Visible and near infrared hyperspectral imaging reveals significant differences in needle reflectance among Scots pine provenances

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Abstract

Genetic diversity is an important indicator of forest sustainability requiring particular attention and new methods to obtain fast and cheap estimates of genetic diversity. We assessed the differences in visible (VIS) and near infrared (NIR) spectral reflectance properties of detached shoots of several distant Scots pine provenances aiming to identify the most informative spectral wavebands and the seasonal time for the genetic diversity scoring. Shoots of five trees per provenance were sampled at two week intervals during the active growth and fall. The samples were scanned using a hyperspectral camera, equipped with a highly sensitive spectrometer capable of covering the spectral range of 400–1000 nm with a sampling interval of 0.6 nm. The ANOVAs revealed significant provenance effects on the spectral reflectance at variable spectral intervals depending on the sampling occasion. During the active growth, PCA identified the most informative wavebands over whole spectral range investigated. During the shoot/needle hardiness development, NIR was the most informative. Provenance ranking in spectral reflectance returned geographically interpretable pattern. We conclude that there are significant provenance attributable and interpretable differences in spectral reflectance of Scots pine needles providing a good opportunity for detecting this spectral variation with the hyperspectral imaging technique.

Key words: Pinus sylvestris, forest inventory, genetic diversity, phenology, remote sensing.

Introduction

Owing to recent indications of global climatic change, genetic stability of forests became an important issue (Savolainen et al., 2007; Kremer, 2006). Genetic diversity being among the main components of the genetic stability may require a monitoring network to reduce the risk of mismanagement. The DNA-based estimates and the on-site phenology-based scoring of genetic diversity are costly, labor demanding and territorially limited methods. New approaches to obtain fast and cheap estimates of genetic diversity would be helpful in developing the forest genetic diversity monitoring network. In this study, we tackle this problem by testing the possibility for using the hyperspectral imaging (HSI) for rapid assessment of genetic diversity in Scots pine chosen for its economic and ecological importance in Eurasia.

The rapid improvement of HSI and invention of the specialized software for processing the hyperspectral images are considered as one of the most significant achievements in remote sensing (Im and Jensen, 2008; Lillesand et al., 2008; Eismann, 2012). In a hyperspectral image, a pixel contains electromagnetic reflectance information about the entire spectral range captured by hyperspectral sensor, producing so called "spectral signature". Hyperspectral sensors, due to high spectral resolution, are capable to detect spectral signatures unique to specific materials: minerals, water, vegetation, etc. Thus, HSI offers the enhanced ability to derive accurate information about internal leaf properties associated with the physiological status of a tree (Kokaly and Clark, 1999; Luther and Carroll, 1999; Carter and Knapp, 2001; Moorthy et al., 2008; Wang and Li, 2012). HSI combines digital imaging and spectroscopy in a single scheme. Thus, hyperspectral cameras are instruments capable to acquire images of an object in hundreds of narrow (nanometer level) contiguous spectral bands. Depending on the construction, hyperspectral cameras can sense the electromagnetic waves in the ultraviolet, visible, near infrared, mid infrared and even thermal ranges of the electromagnetic spectrum (Im and Jensen, 2008). HSI provides a high potential for precise identification, discrimination, and classification of various objects and their features (Treitz and Howarth, 1999; Im and Jensen, 2008). The spectral reflectance properties of trees at the leaf, crown or entire stand level has been studied already for several decades indicating that reflected electromagnetic radiation recorded in narrow spectral bands provides valuable information about the condition and physiological status of plants. Field or laboratory taken hyperspectral measurements at a single plant level can significantly contribute to the discrimination of plants at the species level or reflect physiological and chemical properties of plants (Castro-Esau et al., 2004; Manevski et al., 2011; Vaiphasa et al., 2005; Masaitis and Mozgeris, 2012; Masaitis et al., 2013). Usually, the focus is to identify the portion of the spectrum or even single wavebands markedly contribut-

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ing to the spectral separability of plant species, condition or chemical constituents (ASNER and MARTIN, 2008; MOORTHY et al., 2008; WANG and LI, 2012; MASAITIS, 2013).

Potential application of HSI in the field of forest genetics is diverse. Starting from research, HSI may assist or, in case of routine surveys, even replace the time-and-labor-demanding field measurements of phenology or injury on genetic entries tested in field experiments. In population genetics, HSI may assist in studying species hybridization and introgression or finding rare forms (HODKINSON, 2013; DANUSEVICIUS et al., 2013). Rapid and cost-efficient estimate and monitoring of genetic diversity in natural and artificial forests, especially newly established plantations would allow timely identifying the risk zones and applying the enrichment measures. Early studies spend years of field surveys on within-stand variation in phenology of various forest tree species (GABRILAVICIUS and DANUSEVICIUS, 2003). Finally, the HSI may assist in detecting the illegal imports of forest reproductive material or undesirable hybridization such as in THOMASSET et al., (2013).

The physiological status of trees varies over the annual cycle depending on the phenological stage. The phenology of trees changes markedly from the state of dormancy during the cold period to active growth during the warm period (PERRY, 1971). The well-known patterns of phenology variation among the populations of northerly conifers (EKBERG et al., 1979) constitute the background for our idea to utilize this variation in rapid detection of genetic differences. Northerly conifers are adapted to specific timing of phenology at the original location, so that the onset and cessation of active growth, building up of bud dormancy and shoot frost hardiness, rest, chilling requirement to break the rest and the transition to quiescence exhibit clear patterns of geographical variation depending on the temperature and photoperiodic gradients at the place of origin (EICHE, 1966; ERIKSSON et al., 1980; PERSSON and PERSSON, 1992; DORMLING, 1993; DANUSEVICIUS and GABRILAVICIUS, 2001; HANNERZ, 1998). Therefore, for northerly conifers, the phenology traits being of high adaptive significance are among the best genetic markers revealing the genetic differences between the trees (ERIKSSON et al., 1978; HÄNNINEN and PELKONEN, 1989). Another specific feature of northerly conifers is the large geneflow-mediated variation in phenology within the natural populations constituting the basis for adaptation under changing environment (SKRØPPA, 1982; EKBERG et al., 1985; ERIKSSON, 1991). The content of pigments in Scots pine needles is markedly affected by growing conditions, plant age, needle age and origin of the seed source (LINDER, 1972). Can these phenology-related physiological changes within the needle and shoot meristems of the genetically different trees be detected with the HSI method with an acceptable error? The answer is perhaps as there is a positive indication for HSI capabilities to detect phenological and so genetic variation in forest trees (SUNDBLAD et al., 2001). For instance, the variation in foliar chlorophyll and carotenoids as well as additional constituents within the leaves affect leaf spectral properties in the visible (VIS) and near infrared portions (NIR) of the spectrum and provide the possibility of remotely diagnosing plant stress level (ROCK et al., 1986; CARTER, 1993; MARTIN and ABER, 1997; DATT, 1998; ZARCO-TEJADA et al., 2004). Changes in leaf chlorophyll and water content affect the leaf reflectance and so could be spectral indicators of variation in growth onset in spring (CARTER, 1993). Whereas, the increase of lignin contents in cell walls and structural changes within the meristems towards hardening and dormancy may provide spectral reflectance for detection of variation in growth cessation (SUNDBLAD et al., 2001; ROCK et al., 1994).

The first step in the investigation of the benefits of HSI for estimating the genetic diversity is to identify the informative spectral wavebands and seasonal time when the HSI assessments are the most efficient. The objective of our study was to assess the differences in VIS and NIR spectral reflectance properties of detached shoots and needles of several distant Scots pine provenances aiming to identify the most informative spectral wavebands and seasonal time for the diversity scoring. The ultimate goal is to develop procedure for application of hyperspectral imaging for rapid estimation of genetic diversity in forest trees.

Materials and Methods

Sampling in the field test

Needle samples were collected in the common garden field test of Scots pine provenances belonging the Prokazin series of provenance field tests (SHUTYAEV and GIERTYCH, 1998) established in 1975 in Jūrė forest district Lithuania (WGS coordinates E23.593, N54.791). At the time of the sampling, the trees in the field test reached the age of 39, mean tree diameter of 18.9 cm (std. dev. of 7.3 cm). The following four distant Scots pine provenances were selected in the field test to repre-

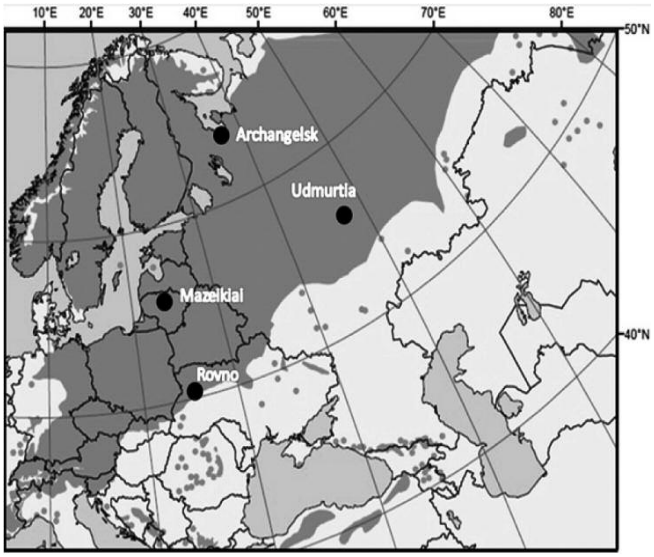

Figure 1. – Location of the provenances. The grey area indicates natural distribution range of Scots 6 pine.

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sent different parts of the species range: Arkhangelsk (north-western Russia N64.533, E40.533), Mažeikiai (Lithuania, E22.333, N56.317), Rovno (western Ukraine, E26.250, N50.617) and Udmurtia (continental Russia, Ural region, E52.750, N57.283) (*Fig. 1*). Five healthy trees of mean height 15 m with no observable foliar loss or other symptoms of stress were randomly selected from each provenance in the field test.

The needles were sampled and hyperspectral scanning sessions were performed at seven occasions during May to September 2012. The sum of mean daily air temperatures exceeding 10 °C (effective temperature sum – ETS) was calculated for each sampling date (data from Kaunas weather station located at E23.835, N54.883, within 18.5 km from the test site). The sampling dates were May 10 (ETS 242.4 °C), May 24 (ETS 405.3 °C), June 11 (ETS 624.8 °C), July 11 (ETS 1165.5 °C), August 22 (ETS 1925.0 °C), September 10 (ETS 2205.2 °C), and September 26 (ETS 2400.8 °C).

Climbing equipment and a telescopic cutter were used for collection of samples. One lateral branch containing several shoots was sampled from the southern, western, northern and eastern sides of the middle-upper part of the crown of each tree. Only the current year's shoots were collected. At each sampling occasion, 20 shoots were collected to represent a provenance (4 shoots x 5

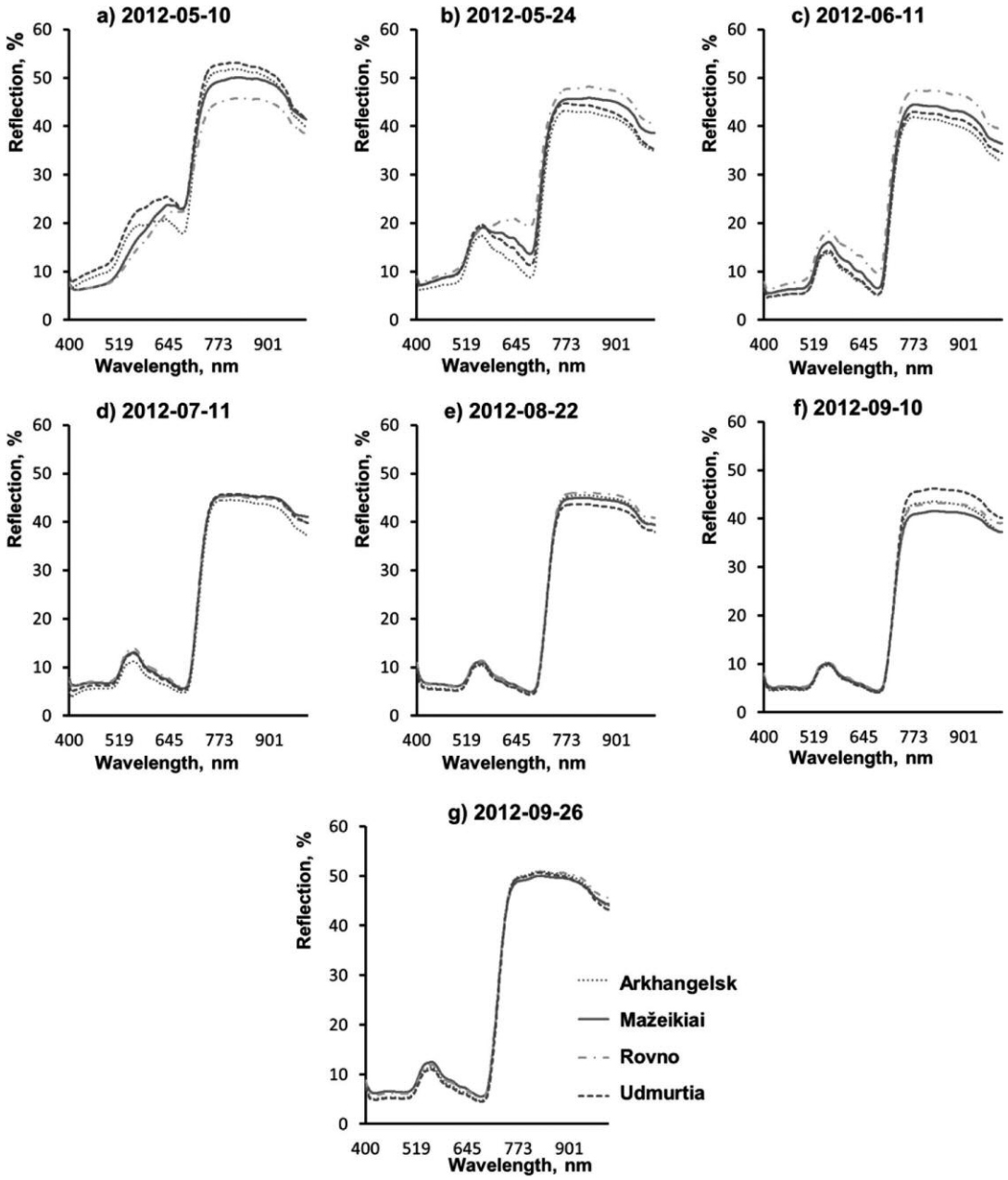

Figure 2. – The provenance mean spectral reflectance at each sampling occasion.

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trees), totaling to 80 samples at each sampling occasion (20 shoots x 4 provenances). The detached branches were cut into segments and immediately packed into plastic bags. The bags were labeled, put into portable cooler bags and transported to the laboratory for immediate spectral measurements during the same sampling day.

The spectral imaging

Sample needles were dissected from the shoots immediately before the scanning session. The scanning process was conducted using Themis Vision Systems LLC hyperspectral camera VNIR400H (Bay St. Louis, MO, USA). This device was equipped with a highly sensitive VNIR spectrometer capable of covering the spectral range of 400–1000 nm with a sampling interval of 0.6 nm, producing 955 spectral bands. The spatial data of each scanned sample was recorded in a charged-coupled device (CCD) array with a 1,392 × 1,000 pixel resolution (pixel size was 6.45 µm × 6.45 µm). The camera, using a field of view of 30 degrees, was mounted on a copy stand and was oriented in the nadir position with the lens fixed at 33 cm above the sample. Two 100 W halogen lamps, which provide stable electro-magnetic radiation in the 400–1000 nm range, were used for sample illumination. The halogen lamps were fixed symmetrically at both sides of the camera's lens and illuminated the sample allowing their light beams to crisscross above the sample. The scanning room was darkened to avoid unrelated spectral signals from ambient light sources.

The needles were spread on top of a plate painted black matt so that the background plate was fully covered by needles. The spectral response of each needle sample was recorded four times. The background plate was rotated 90 degrees horizontally after every hyperspectral sample to correct for the bidirectional reflectance distribution. These steps were repeated for all samples resulting in raw hyperspectral images of needle samples (four for each separate sample). Next, the radiance curve was converted to a reflectance curve for each image pixel. Completed target measurements were compared against the ones of a reference panel of known spectral reflectance (Avian Technologies LLC 99% white reference panel). The spectrometer internal current (dark current) was also corrected. The resulting

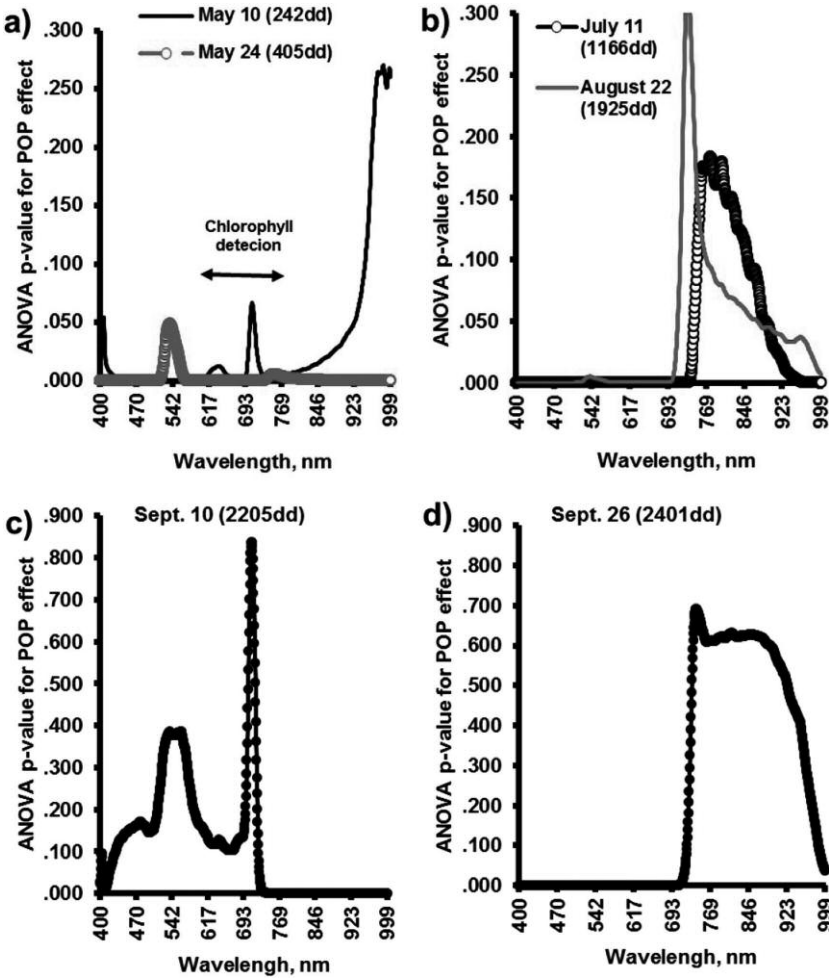

Figure 3. – The variation in the ANOVA p-value for the provenance effect over the spectral interval investigated. The significance values for sampling at June 11 are not shown because they all were highly significant (<0.0001) and overlapping with the X axis.

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spectra were then smoothed using the Savitzky-Golay filter function (Savitzky and Golay, 1964) with a 4th-order polynomial fit and 25 data points. The spectral curves derived from every pixel of each needle sample image were averaged to construct the unique spectral curve of the whole image. Finally, four reflectance curves were derived from the four needle sample images and then averaged to construct a single reflectance curve for each sample. A total of 80 reflectance curves were constructed (20 for each provenance) during one scanning session. Each reflectance curve was treated as a series of numbers (reflectance coefficients) and was used for statistical analyses.

Data analysis

To identify the most informative parts of the spectrum for revealing the provenance differences at each sampling occasion, the analysis of variance (ANOVA) was performed on the branch mean level separately for each wavelength and sampling occasion according to the following model:

$Y_{ij} = P_j + e_{ij}$, where

Y_{ij} is mean reflectance value for branch i within provenance j at a particular wavelength (4 observations per tree, 5 trees per provenance); P_j is the effect of provenance j and e_{ij} is the random error. The wavelength range from 400 to 1000 nm with the steps of approximately 0.6 nm was investigated in 955 separate ANOVAs for each sampling occasion. The effect of branch within tree was not significant and not included in the model (not shown). To meet the assumptions for the ANOVA, the distribution of the spectral responses at each wavelength was tested for normality using the Shapiro-Wilk test ($\alpha = 0.05$) and the homogeneity of the variance by Levene's test ($\alpha = 0.05$). The spectral data at every spectral band and for all investigated provenances was normally distributed with homogeneous variances.

The principal component analysis (PCA) by the non-linear iterative partial least squares algorithm was used on the tree mean level to identify the most informative combinations of the wavelengths to the total variation in spectral reflectance at each sampling occasion separately. The PCA is routinely used in spectral imaging studies as a reliable technique for unsupervised band selection (Bajcsy and Groves, 2004; De Backer et al., 2005; Kalacska et al., 2007; Hesketh and Sánchez-Azofeifa, 2012; Koonsanit et al., 2012). The data was standardized to unit variance and mean of zero. Based on the PCA the reflectance values in the most informative wavebands for each of the first three to five principal components PC depending on the sampling occasion were used as the discriminating variables in the discriminant function analysis (DFA) on the shoot mean level (McLachlan, 2004) aiming to test the accuracy of the classification of the mean reflectance values into four Scots pine provenances. DFA determines a set of linear combinations of the discriminating variables by maximising the between group and minimising the within group variance. These linear combinations of the original variables are called the canonical discriminant functions described by the following formula:

$$f_{cg} = l_0 + \sum_{i=1}^{p} l_i \cdot X_{icg}$$

where:

f_{cg} – the value of the canonical discriminant function for case c in the group g;

l_0, l_i, – function coefficients;

X_{icg} – the value of discriminant variable X_i for case c in group g;

p – number of discriminating variables.

The number of the discriminant functions is equal to the number of groups minus one or equal to the number of discriminating variables, whichever is less. The mean discriminant scores for each grouping variable for the first pair of the canonical discriminant functions (DF1 and DF2) were plotted to visualise how these functions discriminate between the groups (*Fig. 4*). The foliage samples were then classified into four groups (Scots pine provenances). To estimate the classification accuracy,

Table 1. – The percentage of the total variance in spectral reflectance explained by the principal 3 components (PC) at each sampling occasion and the most important wavelengths for each PC. 4 VAR is the percentage of the total the variance explained by the PC. WL is the most 5 informative wave length for a particular PC.

Sampling occasion	PC 1		PC 2		PC 3		PC 4		PC 5	
	VAR, %	WL, nm	VAR, %	WL, nm	VAR, %	WL, nm	VAR, %	WL, nm	VAR, %	WL, nm
2012-05-10	80.97	725.2	10.55	688.9	7.49	681.8	-	-	-	-
2012-05-24	73.11	723.3	20.41	529.0	3.98	682.5	1.84	409.7	-	-
2012-06-11	85.20	724.6	11.30	764.8	1.26	954.6	1.73	543.6	-	-
2012-07-11	67.21	730.3	26.08	821.9	4.96	712.5	1.17	400.1	-	-
2012-08-22	66.86	702.3	28.72	898.2	3.08	715.7	0.69	400.1	-	-
2012-09-10	66.10	528.4	30.31	828.4	2.22	713.8	0.37	400.6	0.63	1000.1
2012-09-26	52.99	696.5	41.57	827.7	3.88	717.6	0.82	1000.1	-	-

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the 4-fold cross-validation (KOHAVI, 1995) was used. The initial sets of 80 samples (per each sampling date) were divided into 4 equally sized (4 × 20) subsets (folds). A single subset was kept as the validation data, and the remaining 3 subsets were used as classification training data. The cross-validation process was then repeated 4 times, with each of the 4 subsets used only once as the classification's validation data. The results from the 4 folds were then averaged to produce a single estimation of the classification accuracy per each sampling date.

The sampling occasion represented by the effective temperature sum (above 5 °C, ETS) for which the best classification accuracy was obtained was considered as an optimal time point during the warm period for spectral determination of Scots pine genetic differences. The statistical analyses were carried out with the Unscrambler X ver. 10.2 (trial version) and SPSS ver. 17.0 software.

Results

Generalized over all the sampling occasions, the provenance mean reflectance followed well known trend of low reflectance in the blue and yellow to red portion of the spectrum and high reflectance in the red edge of the spectrum (*Fig. 2*). The reflectance peak within 400-700 nm wavelength interval was less evident at the first two sampling occasions (May 10 and 24), before and at the beginning the needle elongation (*Fig. 2a* and *b*).

The provenance effect on the reflectance varied markedly depending on the wavelength and the sampling occasion (*Fig. 3*). At the beginning of the growth period during the initiation of needle elongation, the provenance effect was significant at 450–950 nm wavelength interval, with a local peak of the less-significant effect at 710–750 nm and the end of the infrared part above 920 nm (*Fig. 3a*). After the full development of needles during the period of fast shoot elongation (May 11, June 11), the provenance effect was highly significant at the whole spectral range 400–1000 nm, with an exception of a local peak of less significant provenance effects in 500–540 nm for May 24 and 710–750 nm for May 10 (*Fig. 3a*). These 500–540 nm and 710–750 nm intervals with weak provenance effect were associated with the steep increase in reflection (*Fig. 2b* and *c*). At the time of growth cessation and bud set (July 11, August 22), the provenance effect turned to non-significant at the red and infrared spectra, 710–950 nm wavelength (*Fig. 3b*). Whereas, during development of frost hardiness and lignification of needles and shoots (Sept. 10), the pattern of variation in provenance effect with the wavelength contrasted that observed during the growth period: weak effect at below 750 nm and strong effect above 750 nm (*Fig. 3c*). Finally, during the development of shoot frost hardiness and dormancy (Sept. 26), the variation pattern in the strength of the provenance effect resembled that during the growth cessation in July 11 (*Fig. 3d*).

There also was a noteworthy and interpretable ranking shift between the provenances in the reflection percentages during the different developmental stages (*Fig. 2*). In early spring during the budburst, the southern provenance (Rovno) exhibited lower reflection values than northern provenance (Arkhangelsk), especially at the wavelengths exceeding 750 nm (*Fig 2a*). However, later at the end of May to June during the period of fast elongation of both needles and shoots, vice versa the southern provenance had the highest reflection scores over whole VIS and NIR spectrum (*Fig 2bc*). In July to August during growth cessation and rest induction, the among-provenance variation decreased at all wavelengths, with the northern provenance of Arkhangelsk exhibiting the lowest reflectance (*Fig. 2 de*). However, in September, when the among provenance variation peaked to its highest values, the continental provenance of Udmurtia (Ural region) exhibited the highest and the maritime Lithuanian population the lowest reflectance values (*Fig. 2f*).

Depending on the sampling occasion, 3 to 5 principal components (PC) explained more than 99% of total spectral reflectance variance (*Table 1*). Importance of the first PC declined markedly towards the autumn and starting from September 10 more than 30% of the variation was represented by the second PC (*Table 1*). During active growth and initial stages of dormancy development (August 22), the spectral interval of 702 to 730 nm was the most important for the major PC1 (*Table 1*). However, during intensive shoot lignification and needle frost hardiness development, 528 nm was dominating for PC1 and 828 nm for PC2, whereas the wavelengths of ca 700 nm explained less than 1% of the variance (*Table 1*). After the cessation of active growth since July 11, the importance of the NIR spectral interval to the among-tree variation in spectral reflectance increased markedly (referring to the variation explained and the informative wavelength for PC2 in *Table 1*).

At the beginning of active growth on May 10 and May 24, the 4-fold cross-validated DFA mean classification accuracy was moderate (55 to 58%, *Table 2*), then the provenances representing the cold climate with the earliest budburst were most spectrally separable (*Table 2*). The highest classification accuracy was obtained during the period of active shoot elongation on June 11 (89%), when the mean classification accuracy reached 90% for all the provenances but Rovno – the very southern one (*Table 2*). Towards the growth cessation, the mean classification accuracy gradually decreased to 59% at August 22 and rose to 74% during the period of intensive shoot lignification in September (*Table 2*). During the growth cessation and beginning of the lignification (July 11–August 22), the two northern vprovenances, especially the continental Udmurtia, were more separable than the southern provenances (*Table 2*). During the more advanced stages of lignification and frost hardiness development, the classification precision for Udmurtia decreased markedly (September 10, *Table 2*). Over all the sampling occasions, the misclassification percentage between provenances representing different climate was markedly lower than among provenances from similar climates (*Table 2*).

Distribution of the provenance mean scores on the canonical discriminant function plots (*Fig. 4*) indicates that the first canonical discriminant function (DF1,

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Table 2. – The 4-fold cross-validated classification accuracy of Scots pine provenances based on 2 the DFA.

Sampling date (effective temperature sum, day degrees)	Provenance	Classified into groups, %			
		Arkhangelsk (cold)	Mažeikiai (warm)	Rovno (warm)	Udmurtia (cold)
2012-05-10 (242 dd)	Arkhangelsk	65	10	5	20
	Mažeikiai	5	25	30	40
	Rovno	0	35	60	5
	Udmurtia	10	5	15	70
	Mean classification accuracy, %				**55.0**
2012-05-24 (405 dd)	Arkhangelsk	70	0	5	25
	Mažeikiai	20	35	20	25
	Rovno	0	15	80	5
	Udmurtia	25	20	10	45
	Mean classification accuracy, %				**57.5**
2012-06-11 (625 dd)	Arkhangelsk	90	0	0	10
	Mažeikiai	0	90	5	5
	Rovno	0	15	85	0
	Udmurtia	5	5	0	90
	Mean classification accuracy, %				**88.8**
2012-07-11 (1166 dd)	Arkhangelsk	85	0	0	15
	Mažeikiai	0	75	25	0
	Rovno	0	35	40	25
	Udmurtia	0	5	15	80
	Mean classification accuracy, %				**70.0**
2012-08-22 (1925 dd)	Arkhangelsk	65	10	5	20
	Mažeikiai	15	65	15	5
	Rovno	5	50	30	15
	Udmurtia	10	15	0	75
	Mean classification accuracy, %				**58.8**
2012-09-10 (2205 dd)	Arkhangelsk	80	10	0	10
	Mažeikiai	0	80	20	0
	Rovno	0	40	60	0
	Udmurtia	30	20	0	50
	Mean classification accuracy, %				**67.5**
2012-09-26 (2400 dd)	Arkhangelsk	65	10	0	25
	Mažeikiai	5	80	15	0
	Rovno	0	25	75	0
	Udmurtia	25	0	0	75
	Mean classification accuracy, %				**73.8**

Fig. 4) stronger reflects the south to north geographical gradient, where at all the sampling occasions, the provenances are ranked following their original latitude: Arkhangelsk (north), Udmurtia, Mazeikiai, Rovno (south). For DF2 (*Fig. 4*), an interpretable pattern of the provenance mean scores was observed during the growth onset on May 10 and intensive shoot lignification on September 22, when the continental Udmurtia contrasted rest of the provenances especially the maritime Mazeikiai provenance.

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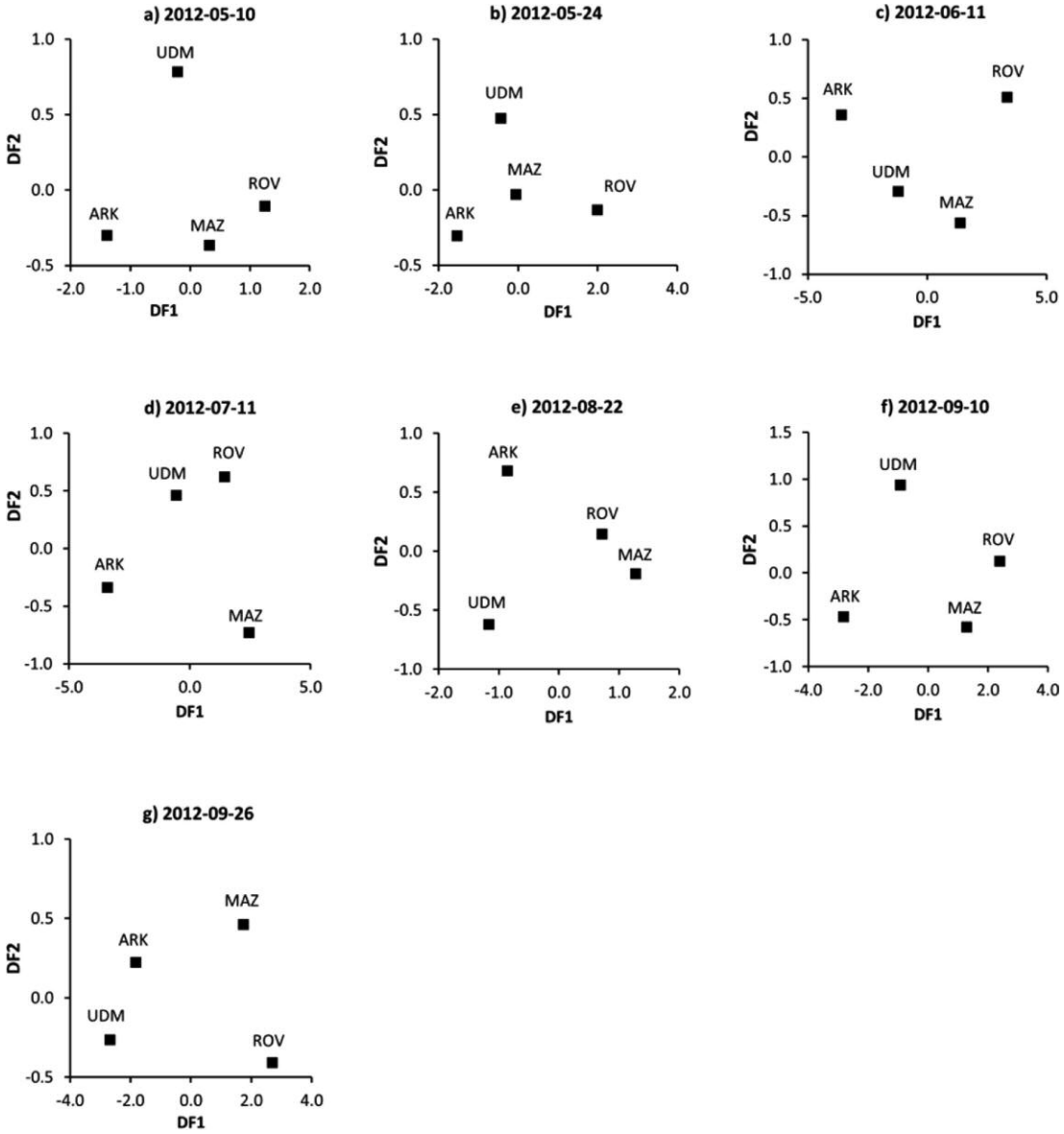

Figure 4. – Plots of the provenance mean scores for the two first canonical discriminant functions 5 (DF1 and DF2) for each sampling occasion. ARK is Archangelsk (north-eastern Russia, the 6 northernmost), UDM is Udmurtia (Ural region, the easternmost), Maz is Mazeikiai (Lithuania) 7 and ROV is Rovno (western Ukraine, the southernmost).

Discussion

The mean reflectance variation over the wavelengths reflects the common properties of green vegetation spectral reflectance (HOFFER, 1978; GUYOT, 1989; LEBLON, 1997). In May, the needles are not well developed and the chlorophyll absorption zone (600–700 nm) was not yet well defined in the spectral reflectance curves that time (*Fig. 2*). During the growing season, as the elongating needles accumulated chlorophyll, the reflection in visible spectra showed a decreasing trend (*Fig. 2c* to *g*). The high reflectance in the near infrared portion of the spectra (750–1000 nm) is a result of the high reflective response of the foliage to the near infrared wavelengths. In this spectral interval, the high reflectance of leaves creates a plateau, where the reflectance level depends on the internal structure of leaves such as more heterogeneous cell shapes and contents, bigger sizes of cells and spaces among them result higher reflection in near-infrared portion of a spectrum (GUYOT, 1989; LEBLON, 1997)

Based on the variation in the significance of the provenance effect over the spectral interval investigated and the changes in ranking between the provenances in the reflectance percentage, the following major provenance-dependent stages of tree physiological status are emerging. The first stage, the growth initiation and active elongation period with significant provenance differentiation and stable ranking at full spectral length, with a

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minor exception in the 710–750 nm (*Fig. 2a*), in which the wavelengths known as most sensitive for detecting the variation in chlorophyll concentration in Scots pine needles are located (KUPKOVA et al., 2012; MASAITIS, 2013). The reflectance peak at about 534 nm wavelength (green spectral zone) also coincided well with the weak provenance effect at the beginning (May 24, *Fig. 3a*) and end of active growth (August 22, *Fig. 3b* (slightly) and Sept. 10, *Fig. 3c*). The second stage, including growth cessation, bud set and building up of bud dormancy, the provenance effect abruptly turning to non-significant at the NIR spectral part in 710–750 nm and with the provenance reflectance ranking remaining similar as during the active growth (*Fig. 3b*). The third stage, the period of intensive development of needle/shoot frost hardiness (shoot and needle lignification) as well as rest induction at the first half of September where, in contrast to the earlier stages, a marked shift in provenance ranking occurred together with provenance differentiation being significant to the NIR spectral part only (*Fig. 3c*). The provenance ranking during the first two of these physiological stages was stable with a clear south to north gradient, where the southern provenance possessed the highest reflectance (*Fig. 1bc*). During the stage of frost hardiness development and shoot lignification, the geographical gradient of the reflectance percentage changed to a clear maritime-continent (west-east) pattern with the continental provenance exhibiting the strongest and the maritime the weakest reflectance (*Fig. 2f*). Evidently, the spectral interval of 710–750 nm represents a physiological threshold important for provenance differentiation as most of the changes in significance of provenance effect occurred within this interval (*Fig. 3*).

The physiological interpretation of such patterns of the provenance effect and ranking is related to phenology variation between provenances. During the growth onset, the temperature has the major effect (EICHE, 1966) causing the south-north gradient in provenance spectral reflectance ranking observed in our study. When moved southwards, due to low requirements of heat to budburst, northern provenances start growth early in spring (HANNERZ, 1998; DANUSEVICIUS and GABRILAVICIUS, 2001). In our study, the northern provenance started active growth early possessing relatively greater concentrations of chlorophyll and carotenoids in the needle mesophyll cells (LINDEN, 1976). During the growth onset at the beginning of May, this presumably was reflected by the higher reflectance values of the northern-most Arkhangelsk provenance within the interval 710–750 nm in which the wavelengths known as most sensitive for detecting the variation in chlorophyll concentration in Scots pine needles are located (KUPKOVA et al., 2012; MASAITIS, 2013; *Fig. 3a*). During the period of active elongation, the seedlings originating from the warmer climates contain higher concentration of chlorophyll than those from colder climates (LINDEN, 1976; BALDWIN, 1955), presumably owing to the competition driven adaption for an intensive growth. Again in our study, the above findings are indirectly supported by the higher reflectance percentage 710–750 nm chlorophyll detection interval of the southern provenance during the active growth (*Fig. 3bc*). Under the effect of short days and chilling, needle chlorophyll content rapidly decreases and intensive accumulation of lignin in cell wall begins (PULKKINEN, 1993), which is accompanied by an increase in thickness of the cell wall and a decrease in the cell lumen (PRAVDIN, 1964; MIIDLA, 1989). In our study, these findings may have manifested by the weak provenance differences in the VIS and the red edge (400–750 nm) together with the significant differences in NIR (7501000 nm) spectral parts (*Fig. 2*), the earlier being good reflector of chlorophyll concentration (DATT, 1998; CARTER and KNAPP, 2001; GITELSON et al., 2003) and the latter being good indicator of cell structural changes such as cell wall lignification and variation in cell wall thickness (ROCK et al., 1994; MARTIN and ABER, 1997; KOKALY and CLARK, 1999; GUYOT, 1989; LEBLON, 1997). The shift to the maritime – continent gradient may be related to the differential dormancy induction and the associated variation in shoot lignification and frost hardiness. Being less adapted to varying temperatures within a season, continental Scots pine provenances require less chilling to dormancy release and are relatively more responsive to warm autumn temperatures than maritime provenances (LEINONEN, 1996).

Our findings are supported by SUNDBLAD et al. (2001), where an attempt was made to investigate VIS and NIR capability to detect variation in forest hardiness of young Scots pine seedlings in nurseries. Significant provenance differentiation in spectral reflectance was detected. The VIS spectral interval centered at 680 nm was good indicator of relative lignin/chlorophyll contents, whereas, the higher bands of NIR represents information from lignin, water contents, carbohydrates and cellulose (OSBORNE and FERN, 1986; CURRAN, 1989).

The PCA based variance portioning maximizing the total spectral variation reflected similar trends as for among provenance variation. Except for September 10, the most informative wavebands for the tree discrimination during the active growth resembled the optimal spectral interval for assessment of chlorophyll contents (700–750 nm, PC1 in *Table 1*). Whereas, after budset the importance of the NIR spectral interval started to increase forming the own component of variation PC2 reflecting us much as 42% of the total variance on frost hardy and lignified shoots and needles on September 26 (uncorrelated with variation in PC1 i.e. during active growth). Consequently, the variation represented by PC1 may reflect the growth related physiological processes and PC2 may represent cell structural changes towards lignification and forest hardiness.

The best classification accuracy of the discriminant function analysis (89%), indicating the biggest spectral discrimination among provenances, was obtained during the intensive elongation of shoots on June 11. Presumably, during that time the provenances were at most different stages as regards both cell structure and chlorophyll contents leading to the significant provenance differentiation at the full spectral interval investigated. Another support for a strong genetic versus random component in the spectral reflectance values is the geo-

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graphically interpretable ranking among the provenances depending on their phenology. Of course, the longer wavelengths of the NIR spectral part need to be investigated, especially considering that the decrease of cell water content during hardiness development (SAKAI and LARCHER, 1987) is better reflected by NIR above 1000 nm (CARTER, 1991). A further development could be to carry out simultaneous spectral and physiological response analysis (SUNDBLAD et al., 2001).

In conclusion, there are significant provenance attributable and interpretable differences in spectral reflectance of the Scots pine needles providing a good opportunity for detecting this spectral variation with hyperspectral imaging. When aiming to estimate the genetic diversity, the choice of most informative spectral interval depends on the phenology development of the material.

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