

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 (PN-III-P1-1.1-TE-2019-1099; Financing Contract for Project Execution TE 75 / 2020) within the **01/01/2021 - 31/12/2021** period

Stage II: study of the three conifer species (**Douglas fir, Norway spruce and silver fir**) and their comparison using the two databases: annual growth data and genetic data

I. RST – RESULTS STAGE II:

A). ISI articles in which the REASONING project was mentioned in the Acknowledgements. Articles 1, 2, 3 and 4 resulted from the collaboration of the project leader Ana-Maria Hereş with international and national research centres. Given that, while working on these articles, the project leader Ana-Maria Hereş was financially supported from the budget of the REASONING project (PN-III-P1-1.1-TE-2019-1099), it was mentioned in the Acknowledgements of these articles.

1. **Hereş A.-M.**, Bragă C., Petritan A.M., Petritan I.C., Curiel Yuste J., **2021**, Spatial variability of soil respiration (R_s) and its controls are subjected to strong seasonality in an even-aged European beech (*Fagus sylvatica* L.) stand. European Journal of Soil Science 72, 1988 – 2005. <https://doi.org/10.1111/ejss.13116>
2. Encinas-Valero M., Esteban R., **Hereş A.-M.**, Becerril J.M., García-Plazaola J.I., Artexe U., Vivas M., Solla A., Moreno G., Curiel Yuste J., **2021**, Photoprotective compounds as early markers to predict holm oak crown defoliation in declining Mediterranean savannahs. Tree Physiology; <https://doi.org/10.1093/treephys/tpab006>
3. Gazol A., Camarero J.J., Sánchez-Salguero R., Zavala M.A., Serra-Maluquer X., Gutiérrez E., de Luis M., Sangüesa-Barreda G., Novak K., Rozas V., Tiscar P.A., Linares J.C., Martínez del Castillo E., Ribas M., García-González I., Silla F., Camison Á., Génova M., Olano J.M., **Hereş A.-M.**, Curiel Yuste J., Longares L.A., Hevia A., Galván J.D., Ruiz-Benito P., **accepted**, Tree growth response to drought partially explains regional-scale growth and mortality patterns in Iberian forests. Ecological Applications
4. **Hereş A.-M.**, Polanco-Martínez J.M., Petritan I.C., Petritan A.M., Curiel Yuste J., **in prep.**, The stationary and non-stationary character of tree-growth climate relationships revealed by heat map analyses based on rolling window correlations
5. Ciocîrlan E., Şofletea N., Petritan I.C., Petrea Ş., Curtu A.L., **Hereş A.-M.**, **in prep.**, Assessing genetic diversity and structure of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] plantations in Romania. **Results regarding the determination of the provenance of the seeds that have been used to plant the three conifer species and their genetic diversity.**

B). Conferences where the REASONING project was mentioned in the Acknowledgements:

1. Petrea Ş., Petritan I.C., **Hereş A.-M.**, **2021**, "Mixed silver fir, Douglas fir and Norway spruce plantations in the SW of Romania – growth and components of tree resilience to severe droughts". 5th Edition of the International Conference "Integrated Management of Environmental Resources"; Suceava, Romania; oral presentation. **Preliminary results regarding the growth performance of the three conifer species (Douglas fir, Norway spruce and silver fir) and their resistance / recovery / resilience to droughts.**

II. ABSTRACT OF STAGE II:

SPECIFIC OBJECTIVES OF STAGE II: study of the three conifer species (Douglas fir, Norway spruce and silver fir) and their comparison using two databases: annual growth data and genetic data.

ACTIVITIES ACCOMPLISHED DURING STAGE II:

Activity 2.1: Completion of the laboratory activities started in Stage I: measurement of annual tree rings (on wood cores) and DNA extraction (from leaves / cambium).

Activity 2.1.1: Due to the large volume of work and to the fact that these activities [i.e., measurement of annual tree rings (on wood cores) and DNA extraction (from leaves / cambium)]

are extremely thorough, requiring a lot of precision and therefore time, they are continued from Stage I.

Completion of the two databases: annual growth data and genetic data for each of the three studied conifer species (i.e., Douglas fir, Norway spruce and silver fir).

Activity 2.2: Carrying out a second field campaign.

Activity 2.2.1: This activity is necessary in order to estimate the regeneration capacity of the three studied conifer species (Douglas fir, Norway spruce and silver fir). At the same time, the regeneration capacity of other species (trees, shrubs) will be also estimated in order to evaluate the diversity of species that emerge and survive in mixed plantations of Douglas fir, Norway spruce and silver fir. Within this activity, we will also collect data (land, management plans) that will allow us to identify possible ecosystem services that may be offered by mixed plantations of Douglas fir, Norway spruce and silver fir. The data resulting from this activity will be analysed in Stage III.

Activity 2.3: Statistical analyses that will be used to write scientific manuscripts (i.e., ISI articles).

Activity 2.3.1: The two databases (annual growth data and genetic data) will be statistically analysed in order to achieve the 1) and 2) objectives of the REASONING project. Based on these statistical analyses scientific manuscripts will be written and published in scientific journals.

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

All the activities proposed for the Stage II of the REASONING project (i.e., see section II above) have been successfully completed, in accordance with the Implementation Plan.

Activities 2.1 and 2.1.1: In 2021, the laboratory activities started at the end of 2020 were completed: the measurement of the annual tree rings (on wood cores) and the extraction of DNA (from leaves / cambium). These activities were carried out on the samples collected during the 2020 field campaign in the mixed plantations of Douglas fir, Norway spruce and silver fir located in the SW of Romania: Ana Lugojana, Rusca Montană and Oțelu Roșu. The two activities took place within the Research - Development Institute of the Transilvania University of Brașov, following standard procedures for each of them. From the laboratory analyses, two databases were obtained: *i*). annual tree-ring data needed to study the historical growth of the three conifer species (**the first specific objective of the REASONING project**) and *ii*). genetic data needed to study the genetics of the three conifer species (**the second specific objective of the REASONING project**).

Activities 2.2 and 2.2.1: From 27.09.2021 to 03.10.2021, the members of the research team of the REASONING project carried out a second field campaign. During this field campaign, all 225 trees (Douglas fir, Norway spruce and silver fir), from which wood cores were extracted and leaves / cambium samples were collected in 2020, were revisited at the Ana Lugojana, Rusca Montană and Oțelu Roșu study sites. Thanks to the fact that during the 2020 field campaign all 225 trees had been geolocated, they were easy to be found again in 2021.

Following the methodology described by Curiel Yuste et al. 2019, a circle of 5 m diameter was established around each of the 225 selected trees and a detailed understory and competition survey was carried out within this circle (**the third specific objective of the REASONING project**). Specifically, all seedlings were counted and identified at the species level. At the same time, all the dominant competing trees were also established, identifying them at the species level and measuring their diameters and their distances to the 225 trees (i.e., the reference trees).

This field campaign was therefore necessary to estimate the regeneration capacity of the three studied conifer species (Douglas fir, Norway spruce and silver fir). The data collected during this field campaign will be used to also estimate the regeneration capacity of other tree and shrubs species. Thus, it will be possible to evaluate the diversity of species that emerge and survive in mixed plantations of Douglas fir, Norway spruce and silver fir. Regarding the competition data, this data will be used to understand the performance of the 225 sampled trees.

The data resulting from the activity will be analysed in Stage III.

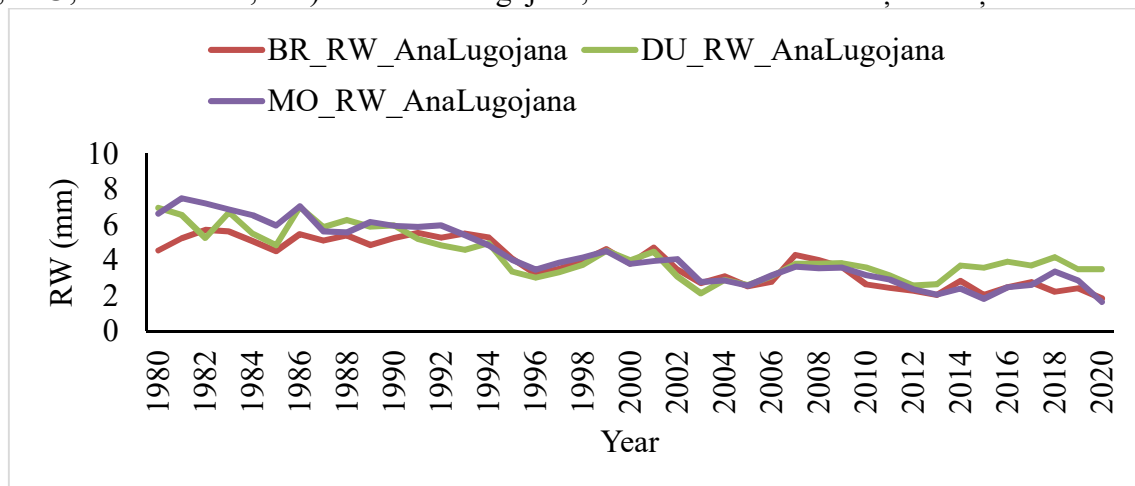
Activities 2.3 and 2.3.1: In 2021, the statistical processing of the two databases started: annual growth data (i.e., dendrochronological data) and genetic data. Specifically, after all the extracted wood cores were processed and measured, the resulting dendrochronological database was cleaned. Following this data cleaning process, out of the 225 trees from which wood cores were extracted, we were left with a total of 215 trees (Table 1), which means a very high crossdating rate (95%). The correlation with the master series, an indicator of the quality of the dendrochronological data, was always over 0.5 (i.e., according to the standards) (Table 1).

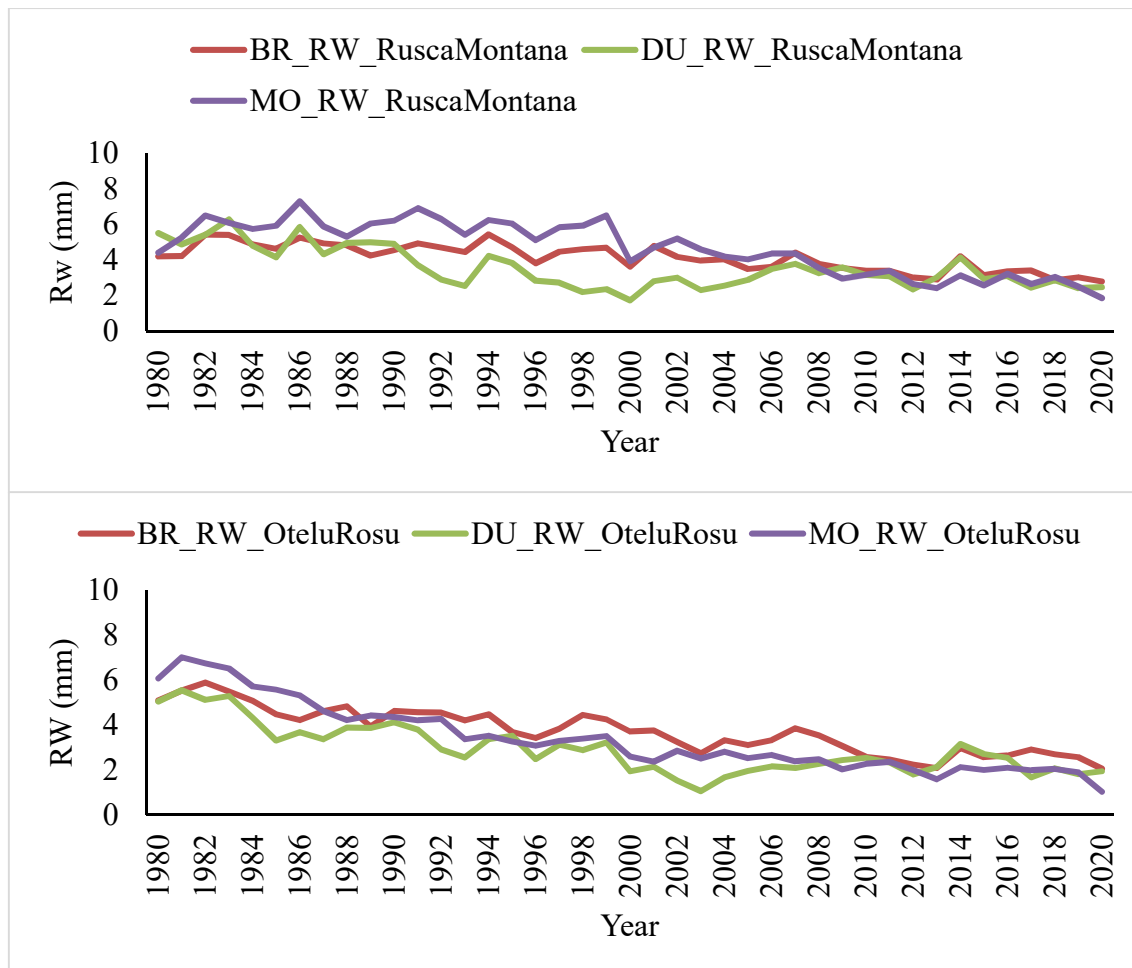
Table 1. Main characteristics of the 225 selected trees from Ana Lugojana, Rusca Montană and Oțelu Roșu

Species	silver fir			Douglas fir			Norway spruce		
	Ana Lugojana	Rusca Montană	Oțelu Roșu	Ana Lugojana	Rusca Montană	Oțelu Roșu	Ana Lugojana	Rusca Montană	Oțelu Roșu
No. of crossdated trees	23	24	24	25	25	24	24	24	22
Correlation with the master series	0.549	0.557	0.499	0.680	0.644	0.629	0.598	0.586	0.505
Age (years)	46	42	45	39	45	48	40	39	46

Preliminary results show that the average age of the Douglas fir, Norway spruce and silver fir trees from the mixed plantations Ana Lugojana, Rusca Montană and Oțelu Roșu is around 43 years (Table 1). Regarding their annual growth rates (i.e., RW), a slight negative trend was found (Fig. 1).

Fig. 1. Annual growth rates of the three studied conifer species (Douglas fir, **DU**; Norway spruce, **MO**; and silver fir, **BR**) from Ana Lugojana, Rusca Montană and Oțelu Roșu





These statistical analyses and preliminary results were used in the oral presentation Petrea Ș., Petritan IC, Hereș A.-M., 2021, "Mixed silver fir, Douglas fir and Norway spruce plantations in the SW of Romania - growth and components of tree resilience to severe droughts ". 5th Edition of the International Conference "Integrated Management of Environmental Resources"; Suceava, Romania. Further on, an ISI article will be prepared and published in an international journal.

Regarding the genetic database, it was compiled following the DNA extraction from the leaves / cambium and quality and concentration controls of the extracted DNA. In 2021, it was necessary to purchase laboratory materials to finish and therefore complete the genetic analysis. Subsequently, the obtained genetic data were analysed. These data will be used for the article: Ciocîrlan E., Șofletea N., Petritan IC, Petrea Ș., Curtu AL, Hereș A.-M., in prep., Assessing genetic diversity and structure of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] plantations in Romania.

IV. THE MANAGEMENT OF THE REASONING PROJECT:

In 2021, it was necessary to purchase laboratory materials to finish and complete the genetic analysis. Thus, the following materials were purchased: *i*). two pairs of genetic markers (V7-3 set of synthetic oligonucleotides (100 nm scale) and NAD711-2 set of synthetic oligonucleotides (100 nm scale) and *ii*). separation gel for Dual Plate GeXP and CEQT 8800 Systems (20 mL), Ceq DNA Separation Capillary Array (33 cm x 75) and Ceq DNA Size Standard Kit (400 bp, 96 sep).

V. CONCLUSIONS OF THE STAGE II OF THE REASONING PROJECT:

All activities (2.1, 2.1.1, 2.2, 2.2.1, 2.3 and 2.3.1) proposed for the Stage II of the REASONING project have been successfully completed during 2021, in accordance with the Implementation Plan. The main results of this stage are the two databases (dendrochronological and genetic), as well as the statistical analyses and preliminary results obtained (see Chapter I. **RST – RESULTS STAGE II** of this report).

VI. BIBLIOGRAPHY:

Curiel Yuste J., Flores-Rentería D., García-Angulo D., Hereş A.-M., Bragă C., Petritan A.-M., Petritan I.C., 2019. Cascading effects associated with climate-change-induced conifer mortality in mountain temperate forests result in hot-spots of soil CO₂ emissions. *Soil Biology and Biogeochemistry* 133:50-59; <https://doi.org/10.1016/j.soilbio.2019.02.017>

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