"Blue rings" in Scots pine (*Pinus sylvestris* L.) roots and stems as a proxy of cooling events in boreal and temperate zones of Europe (bROOT)

With climate change, the growing season is lengthening and temperature fluctuations at the beginning and end of the period are increasing. This means that trees are increasingly exposed to climatic extremes, including severe cooling. Trees play a vital role as natural archives of climate changes. Studying how trees respond to extreme climate conditions, including cooling events, is crucial to understanding the impact of global climate change on the biosphere. Tree rings, which serve as a unique marker of paleoclimatic conditions, allow us to track extreme events at high resolution. Moreover, in recent decades, quantitative analysis of wood anatomy has become increasingly important and can provide even seasonal solutions to these events.

The project aims to accurately identify cooling events through high-resolution analysis of tree rings in Scots pine (*Pinus sylvestris* L.) in boreal and temperate zones of Europe. We aim to perform our analyses over 250 long time period, which is much longer than instrumental meteorological data for the study zones. The final time span for our analyses will depend on the mean age of the tree sampled.

The first novelty of our research is that to obtain a holistic response of tree growth to cooling events, we will study both tree stems and roots. The second novelty is that we will focus on "blue rings" (BRs), i.e., not fully lignified tree rings. Their blue colour is the result of a wood preparation technique, in which not fully lignified wood cell walls are dyed blue. The occurrence of BRs is associated with cooling events. We will try to identify different types of BR in *Pinus sylvestris* L. stems and roots, which will enable recognition of the frequency and timing of cooling events with intraseasonal resolution.

Our research will be conducted mainly in the latitudinal and elevation treeline, i.e. in the northern part of the Scandinavian boreal zone, where trees are exposed to some of the most extreme climatic conditions and are very sensitive to temperature. Two study sites were identified: Iškoras in Norway (69°N) and Värriö in Finland (67°N), where there are over 250-year-old pine trees with exposed roots. In parallel, research will be carried out in the temperate zone in Poland in the Brodnica Lakeland (53°N), where we recently identified a high frequency of BR in exposed pine roots. To date, this is the only documentation of BRs in tree roots in the world.

Roots are the "holy grail" of dendrochronology. Dating roots is a big challenge for researchers due to many factors, such as their irregular growth, which is why they are rarely studied. However, recent research indicates that tree roots are a valuable bioindicator of environmental changes. The potentially longer growth period of tree roots during the year than tree stems and their high climatic sensitivity will allow for accurate identification of cooling events. Additionally, analysis of roots and stems will allow for a full understanding of the impact of soil conditions on tree growth. We anticipate that information obtained from roots about cooling events will not only complement but also enhance signals identified from tree stems.

As part of the bROOT project, all BRs analysis will be performed on high-resolution digital images which will enable accurate measurement of anatomical changes at the level of individual wood cells. We will link BRs occurrence in roots and stems to daily climatic data. Additionally, we will determine i) the characteristics of the pine stem and root growing season, including its start and end, and ii) the thermal and soil conditions affecting pine growth in the boreal and temperate zones.

Research will be undertaken on the lignification of cell walls in trees, i.e., the process responsible for the formation and resistance of wood. This study aims to advance our understanding of the physiological mechanisms behind wood lignification and its relation with temperature. As a result, the project will make a significant contribution to the broader discussion on thermal thresholds influencing tree ring formation. Moreover, the results of our research will constitute the first complete documentation of BRs in tree roots and stems. This will enable the creation of a quantitative repository detailing cooling events and tree growth responses with high temporal resolution.

Discovering the intricate 'blue rings' in Scots pine roots and stems will contribute significantly to advancing our understanding of how trees respond to extreme climate conditions. Additionally, comprehensively documenting their response to cooling events will highlight trees' key role in unraveling the effects of global climate change on the biosphere. The research topic of the project may be extremely interesting for dendrochronologists, climatologists, ecologists, and companies producing wood materials or forest services that develop strategies for the protection of forest ecosystems.