

RST – Scientific report regarding the implementation of the "**REASONING – Drought resilience and ecological value of planted Douglas fir, Norway spruce, and Silver fir conifers in the face of climate change**" project (code: PN-III-P1-1.1-TE-2019-1099; Financing Contract for Project Execution NO. TE 75 / 2020) within the 15/09/2020 – 31/12/2020 period

I. ABSTRACT - STAGE I:

SPECIFIC OBJECTIVE OF THE STAGE I: realisation of two databases: growth and genetics of mixed Douglas fir, Norway spruce and Silver fir forests from the SW of Romania

ACTIVITIES OF THE STAGE I:

Stage I: Dendroecological and genetic studies in mixed Douglas fir, Norway spruce and Silver fir forests

Activity 1.1: Selection of mixed Douglas fir, Norway spruce and Silver fir forests in the SW of Romania.

Activity 1.1.1: The selection of the mixed Douglas fir, Norway spruce and Silver fir forests will be done based on the information extracted from Local Forest Management Plans. In this regard, the Ana Lugojana (Timiș), Rusca Montană (Caraș-Severin), and Oțelu Roșu (Caraș-Severin) Local Forest Administrations have been already contacted and a collaboration has been already established with them.

Activity 1.2: Field campaigns in order to sample the mixed Douglas fir, Norway spruce and Silver fir selected study sites.

Activity 1.2.1: Wood cores (to measure the annual tree rings) and leaves / cambium (to extract DNA) need to be sampled from 225 Douglas fir, Norway spruce, and Silver fir trees. The realisation of this activity was subjected to the COVID-19 situation, taking care not to risk the health of the team members. In case of such risk, the activity would have been postponed for the 2021 spring.

Activity 1.3: Processing of the samples collected during the field campaign.

Activity 1.3.1: Tree rings measurements and DNA extractions following specific dendrochronological and genetic protocols and software. Due to the fact that these activities are very precise and require thus a huge amount of work and time, they will be continued in the second stage (2021) of the REASONING project. This activity was subjected to the realisation of the activity 1.2 (field campaigns), but finally could be realised. The final result of these work will be two databases: tree growth and genetics.

II. SCIENTIFIC AND TECHNICAL DESCRIPTION, HIGHLIGHTS OF THE STAGE I RESULTS, AND THE DEGREE OF ACHIEVEMENT OF THE OBJECTIVES:

All the activities proposed for the stage I of the REASONING project have been realised successfully, in accordance with the implementation plan.

The REASONING project started in September 2020 (i.e., 15/09/2020). At the beginning of October 2020, the field campaign was already organized and could be realized with precautions considering the COVID-19 situation in Romania at that point.

Activities 1.1 and 1.1.1: after carefully studying the Local Forest Management Plans of the Ana Lugojana (Timiș), Rusca Montană (Caraș-Severin), and Oțelu Roșu (Caraș-Severin) Local Forest Administrations, we selected three different mixed Douglas fir, Norway spruce and Silver fir plantations located in the SW of Romania. This part of Romania was preferred as here the climate has a Sub-Mediterranean influence and the purpose of the REASONING project is to study, among others, these three species' drought-related performance considering their provenance and genetic diversity. The three mixed Douglas fir, Norway spruce and Silver fir study sites were named Ana Lugojana, Rusca Montană and Oțelu Roșu, in order to be easy to identify, and were selected to all have a north-facing exposition.

Activities 1.2 and 1.2.1: the field campaign took place at the beginning of October 2020. Field sampling consisted in sampling wood cores and leaves / cambium in order to do the dendrochronological and genetic proposed analyses. At each of the three study sites a number of 75 mature trees were randomly selected and sampled: 25 Douglas firs, 25 Norway spruces, and 25 Silver firs. All selected trees (75 trees x 3 sites = 225 trees) were dominant, with similar diameter at breast height (DBH), in order to avoid introducing unwanted noise into the analyses. Only living, healthy trees, with no signs of biotic attack (i.e., insects, fungi), nor wind- or snow-throw affected have been sampled. All sampled trees were situated along the same slope, so micro-site conditions should not largely vary between them either. No mortality events among the studied species were observed during the field campaign at any of the three study sites.

From each of the selected trees (i.e., 225), two wood cores were extracted at standard breast height (i.e., 1.3 m above ground), perpendicular to the slope to avoid compression wood, using increment borers (5 mm diameter; Haglöf, Sweden). These samples will be used to accomplish the **first specific objective of the REASONING project**: study the historical growth rates (i.e., tree rings) of the Douglas fir, Norway spruce, and Silver fir studied species from the Ana Lugojana, Rusca Montană and Oțelu Roșu study sites located in the SW of Romania.

From exactly the same 225 selected trees, we also took samples of leaves / cambium. This was done either using a telescopic scissor, needed to reach the leaves situated up in the crown, either a very resistant metallic cylinder, needed to extract cambium. These samples will be used to accomplish the **second specific objective of the REASONING project**: study the genetics of the Douglas fir, Norway spruce, and Silver fir studied species from the Ana Lugojana, Rusca Montană and Oțelu Roșu study sites located in the SW of Romania.

Activities 1.3 and 1.3.1: following the field campaign, we started to process the collected samples (i.e., wood cores, leaves, cambium). Specifically, the wood cores were air-dried, glued to special wood supports, and polished using a series of sand-paper grids until all tree-rings boundaries were clearly visible. The wood cores were then scanned at 1200 dpi (Epson Expression 11000XL) and started to be measured to the nearest 0.01 mm using the CooRecorder software (Cybis Elektronik & Data, Saltsjöbaden, Sweden). Tree rings measurements are a time consuming activity as it is a very precise process that needs special attention. Thus, this activity will be continued in the second stage (i.e., 2021) of the REASONING project. The final result will be a tree-rings database that will be used to perform dendrochronological and dendroecological analyses in order to compare the growth patterns of the three species (i.e., performance), and to evaluate their drought resistance/tolerance/resilience to past severe drought events (i.e., tree-rings faithfully register the climatic conditions to which the trees are being exposed to during their life). All these analyses could help us understand how these species have historically grown considering that they have all had the same environmental conditions. Furthermore, they will allow us to make estimations on how they will respond in the future to upcoming severe droughts considering the (different) strategies that they have used in the past to cope with such stressful events.

The leaves / cambium were used to extract DNA. For this, 60-70 mg of biological tissue (i.e., leaves / cambium) were first introduced in a 2 ml cylinder together with 2 tungsten balls and milled (30 Hz) for 2-3 minutes using the Tissue Lyser Roche MM400. DNA extraction was then performed following the CTAB protocol [19, 20] that consists of several steps. First step: in order to break the cellular membranes, 1000 μ l 2 x CTAB Extraction Buffer (containing CTAB, PVP, and β -mercaptoethanol) and 10-15 μ l of Proteinase K, were added to the milled samples that were then introduced into a Thermomixer for 30 minutes at 65°C and 550 rotations / minute. Second step: in order to separate and denature some specific proteins, 200 μ l of Wet Chloroform were added, and then, in order to eliminate residuals of CTAB and to purify the extracted DNA, 600 μ l of Isopropanol (-20°C) and 1000 μ l of Wash Buffer (i.e.,

50 µl ammonium acetate + 40 ml ethanol 76% + 10 ml of H₂O) were added. Finally, to the resulted solution, 50 µl of Elution Buffer were added. Following DNA extraction, its quality and concentration were tested using a Nanodrop 8000 spectrophotometer. To do so, 1 µl of the DNA stock solution was used. The concentrations resulted to be high enough, but the quality of the DNA, estimated considering the 260/280 ratio for which the optimal values range between 1.8 and 2, was in some cases low due to impurities. Thus, depending on the DNA quantity and quality, we needed to make different dilutions (i.e., 1:10 - 1:30) in order to obtain a concentration of approximately 20 – 30 ng/µl. The final result will be a genetic database that will be used to identify the putative geographic origin of the seeds that have been used to plant Douglas fir, Norway spruce, and Silver fir. The genetic analyses proposed for the REASONING project would also allow us to further evaluate the genetic diversity of the selected mixed plantations, information that would allow us to better understand local adaptation, and the fate of these conifer plantations considering climate change.

III. THE MANAGEMENT OF THE REASONING PROJECT:

In 2020, we needed to buy some materials in order to perform the dendrochronological and genetic analyses. Specifically, a wood grinding machine and accessories (i.e., protection audiphones and vices) were needed to polish the sampled wood cores. Then, for the DNA extractions and genetic analyses, different laboratory kits were also needed (i.e., genetic markers, Eppendorf tubes, microplates, pipette tips, etc.). Finally, the team also needed to buy printer toners in order to properly carry on its laboratory and desk work.

IV. CONCLUSIONS OF STAGE I OF THE REASONING PROJECT:

All activities (i.e., 1.1, 1.1.1, 1.2, 1.2.1, 1.3, and 1.3.1) proposed for the Stage I of the REASONING project were successfully completed during 2020, according to the working plan. The main results of this Stage I will be two databases (dendrochronological and genetic) that will serve as a base for further analyses on the Douglas fir, Norway spruce, and Silver fir planted trees sampled in the SW of Romania.

V. SCIENTIFIC ACHIEVEMENTS OF THE PROJECT LEADER DURING STAGE I OF THE REASONING PROJECT:

Since the beginning of the REASONING project, the project leader Ana-Maria Hereş has managed to publish one ISI article: Gazol A., **Hereş A.-M.**, Curiel Yuste J., Land-use practices (coppices and dehesas) and management intensity modulate responses of Holm oak growth to drought. *Agricultural and Forest Meteorology*, *in press*, <https://doi.org/10.1016/j.agrformet.2020.108235>

This article represents the result of her collaboration with international institutions. As during this period, the project leader was financially supported by the REASONING project, the project (PN-III-P1-1.1-TE-2019-1099) is mentioned in the Acknowledgement section of the published article.

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IP project,
dr. Ana-Maria Hereş

