IMPROVING SKILLS FOR ECOPHYSIOLOGICAL AND METEORORLOGICAL RESEARCH APPLICABLE TO POPLAR SRC

Scientific report

Short Term Scientific Mission, COST FP1301

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INTRODUCTION

Fitting into the pattern of the recent EC directives about the use of renewable energy resources, the National long-term strategy for development of the forest branch in Bulgaria declared the use of biomass as priority for the years to come. As an alternative to the use of remnants from timber (forest) harvesting and wood-processing industries, the establishment and use of biomass from energy plantations from fast growing woody species has a promising potential. Bulgarian coppices occupy area of about 2 million ha, which amounts approximately 50% from the total forest area in the country. Moreover, there are roughly about 1 million ha low and unproductive agricultural lands potentially usable for further afforestation. From this point of view, there are attractive opportunities for broadening the total area of the SRC managed woody plantations for biomass production.

From 2012 on the Department of "Genetics, physiology and plantations" of the Forest Research Institute, Sofia has been involved in the implementation of three nationally funded projects (two finished, one in progress now), which might be united under the global objective of assessment of general biomass productivity of some fast growing forest species (*Populus* spp. *Salix* spp., *Robinia pseudoacacia*, *Paulownia* spp.) (Stankova et al., 2016a; Stankova et al., 2016b). Within the ongoing project, the accent was put on biometric measurements, but pilot physiology studies of some photosyntetically related parameters (as net photosynthetic activity, transpiration, stomatal conductance, etc.) have also been carried out by using a portable infrared gas analyzer Li-COR 6400. We have been doing this research on a collaborative basis with colleagues from the University of Forestry, Sofia.

OBJECTIVE

The mission was generally aimed at getting knowledge about the functioning and use of instrumentation and methodologies for (eco)physiological and partly meteorological studies related to poplar SRC. Due to the specificity of the STSM application process, the shortage of time remaining to the common deadline of the mission within the COST action and the pre-planned coppicing of the Flemish SRC poplar plantation (in Lochristi) in February 2017, the primary idea for implementation of measurements on the field was transformed into work demonstration of different devices for (eco)physiological studies and discussion about their

specific practical use in (eco)physiological research and especially in SRC experimental plantations.

HIGHLIGHTS OF THE VISIT

The basic scientific mission of the Center of Excellence (Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium, PLECO) is to improve the knowledge and basic understanding of the general impact of global changes on plants, vegetation and ecosystems at different hierarchical levels. Based on a solid experience in different sub-disciplines in the domains of ecology (ecology of plants and vegetation) and biogeochemistry (carbon cycle and its relations with water and nitrogen) the nowadays scientific activity of PLECO (more than 50 persons) covers studies on a broad range of climatic gradients (projects from the tundra to tropical forests) and management regimes and intensities, including also agricultural crops and SRC plantations from fast growing woody species (*Populus* spp.).

The essentials of my stay in PLECO were:

1) Visits of three experimental sites (ecosystem stations, ES) for active monitoring of a number of ecological processes, covering (eco)physiological and meteorological research;

2) Attendance at demonstration of technical equipment and practical use of different instrumentation for field (eco)physiology research;

3) A series of meetings with experienced researchers, PhD students, postdocs and experts in plant (eco)physiology for getting acquainted with their research activity and especially for sharing their experience in using different kind of instrumentation for plant (eco)physiological studies.

1. Field trips for visiting ecosystem stations (ES)

With the aim of getting acquainted in more detail with the PLECO research activity and the ongoing projects including (eco)physiological and meteorological measurements, a couple of visits to experimental plots were kindly offered by Prof. R. Ceulemants. The visiting group was also joined by some recently arrived PhD students and post docs, who were to join the PLECO team in different projects.

The rallying point between the visited places (Ecosystem Stations, ES) was that they are ecosystem observation stations of the Flemish *in situ* network of the ICOS (Integrated Carbon Observation System; www.icos-ri.eu). The research infrastructure has been established for providing continuous information about greenhouse gases based on the implementation of a series of meteorological and ecological (including ecophysiological) observations, their further integration and improving the knowledge for policy making in climate change mitigation and adaptation. The monitoring of the global cycle of carbon and other greenhouse gases is of high importance because of their relation to human emissions and to the existing carbon balance. This was the idea of establishing a Pan-European integrated infrastructure network for monitoring fluxes (of CO_2, CH_4, N_2O, H_2O, O_3) and energy between the atmosphere and ecosystems as well as ocean surfaces in order to qualify and to better understand and predict the behavior and trends of greenhouse gases of Europe and neighboring regions. Due to the established uniform infrastructure, the standardized instrumentation, the uniform methodology and the automated high precision measurements, the generated data are comparable and highly reliable.

LOCHRISTI, EAST FLANDERS

The site was visited on 5 April 2017 and was the focal point of the visit. The experimental SRC plantation of 14.5 ha was established on previous agricultural and pasture land in 2010 in connection to the POPFULL project of the European Research Council. Coordinates of the site are 51° 06' 44''N, 3°51' 02''E, elevation 6.25 m, average annual temperature 9.5 °C, and average annual precipitation 726 mm (**Fig. 1**).

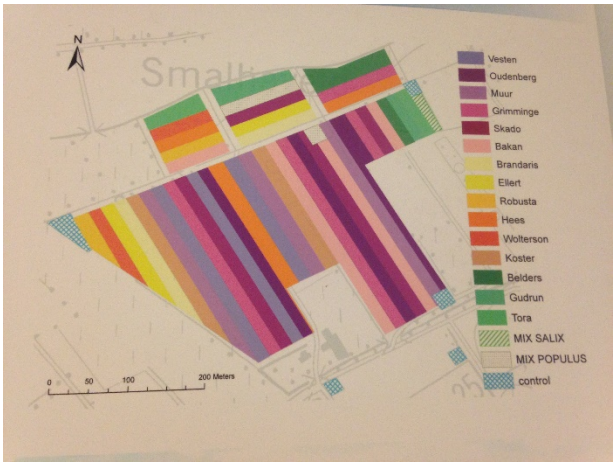

Fig. 1. Layout of the experimental poplar SRC plantation in Lochristi.

The project was aimed at making a full balance of the most important greenhouse gases (CO_2, CH_4, N_2O, H_2O and O_3), a full energy and economy accounting and performing a full life

cycle analysis (LCA). The plantation consisted of 12 different poplar clones (hybrids form Europe, North America and Asia) and was managed under a 2+2+3 coppicing scheme. A density of 8000 plants/ha was achieved by a double row planting scheme. Hardwood cuttings were planted 1.1 m apart with alternating inter-row densities of 0.75 and 1.5 m and 1.1 m between the individuals into each row. (Verlinden et al, 2013; Verlinden et al., 2015) A telescopic mast (3-15 m) is equipped with various instruments for continuous eddy covariance monitoring of ecosystem level fluxes (of carbon, water and energy) and continuous measurements of meteorological variables (quantum sensor for measurements of photosynthetically active radiation (PAR), Vaisala probes mounted on the telescopic mast for measurements of air temperature, sonic anemometers, etc.). Additionally, volumetric soil water content is measured at specific depths (1 m, 0.8 m. 0.6 m, 0.4 m, 0.2 m below the soil surface) by using soil moisture probes. The SRC poplar plantation is the only bioenergy plantation and, together with the Maasmechelen heather ecosystem, they are representing unique ecosystems within the ICOS infrastructure network.

BRASCHAAT – Province of ANTWERP

The site was visited on 5 April 2017. The Ecosysytem Station is located at "De Inslag", which is a Scots pine forest planted in 1929 especially for providing piles for building supporting constructions in the mine galleries. The measurements are taken at different heights of a 40 m scaffolding tower. (**Fig. 2**). The station is operated jointly by PLECO and the Research Institute for Nature and Forest (INBO).

Fig. 2. The scaffolding tower at the Braschaat ecosystem station.

The Ecosystem Station was visited on 12 April 2017. It is located in Belgium's National park "Hoge Kempen", Limburg. The park covers an area of some 5700 ha and is famous thanks to the large abundance of heather vegetation (mostly *Calluna* spp.), thus forming a unique and worthy to study ecosystem.

2. Instrumentation

Sap Flow meter – (Dynamax Inc., Houston, TX, USA)

The sap flow meter devices are used for measuring the sap flow rates in connection with studying the plant-water relations (whole plant water flux, daily transpiration rate, canopy/ stand transpiration, etc.). A detailed explanation about technical characteristics of Dynamax device (**Fig. 3**) and use of sensors as well as some practical tips for their mounting on the tree and insulation, were kindly shared by Dr. Alejandra Navarro.

Fig. 3. Dynamax Sap flow meter mounted on a poplar stem.

Diffusion porometer – (AP4 Leaf porometer, Delta T devices, UK)

The device is broadly used in different types of (eco)physiological research for measuring the leaf stomatal conductance. (**Fig. 4**) The stomata of leaves are responsible for both water loss and CO_2 uptake. The plant water status is also closely dependent from the stomatal dynamics since the stomatal conductance is strongly correlated with transpiration. The discrete leaf measurements could be easily upscaled by using different approaches to the whole tree or the stand.

Fig. 4. Measurements of stomatal conductance with the AP4 porometer.

Pressure chamber (ARIMAD-2, A.R.I KFAR Charuv Water-Supply Accessories, Israel) (**Fig. 5**) The pressure chamber is broadly used for measuring the plant water potential. This parameter is closely connected with the drought stress-tolerance of different species.

Fig. 5. Pressure chamber ARIMAD-2

Devices for Indirect LAI measurements - SunScan (Delta T, UK), Li-Cor LAI 2200C Plant Canopy Analyzer (Linkoln, Nevada, USA), Hemispherical Photographs

All of the above mentioned tools are used for leaf area index (LAI) measurements in function of the research requirements. Due to the standardization restrictions imposed from the ICOS infrastructure network, the most frequently used device is the Li-Cor 2200C. Some technical details, principle of action and practical tips connected with the real use of the instrumentation, were provided by Cristina Ariza Carricondo.

Tools used for tree biometrics

Coupled with plant ecophysiological measurements, some other tools are routinely used for collecting biometrical data. These include:

- ✓ Different brands of digital calipers are used for ad-hoc tree diameter measurements;
- ✓ A Nedo Messfix-S (Nedo GmbH & Co. KG, Germany) telescopic measuring rod is used for accurately measuring tree heights. The model in use has a limit of 8 m;
- ✓ Two types of dendrometer devices are used for precisely following the tree diameter, resp. increment dynamics – an old fashion metal belt-based device and more sophisticated T-shape point dendrometers (Natkon, Switzerland) (**Figs 6 and 7**).

Figs. 6 and 7. Metal-belt and T-shape point dendrometer (resp. left and right)

3. Personal meetings with scientists and experts in plant (eco)physiology

During the mission a series of useful meetings and discussions with colleagues (experts, senior and postdoc scientist, PhD students) were realized, namely:

Dr. Miguel Portillo Estrada – He is involved in Volatile Organic Compounds emission measurements (Copolovici et al, 2014) and their quantification within the ICOS network by using a highly sophisticated technique (Proton-Transfer-Reaction Time-of-Flight Mass Spectrometer, PTR-TOF-MS). Miguel shared an interesting novelty designed to adapt the Li-Cor device for measuring leaf isoprene fluxes as an addition to the routine measurements of net photosynthetic activity, transpiration and stomatal conductance. The 'invention' consisted of a plastic bag fitted to an inlet of the device and intended for the collection of isoprene for further analysis under lab conditions.

Dr. Matteo Campioli – He is the principal investigator of a new project of the European Research Council (ERC Starting Grant) devoted on studying the factors controlling leaf senescence in some broadleaved forest species. He has planned various experiments to support the novel hypothesis that in the presence of growth-limiting conditions (environments) the photoperiod is the factor controlling the onset of senescence.

M.Sc. Joanna Horemans, PhD. student – She is involved in ecological modelling, which is an useful tool for the prediction of ecosystem reactions to different scenarios of climatic challenges. The dominantly used tool for SRC is the AquaCrop model. This FAO-offered modelling software is intended to monitor crop productivity and yield response; it is able to predict the daily biomass productivity together with the final crop yield. It appeared that the model, when coupled with other techniques for ecological studies, could be useful even for prediction of some stand-level variables, like water balance for instance (Bloemen et al, 2016).

Dr. Manuela Balzarolo – She is the scientist working on the ongoing HYPI project. This project is aimed at linking isoprene (as one the most important Biogenic Volatile Organic Compounds) and flux measurements at leaf and canopy levels to hyperspectral vegetation indices. By doing this she tries to check the opportunity to use the latter as an indicator of temporal and spatial deviations of isoprene emissions from ecosystems. Manuela provided valuable advices towards the possibility of using remote sensing imagery for monitoring of the phenology in poplar SRC plantations.

Nerea De Oliveira Rodrigues – Nerea is involved in a (Spanish) PhD study on assessing the efficiency of different irrigation and fertilization regimes on the productivity of several poplar clones in Spain. The methodology, experimental design and some specific challenges she faced with during the implementation of the field experiments were discussed. Since it appeared that one of the most popular clones (‘I-214’) was used in the SRC poplar experiments in both countries (Spain and Bulgaria), we decided that it would be a good idea to look for options to compare its early growth performance in different climatic environments. Therefore, the compilation of existing data is forthcoming.

Dr. Alejandra Navarro – She is a postdoc deeply involved in studies linked with measuring sap flow with an idea to upscale the final analyses from the stem to the stand level. The poplar SRC in Lochristi is the experimental plot where she has done long-term monitoring of sap flow in some of the poplar genotypes. Alejandra provided valuable advices concerning the use of devices for sap flow measurements.

M.Sc. Stefan Vanbeveren, PhD student – Stefan has been deeply involved in various studies on the poplar SRC in Lochristi and we had very productive discussions about the applicability of the integrative approaches (including plant physiological research) for more complex general assessments of growth performance and biomass productivity of this type of bioenergy SRC plantations.

Dr. Jasper Bloemen – He is a postdoc recently recruited in connection with a project on "Drought legacies in the carbon cycle of forests across the globe". During his previous stay at PLECO, University of Antwerpen he produced – in co-authorship with Stefan and other colleagues from PLECO – an interesting comparative study on phenological assessments of the poplar SRC in Lochristi based on four different approaches for the quantification of phenology before and post coppicing (Vanbeveren et al., 2015). Since the phenological studies are directly related to the growth performance of poplar SRC, resp. to the bioproductivity, we are highly interested in the potential inclusion of such kind of research in our research in Sofia. The four types of approaches included: 1) visual observations of bud phenology; 2) LAI measurements; 3) webcam images; and 4) satellite images. The most essential conclusion from the study is that the final results were rather similar showing at the same time the effect of coppicing.

M.Sc. Cristina Ariza Carricondo – She is responsible for part of the field ecophysiology studies within the ICOS network, especially LAI measurements. Three approaches for indirect LAI measurements using different instrumentation were demonstrated, namely the SunScan (Delta T, UK) and Li-Cor 2200C (Li-Cor Biosciences, USA) canopy analyzers as well as a conventional reflex camera equipped with an objective with a fish-eye lens. She presented the draft results of a paper in progress on comparing the efficiency of the different above mentioned approaches for indirect LAI measurements performed in various environments, i.e. SRC poplar plantation, oak (*Quercus* spp.) and pine (*Pinus* spp.) forest stands. A rough preliminary conclusion is that the applied approaches provide similar results. She also compared direct (destructive) and indirect measurements on coppiced poplar plants. The results showed some overestimation of the data coming from the indirect measurements as compared with those calculated after collecting leaves with litter traps. We also discussed the affordability of the instrumentation used; we agreed that likely the use of a conventional reflex camera with a 'fish-eye' objective combined with an Open source software for analysis of the hemispherical pictures could be a good initial choice for LAI measurements when specific more sophisticated instrumentation are not available.

Dr. Joke Van den Berge - She is the manager of the PLECO part of the IMBALANCE-P project within a partnership with teams from four other European countries. The project is funded by the European Research Council (ERC) Synergy Grants of the EC and is aimed at assessing the impact of the imbalance between carbon, phosphorous and nitrogen on life in different terrestrial ecosystems of the planet.

Dr. Nicola Arriga – We visited together the Ecosystem Station situated in the National Park "Hoge Kempen", close to Maasmechelen, region Limburg (**Fig. 8**). He is responsible for the technical support of the instrumentation on site as well as for management of the ICOS data generated from the instrumentation. While at the site, he explained the functioning and the specificity of the installed devices for the continuous collection of ecological data.

Fig. 8. The unique heather vegetation in the Natural Park 'Hoge Kempen'

CONCLUSIONS

I greatly acknowledge the COST FP1301 action for giving me the grant to visit the Center of Excellence (PLECO) of the University of Antwerpen. I would like to cordially thank Prof. R. Ceulemans and all colleagues I met at the Drie Eiken campus of the University of Antwerpen for their friendliness and kind hospitality. I think my research visit in the Center of Excellence PLECO was extremely useful in terms of my further activity in plant ecophysiological research. Generally, I see the future benefits from my research visit in several directions. The accumulated new knowledge about the technical capacity and practical use of contemporary technical devices for plant (eco)physiology upscaled my professional skills and broadened my expertise in the field of plant (eco)physiology. Additionally, exploiting the opportunities of various hi-tech sophisticated devices for plant (eco)physiological research will create opportunities for a more profound understanding of the physiological processes not only at plant level, but within an integrative approach, to extend this knowledge to the ecosystem level. During my stay, I had the possibility to meet many colleagues dealing with various kinds of research in the field of ecology. All meetings were quite useful in terms of mutual introduction of the research activity, information exchange and discussion of research issues. As a result, opportunities for the establishment of new or for expanding existing thematic

research networks appeared. Moreover, the successful networking would be a good ground for collaborative work and coping with scientific challenges towards the improvement of life quality and mitigating the impact of global climatic changes.

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