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Chlorophyll content and chlorophyll fluorescence analysis in *V. myrtillus*, *V. vitis-idaea* and *V. uliginosum* populations growing in different forest types in Latvia

Agnese GAILĪTE*, Anita GAILE, Dainis RUŅĢIS

Genetic Resource Centre, Latvian State Forest Research Institute "Silava", Latvia

*Corresponding author: <u>agnese.gailite@silava.lv</u>

Introduction

Non-destructive measuring methods are useful for recurring *in situ* plant monitoring to study plant responses to environmental changes as well as seasonal dynamics. Chlorophyll fluorescence methods are simple and rapid (Kalaji et al. 2016) and are mostly used in combination with other methods. Nevertheless, they provide useful information about photochemical function and protection, and indirectly show photosynthetic capacity. Fluorescence parameters reflect plant health status, acclimatization to various environmental conditions, daily and seasonal dynamics (Pieruschka et al. 2014) as well as leaf traits (Pollastrini et al. 2016). They also depend on abiotic factors such as temperature, drought, nutrient level, soil properties, as well as biotic factors.

Chlorophyll content in leaves determining photosynthetic capacity (Li et al. 2018). Changes in chlorophyll content may be a part of adaptive responses.

The most commonly used chlorophyll fluorescence parameters measured using a fluorimeter are:

 $\mathbf{F}_{\mathbf{v}}$ / $\mathbf{F}_{\mathbf{0}}$ - indicates maximum yield of primary photochemistry.

Fv/Fm - indicates the maximum quantum efficiency of photosystem II. This parameter is more stable and a value of 0.83-0.76 is defined as excellent for northern temperate tree species on a sunny day during growing season (Mohammed et al. 2003).

PI (Performance Index) is a sensitive parameter indicating plant vitality and consists of three independent characteristics: the concentration of reaction centres per chlorophyll, a parameter related to primary photochemistry and a parameter related to electron transport (Pinior et al. 2005, Strasser et al. 2000). PI_{ABS} is a PI for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors (Pollastrini et al. 2016).

The aim of the study was to determine chlorophyll content and chlorophyll fluorescence parameters of *V. myrtillus* L., *V. vitis-idaea* L. and *V. uliginosum* L. plants with non-destructive methods in different forest types.

Materials and Methods

Leaf chlorophyll content was measured using a SPAD-502 chlorophyll meter (Konica-Minolta, Osaka, Japan), and fluorescence parameters using a Handy PEA Chlorophyll Fluorimeter (Hansatech Instruments Ltd, Norfolk, England) during the growing season in 2018 and 2019. Leaves were dark adapted for 20 min. Measurements with all three species were performed in *Vacciniosa, Myrtillosa* forest types, and in addition for *V. myrtillus* and *V. vitis-idaea* measurements were made in *Hylocomniosa* forest type. Measurements were performed on 2-5 leaves from 10 individual plants.

Fluorescence parameters

 $\mathbf{F_v}/\mathbf{F_0}$ showed significant differences in different months in *V. vitis-idaea*. This parameter did not differ significantly in the other two species (Fig 2).

Results

Leaf chlorophyll content

Leaf chlorophyll content varied significantly in different months, but there were no significant differences in leaf chlorophyll content in the different forest types for all three species.

Leaf chlorophyll content was lowest in June, as this was the beginning of the growing season and slightly decreased in September in bilberries and bog bilberries (Figures 1A, 1C), as these species are deciduous. Chlorophyll content in the evergreen species *V. vitis-idaea* increases during growing season and continued to increase in September (Fig. 1B).



Fig. 2. F_v/F_0 in different forest types during the growing season A - V. myrtillus, B – V. vitis-idaea, C - V. uliginosum.

ANOVA Single Factor Analysis did not show differences in F_v/F_m between different forest types for all species, but was significantly different at different time points in *V. vitis-idaea*. F_v/F_m values varied between 0.83-0.76 which is defined as excellent (Mohammed at al. 2003) (Fig. 3).



Fig. 3. F_v /Fm in different forest types during the growing season A - V. myrtillus, B – V. vitis-idaea, C - V. uliginosum.

PI_{ABS} varied significantly in *V. myrtillus* and *V. vitis-idaea* in different months, but in *V. uliginosum* significant differences were not observed (Fig. 4). Significant differences between different forest types were not observed in any of the analysed species.



Figure 1. Leaf chlorophyll content in different forest types during the growing season A - V. myrtillus, B –V. vitis-idaea, C –V. uliginosum.

SPAD measurements for different species could vary due to leaf morphology or other traits. Therefore, data from different species are not comparable. Leaf water status, irradiance and time of the day may affect leaf chlorophyll content (Samsone et al. 2007).

Conclusions

The examined species are well adapted to all investigated forest types. This supports previous genetic analysis results, which found that Baltic populations of these species were not genetically differentiated, and that there are no significant barriers to gene flow between these populations. Nevertheless, our results indicate that species biology and seasonal conditions affect leaf chlorophyll content and chlorophyll fluorescence parameters.



Fig. 4. PI_{ABS} in different forest types during the growing season A - V. myrtillus, B – V. vitis-idaea, C - V. uliginosum.

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