamber technique is very useful in determining tree water stress, especially when estimating the stem water potential. It is relatively convenient method to assess tree water status in the field conditions allowing for numerous measurements over the course of a day. The difficulties of this technique are connected with the large weight of a typical pressure chamber: the weight of

Fig. 19. Young trees are particularly sensitive to water shortage in urban soils. Restricted soil volume and soil compaction offen increase water deficit in street soils. (TS)

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simpler devices without a gas tank (which must be carried separately) is about 7 kg, while a set consisting of a chamber and a gas tank including the case may weigh around 20 kg or more. Re-filling of a gas tank must be considered during the use of the device.

Special aluminium-wrapped, hermetic plastic bags preventing transpiration (necessary for determining Ψ_{STEM}) are both usually included in the set and possible to purchase separately (Fulton 2018).

SUMMARY:

1. The measurements of stem water potential performed at midday reflect water deficit affecting the whole tree (plant).

2. The pressure chamber method enables the assessment of the plant water potential in field conditions.

3. The pressure chamber instruments and portable gas tanks are relatively heavy and the tanks require refilling periodically.

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TREE ASSESSOR

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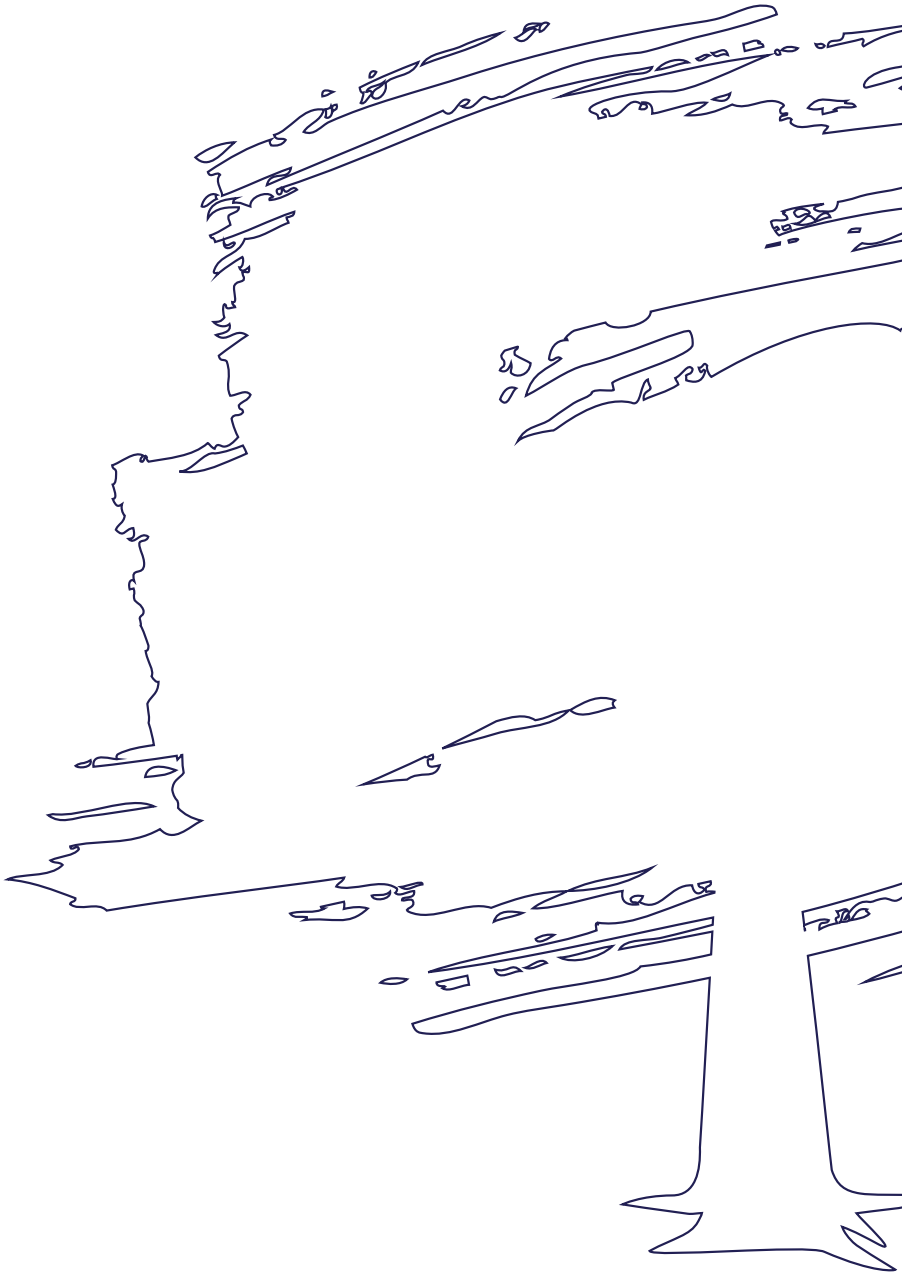

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