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Non-destructive eco-physiological paramaters of evergreen ornamental shrubs under temperate zone climatic conditions

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ABSTRACT

Owing to geohistorical- and climatic conditions, the flora of Hungary is poor in broadleaf evergreens. According to the climate change scenarios, Hungary will be drier, richer in sunshine and winters are expected to become warmer because of the Mediterranean effect. Therefore, thermophile broadleaf evergreen ornamental shrubs were examined for their adaptation capability to the continental conditions. The species selected for the study were from two different habitats, *i.e.*, field and on hillside, namely *Aucuba japonica* 'Rozzanie', Cotoneaster *franchettii*, *Elaeagnus pungens* 'Maculata Aurea', *Ilex cornuta*, *Ligustrum sinense*, *Nandina domestica*, *Osmanthus heterophyllus*, *Phillyrea angustifolia*, *Photinia* x *fraseri* 'Red Robin', *Prunus lusitanica*, *Sarcococca hoockeriana*, and *Viburnum cinnamonifolium*. Our aim was to enrich the offering of nursery-gardens with these yet barely known taxa and the other purpose of this research was to promote the usage of portable *in situ* eco-physiological devices in various branches of horticulture especially in nursery-gardens. *C. franchettii*, *E. pungens* 'Maculata Aurea' and *L. sinense* are expected to adapt well to temperate conditions.

Key words: Chlorophyll contents, photosynthetic rate, thermophile, evergreen ornamental shrubs.

INTRODUCTION

Climate change has always been of great importance, but these changes are caused in large part by human activities. The elevated atmospheric CO_2 concentration and the increased amount of greenhouse gases have led to increased temperature, associated with drought and extremities of the weather. Hungary is poor in broadleaved evergreens because of geo-historical- and climatic conditions and it is very important. It is well known that broadleaved evergreen shrubs originate from generally slightly warmer and milder winter areas. Reading the different climate scenarios, it was thought that the climate in Hungary (USDA Hardiness Zone 5-7) could change in the future. Thermophile varieties were trying up, which can enrich the domestic ornamental's offering of nursery-gardens. To study the behaviour of these thermophile evergreens on our still continental climate, non-destructive ecophysiological tools were used in the long-term experiment. The aim of the current work was to show, that these measurements are not only used in cereal crop research, but also in ornamental horticulture to monitor the health of stand.

Chlorophyll fluorescence is based on the principle that photosynthesis is one of the core functions in the physiology of plants and plant growth is dependent on photosynthesis. Pulse-amplitude-modulation chlorophyll fluorescence (Schreiber *et al*., 11) can give rapid, non-invasive measurements of changes in Photosystem II (PSII) and also possibility to evaluate the effects of environmental factors' changes on PSII performance (Lichtenthaler, 8; Makarova *et al*., 9; Clark *et al*., 4; Kasturi *et al*., 7; Ashraf and Harris, 2).

The leaf chlorophyll concentration is usually determined by extraction from leaf samples and subsequent spectrophotometric measurements. Such *in vitro* determinations are destructive, expensive, and time consuming, and may therefore not be applicable for all purposes. A recently developed device to quickly and non-destructively measure foliar chlorophyll concentration is the chlorophyll meter where the value should correspond to the amount of chlorophyll present in the sample leaf and the meter calculates a unitless "SPAD" value (an index from 1 to 100).

MATERIALS AND METHODS

During the experiment, the following 14 species or varieties of evergreen ornamental shrubs have been examined in collection plot of University Pannonia Georgikon Faculty, Place since 2007: *Aucuba japonica* Thunb. 'Rozzanie' (Cornaceae), *Cotoneaster franchettii* Bois (Rosaceae), *Elaeagnus pungens* Thunb. 'Maculata Aurea' (Elaeagnaceae), *Ilex cornuta* Lindl. and Paxt. (Aquifoliaceae), *Ligustrum sinense* Lour. (Oleaceae), *Nandina domestica* Thunb. (Berberidaceae), *Osmanthus heterophyllus* (G. Don) P.S. Green (Oleaceae), *Phillyrea angustifolia* L. (Oleaceae), *Photinia* x *fraseri* Dress. 'Red Robin' (Rosaceae), *Prunus lusitanica* L. (Rosaceae), *Sarcococca hoockeriana* Baill. (Buxaceae), *Viburnum*

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cinnamonifolium Redh. (Caprifoliaceae). These taxa are originated from USDA Hardiness Zone 6-9.

For the experiment, a 4,250 m^2 area was used on two habitats, on the experimental farms of the University of Pannonia, Georgikon Faculty. In Hungary, there is a considerable difference between winter and summer. Daily average temperatures vary from -2°C in January to 22°C in July. Spring and summer are the wettest periods, much of the rain comes in frequent heavy downpours. Summer weather is pleasantly warm or hot, whilst the winters are cold with snow and fog. The annual precipitation varies between 650-700 mm, and the yearly average hours of sunshine amount to 2000.

Habitat 1 was of 1,750 m^2 located on the Experimental Station for Fruit Cultures of the University, Keszthely (46°44' 46" N and 17° 14' 19" E). The conditions of the plot are plane and medium compacted brown forest soil. The groundwater level is below 200 cm of the surface. The height of tilth is above 150 cm. The top-soil is shallow. The soil is acidic-neutral, its physiological hardness as the humus content is low. The soil analysis data indicate a weakly good phosphorus and potassium supply. The supply of magnesium is good, of manganese and copper it is abundant, while the supply of zinc is optimal. The soil is poor in mineral nitrogen. Thirty pieces of each taxa were planted and according to their viability the plant distance was 2.5 m x 1.5 m, 2.5 m x 1.0 m and 1.0 m x 1.0 m.

Habitat 2 was of 2,500 m^2, is located on Cserszegtomaj, on the Experimental Station for Wine Cultures of the University (46° 47' 44" N and 17° 15' 46" E). On the hillside the groundwater level was below 200 cm. The height of tilth is changeable and stony. The top-soil is shallow-medium. The field condition is brown forest soil on dolomite bedrock. The soil is weakly alkaline, its physiological hardness is medium-high, its humus content is low. The concentration of phosphorus and potassium in soil is very good-good. The supply of magnesium is good, of manganese, copper and zinc is abundant. The level of mineral nitrogen is medium. There were planted 10-15 pieces of each taxa in park-like arrangement. The examined species were planted in the first half of June 2007.

In both experimental areas there was an "iMETOS" (Pessl Instruments GmbH, Weiz, Austria), a mini weather station, which allowed for permanent evaluation of weather data, which were on the www.fieldclimate.com directly and any time accessible. The first experiment was performed between 20 and 22 July, 2010 and the second between 22 and 23 September, 2010. The summer measurements were performed in sunny weather with cloud-free sky, at noon between 11:00 and 14:00 h, and the temperature was 29-30°C. The September measurements were also carried out at noon in the same time. The temperature was only 21°C. The effective quantum yield in a light adapted state was measured in the different habitats on the upper surface of mature, visible healthy, current year leaves according to Van Kooten and Snel (14) using a portable pulse-amplitude-modulated fluorometer (PAM-2000, H. Walz, Effeltrich, Germany). Measurements were randomised according to plants. The photochemical efficiency of PSII (ΦPSII or yield or Y or the actual quantum yield*)* in light adapted state was calculated according to Genty *et al*. (5): $\Phi PSII = Y = \Delta F/F\text{m}' = (F\text{m}' - F\text{s})/F\text{m}'$, where Fm' is the maximum fluorescence yield in light-adapted state and Fs is the fluorescence in steady-state. Measurements were made in twenty replicates in each population. Leaf chlorophyll content was measured in natural conditions by soil plant analysis development chlorophyll meter (SPAD-502 chlorophyll meter Konica-Minolta, Osaka, Japan). The measurements were carried out in 2010, six times on the experimental areas.

For the measurements only visibly healthy leaves were used. Five consecutive readings were made across the adaxial surface of each leaf avoiding the main veins. The mean value from the five readings was calculated using the internal function of the chlorophyll meter. Measurement was performed on 30 intact leaves from at least five individual plants.

Statistical analysis was performed using ANOVA for comparisons of means. In case of significant interactions between factors, monofactorial effects were analysed separately. For testing the difference between sample means, Student's t-test was used. MS Office Excel 2003 (Microsoft, Redmond, Washington) was used for the statistical analysis.

RESULTS AND DISCUSSION

The results of the actual quantum yield measurements in the two different habitats can be found in Tables 1 & 2. First the actual quantum yield of PS II ($Y = \Delta F/F\text{m}'$) was analysed. On the basis of the Two-way ANOVA both the habitats and the species/varieties had a significant role in which habitats preferred the observed species/varieties. Because of a significant interaction between species and the experimental area, One-way ANOVA was carried out, the habitats were compared with the species and the species/varieties were compared with the habitats.

The species/varieties by comparing the measurements in July 2010 showed that *C. franchettii* and *P. angustifolia* were not sensible to the different experimental areas. This time were not any sensitive taxa (R^2 % > 50) found to the different habitats. By autumn *S. hoockeriana* was destroyed because of the summer-drought, therefore it was no longer included in the investigation. In September 2010 the following species were insensitive to the different soil and terrain

Table 1. The results of the photochemical efficiency ($\Delta F/F_m$) of PSII in light adapted state of thermophile ornamental shrubs on two different habitats in July 2010.

Plant species	Field	Hill side	Mean	LSD (P = 0.05)	R^2	p-value
A. japonica 'Rozzanie'	0.52	0.67	0.60	0.07	44.5	***
C. franchettii	0.72	0.74	0.74	0.03	2.9	0.37
E. pungens 'Maculata Aurea'	0.72	0.68	0.70	0.04	13.0	0.05
I. cornuta	0.65	0.55	0.60	0.06	32.2	**
L. sinense	0.68	0.61	0.65	0.03	39.9	***
N. domestica	0.58	0.63	0.61	0.05	12.3	0.07
O. heterophyllus	0.61	0.52	0.57	0.05	31.9	**
Ph. angustifolia	0.66	0.66	0.66	0.04	0.5	0.72
Ph. x *fraseri* 'Red Robin'	0.65	0.53	0.60	0.06	35.8	***
P. lusitanica	0.69	0.64	0.67	0.04	14.1	*
S. hoockeriana	0.60	0.54	0.57	0.06	11.4	0.07
V. cinnamonifolium	0.62	0.54	0.59	0.04	37.8	***
Mean	0.64	0.61				
LSD (p < 0.05)	0.04	0.05				
R^2	49.20	49.9				
p-value	***	***				

*p <0.05, **p <0.01 and ***p <0.001.

Table 2. The results of the photochemical efficiency ($\Delta F/F_m$) of PSII in light adapted state of thermophile ornamental shrubs on two different habitats in September 2010.

Plant species	Field	Hill side	Mean	LSD (p < 0.05)	R^2	p-value
A. japonica 'Rozzanie'	0.55	0.60	0.57	0.03	31.5	**
C. franchettii	0.62	0.61	0.62	0.02	5.9	0.20
E. pungens 'Maculata Aurea'	0.66	0.56	0.61	0.06	29.8	**
I. cornuta	0.67	0.57	0.62	0.05	35.0	***
L. sinense	0.66	0.66	0.66	0.03	0.1	0.90
N. domestica	0.43	0.61	0.52	0.05	67.4	***
O. heterophyllus	0.62	0.59	0.61	0.04	4.7	0.25
Ph. angustifolia	0.60	0.62	0.61	0.02	12.5	0.05
Ph. x *fraseri* 'Red Robin'	0.67	0.50	0.59	0.03	86.4	***
P. lusitanica	0.62	0.63	0.63	0.04	0.8	0.64
S. hoockeriana	0.61	0.54	0.58	0.08	41.4	***
Mean	0.61	0.59				
LSD (p <0.05)	0.04	0.04				
R^2	60.8	33.8				
p-value	***	***				

*p <0.05, **p <0.01 and ***p <0.001.

conditions: *C. franchettii*, *L. sinense*, *P. lusitanica* and *O. heterophyllus. N. domestica* and the *P.* x *fraseri* 'Red Robin' were sensitive to different habitats. In September 2010, the good quality field conditions shared the plants to a greater extent than the dolomite bedrock of the brown forest soil on the hillside. If the cumulated means of the two habitats are compared, it can be seen that the examined plants favoured the field conditions with high-quality brown forest soils, in contrast to the hillside with dolomite bedrock. However, it could be noticed that the results of the measurements in September were lower in both habitats. This could be related to the sudden cooling in September. During the winter 2009-2010, *V. cinnamonifolium* suffered serious frost damages and as a result had to be cut back to ground level. During the spring, it began to sprout out again and the measurements were performed on these new leaves. It clearly showed that the examination gave a current picture of the photosynthetic performance, rather than of the whole plant's condition and of the lived stress, from which it regenerated. Since it required visual inspection, it should be cautioned that chlorophyll fluorescence rather than being used alone is usually best applied within the context of a broader physiological analysis (Mohammed *et al*., 10). The chlorophyll fluorescence measurements provide a powerful non-destructive method for fast out-door screening of trees (Lichtenthaler, 8) and it is useful also for gardeners to monitor the health and vitality of plants (Clark *et al*., 4). It was also useful in our experiment to determine the physiological conditions of these evergreen shrubs. Summarized average was calculated from the measured data, so the lost species reached low places, therefore they could not be suitable to plant in our country without any protection in winter. *S. hoockeriana* should overwinter in a protected place, the simplest being a portable container for planting. On the basis of the aggregate data from the other species/ varieties *C. franchettii*, *P. lusitanica*, *E. pungens* 'Maculata Aurea' and *L. sinense* had high ($\Delta F/F_m > 0.6$) values on both habitats at the measurement times, meaning that they probably adapted well to our climate. According to our observations the forest soil with good quality and water storage ability could be more suitable for the other taxa (*I. cornuta*, *O. heterophyllus* and *P.* x *fraseri* 'Red Robin').

Comparing the two habitats, Cserszegtomaj showed a still lower actual quantum yield value at the given time of the examined taxa, this is probably due to the rapid drainage of rainfall and a few tenths of degrees lower temperatures. This experimental area has a declivity with a southern exposure, which has stronger irradiation - a higher solar radiations angle of incidence - the soil more heated, often more dehydrated (Stefanovits *et al*., 13) because the better warming up slope has higher evaporation, so lower average soil moisture can be expected on such a slope (Anda and Dunkel, 1). Applications of chlorophyll fluorescence in forestry and ecophysiology have expanded markedly within the last decades, owing to the non-destructive nature of the technique, its sensitivity to stress effects and its portability. It is now commonly used in laboratory and ecophysiological research in forestry (Mohammed *et al*., 10), but as yet it has not been widely applied in ornamental plant nurseries; however it would be useful to get to know the environmental demands of the various taxa.

The results of SPAD measurements in the two different habitats can be found in Tables 3 & 4. A large body of literature demonstrates that the optical method does not allow for a direct comparison of chlorophyll contents between different plant species because of the different leaf texture and surface (wax, trichomas), therefore only the same species/varieties

Table 3. Chlorophyll content in thermophile ornamental shrubs growing under field conditions.

Plant species	1st measurement	2nd measurement	3rd measurement	4th measurement	5th measurement	6th measurement
A. japonica 'Rozzanie'	29.04	46.91	48.99	60.17	60.58	73.66
C. franchettii	50.39	55.51	56.26	59.64	66.22	68.22
E. pungens 'Maculata Aurea'	37.02	37.72	42.86	43.07	43.08	35.18
I. cornuta	40.23	56,04	69.75	69.81	70.60	83.47
L. sinense	43.56	51.33	52.42	63.18	65.68	57.66
N. domestica	36.15	45.79	50.94	39.05	35.03	34.20
O. heterophyllus	52.14	71.43	61.69	77.35	81.75	83.73
Ph. angustifolia	46.35	58.26	60.23	67.94	74.03	85.72
Ph. x *fraseri* 'Red Robin'	32.57	46.80	54.43	59.27	59.80	80.92
P. lusitanica	33.67	56.97	59.57	71.98	71.80	82.07
S. hoockeriana	46.39	59.07	60.63	no data	no data	no data
V. cinnamonifolium	35.83	49.24	64.00	65.59	66.14	79.85

Table 4. Chlorophyll contents in thermophile ornamental shrubs growing on the hillside.

Plant species	1st measurement	2nd measurement	3rd measurement	4th measurement	5th measurement	6th measurement
A. japonica 'Rozzanie'	31.28	44.35	47.22	53.41	63.84	51.25
C. franchettii	46.05	50.74	55.43	58.63	61.44	63.10
E. pungens 'Maculata Aurea'	33.76	36.45	43.07	46.11	39.49	20.77
I. cornuta	32.21	44.31	49.97	60.34	62.72	72.90
L. sinense	35.91	46.01	52.06	53.01	54.05	64.90
N. domestica	30.84	35.75	44.70	45.69	43.39	49.86
O. heterophyllus	39.56	57.76	71.18	71.89	73.22	83.35
Ph. angustifolia	44.12	54.43	56.08	65.70	66.44	68.48
Ph. x *fraseri* 'Red Robin'	26.11	33.78	37.63	39.79	40.57	44.79
P. lusitanica	32.21	44.60	45.90	57.30	57.86	65.13
S. hoockeriana	43.85	48.59	49.97	no data	no data	no data
V. cinnamonifolium	37.48	38.57	47.85	60.81	61.58	75.23

were compared in two habitats. The measurements were analyzed and it was found that the field-planted studied taxa had higher SPAD values than the one on hillside. Using a matched pairs t-test it was found that the SPAD values in the field conditions to the hillside conditions were significantly higher of the following species/varieties: *I. cornuta*, *P. angustifolia*, *P.* x *fraseri* 'Red Robin', *P. lusitanica*, *S. hoockeriana*. According to the graphs for *C. franchettii* there was no difference between habitats, but using Student's t-test the averages were significantly different from each other for the benefit of the arable land, contemporaneously R^2 % = 36.6 was in the field and on the hillside R^2 % = 60.5, that is, over time chlorophyll content varies in higher degree in Cserszegtomaj than in Keszthely.

Viewing the graphs, the SPAD reading changes of *E. pungense* 'Maculata Aurea' drew a very interesting curve, because the results of the last two measurements decreased. Since this is a yellow spotted leaf variety, it is possible that increased autumn yellowing of leaves. *N. domestica* was also found to have experienced a similar curiosity, but the decline was observed only in the field. *L. sinense* may show semi-evergreen tendencies but it is evergreen in mild climate and deciduous in cold climates. At the last measurements of *L. sinense* the SPAD value fell on the good quality arable land, because it seems to be semi-evergreen losing its leaves, which have also been experienced in colder winters. At *A. japonica* 'Rozzanie' in the experimental plantation located on the hillside, a similar decline was found, which is similar to the results reported by Hiyama *et al.* (6). During third occasion of measurement on *O. heterophyllus* it was found, that the SPAD readings decreased in field, which could have been caused by a possible stress, which lasted only for a short time, because it did not cause any permanent damage, but just long enough to be detected. The meteorological data was examined and it was found that in the days before the measurement the maximum temperature reached 34.73°C at the departmental trial area, which was higher than on to the sunlight exposed hillside. Taking a closer look at the data, the deviation was also very high at this time, which could reflect the different sensitivity of each plant. Among the SPAD-values of *V. cinnamonifolium* it was found that the data were higher on field, while the averages of these populations with the Student's t-test were not different.

One-way ANOVA was carried out and it was found, that *E. pungens* 'Maculata Aurea' (R^2 = 2.6% in the field; R^2 = 14.5% on the hillside) and *N. domestica* (R^2 = 18% in the field; R^2 = 13.7% on the hillside) both were quite low, *i.e.*, during the examined period the chlorophyll content changed less, the passage of time had little effect on these taxa. This is probably associated with the coloured leaves.

Several studies reported that the SPAD-502 enables to measure chlorophyll contents simply, rapidly and non-destructively in the field which was confirmed with this study. Hiyama *et al.* (6) observed seasonal variation in deciduous plants and evergreens. SPAD showed a clear seasonal change year-round in the examined plants. SPAD was low during leaf expansion, high when the leaves were mature and dropped suddenly with senescence. In agreement with the finding of Silla *et al*. (12) about evergreen *Quercus* species, it was found, that the chlorophyll content depends on leaf age, the leaves grew older and, as we expected, it changed the SPAD readings. Measurements were performed in the natural habitats of plants, they were non-destructive, compared with the traditional destructive methods, this equipment might provide a substantial saving in time, space and resources (Chang and Robison, 3), they were rapid which allows a large number of repeating, that is a major requirement of statistical analysis.

The interest of nursery-gardeners in new ornamental species is always enormous, but it is necessary to know mechanisms of the plant responses to environmental factors, their acclimation during nursery production. Portable devices for ecophysiological measurements on ornamental plants in their natural habitat have been used for the first time in Hungary in this study because so far these instruments have been mainly used in laboratory and on cereal crops for tolerance in controlled stress situations. Our results support the usage of non-destructive measurements in various branches of horticulture to meet the environmental requirements of the different plant populations.

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