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Use of a Common Garden Experiment in Selecting Adapted Beech Provenances for Artificial Stand Restoration

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ABSTRACT

Increased frequency of extreme weather events has seriously affected forestry operations in south-eastern Europe. A precondition for effective artificial restoration of disturbed forest stands is site-adapted forest reproductive material (FRM). Common garden experiments (provenance trials) may assist in selecting such FRM. The main objective of this study was to establish among-provenance variation pattern using data from a beech provenance trial. Usefulness of the results in selecting seed sources for restoration of European beech stands is discussed. The trial was set up in 2007, at a slope of Medvednica mount facing north-west at 730-750 m above sea level. Plant heights were measured and survival scored in 2008 and 2015. Height increments were calculated and processed to determine variance components due to various effects. Highly significant provenance-by-block interaction was revealed, indicating strong microsite effects on provenance performances. Therefore, corrections were made and provenance mean height increments recalculated. Provenance mean height increment multiplied with survival was used as a measure of a provenance's adaptedness. Regression tree (RT) analysis was used to determine the pattern of among-provenance variations. A set of provenance clusters was grown using climatic variables related to the provenance stands of origin as criteria. All analyzed effects were significant (provenance: F=2.07, p<0.05; block: F=5.07, p<0.05; provenance by block interaction: F=7.32, p<0.001). Data corrections reduced the interaction effect, thereby increasing reliability of calculated provenance adaptedness indices (AI). Provenances were grouped into 4 clusters due to elevation, mean July temperature and summer heat-to-moisture index (SHM). Cluster 4, containing provenances from the highest altitudes (>750m), had the highest mean AI (143.9±8.4 cm). The lowest mean AI (106.7±14.8 cm) had cluster 1, containing provenances from lower altitudes with lower mean July temperatures (≤18.4°C). Provenances originating from lower elevations with higher mean July temperatures (>18.4°C) were further divided into two clusters due to the SHM variable. Cluster 2 had the second highest mean AI (141.2±1.5 cm) and contained provenances from relatively wetter habitats (SHM≤48.2). Cluster 3, containing provenances from relatively arid habitats (SHM>48.2), had significantly lower mean AI (116.8±8.6 cm). Established among-provenance variation pattern might be used as a tool in selecting seed sources for artificial restoration of beech stands at mount Medvednica. It is advisable to use FRM from higher altitudes and/or from slightly lower altitudes (up to 150 m lower than a restoring site) but featured with warmer and drier conditions compared to the trial. Generally, provenance trials should be utilized as a valuable decision tool in restoring disturbed forest stands but may also be misleading if not well designed and analyzed.

Keywords: provenance trial; microsite variation; adaptedness; ecotype variation; natural selection; regression tree analysis

INTRODUCTION

Frequency of extreme weather events has recently increased at wide-European scale affecting forest ecosystem functioning and services provisioning to society (Neumann et al. 2017). Artificial restoration of forest stands disturbed by such events has become important issue, especially in southeast Europe where natural forest stand regeneration has been traditionally practiced. Nevertheless, artificial restoration (by planting saplings) of disturbed forest stands is often the only option. Long-term restoration success depends on appropriate seed source (provenance) selection (Ivetić and Devetaković 2016). The availability of quality forest reproductive material (FRM) is a prerequisite for efficient and successful artificial stand restoration. Quality FRM means, among other things, seeds or seedlings that are well adapted to habitat conditions prevailing in stands to be restored. Theoretically, an adapted FRM is one that originates from one or more provenances of a target species that are adapted to specific environmental conditions because their genetic constitution has been shaped by natural selection (Epperson 1992, Savolainen and Pyhäjärvi 2007). Therefore, obtaining FRM from provenances thriving in the same environmental conditions as a local provenance (the one we want to artificially restore) seems like a simple and quick solution. However, identifying such provenances is by no means an easy task. On the one hand, it is difficult to recognize the complexity of environmental conditions (their changes and interactions) that prevail in a specific provenance area. On the other hand, there is no perfect adaptation because various factors affect genetic constitution of a provenance (not only natural selection), thus reducing the adaptedness of FRM (Kremer et al. 2012). Common garden experiments (provenance trials), where progenies from different provenances are tested under similar environmental conditions, are a useful tool for selecting FRM adapted to a habitat (Mátyás 1996). Ranking progenies due to its performance in provenance trials has been frequently used as a mean of determining seed zonation and seed source selection (Westfall 1992, Rehfeldt et al. 1999, Kleinschmit et al. 2004, Hamann et al. 2011).

However, the usefulness of a provenance trial largely depends on the representativeness of sampled progenies as well as on the effects of environmental variation within a trial site on the assessment of among-provenance variation. If progenies included in a trial are not a representative sample of within-species variation (both at provenances and a species level), then the trial results are of reduced utility. In addition, homogenization of environmental variations in a trial is the basis for reliable genetic evaluation of provenances. The significant influence of micro-site variations on genetic evaluation has been a common problem of forest tree provenance trials (Magnussen 1993, Dutkowski et al. 2002, 2006, Funda et al. 2007, Gömöry et al. 2011). The problem can be addressed prior to establishment of experimental plantations, by considering and using alternative planting designs (Libby and Cockerham 1980, Coterill and James 1984). However, most forest trials have been traditionally set up according to randomized complete block design with large contiguous plots and at relatively large areas with pronounced micro-site heterogeneities. It is very difficult to reset such a trial by subsequent blocking in order to reduce micro-site

variations (Gömöry et al. 2011). Despite mentioned problems, common garden trials are still the best available source of information on provenance adaptedness to specific habitat conditions.

Like in most parts of the region, forests in the Republic of Croatia are generally managed based on close-to-nature paradigm, i.e. forest stands are preferably naturally regenerated (Matić 1999, Anić 2007). However, increased incidence of extreme weather events during last decade (e.g. windstorms) has significantly increased a need for artificial restoration of forest stands (Vuletić et al. 2014). Among many examples, the mount Medvednica was hit by two strong and several weaker windstorms damaging many trees, mostly of European beech (Balija 2015, HINA 2019). Therefore, artificial restoration of devastated beech stands at mount Medvednica has become the only option. Therefore, new approaches should be considered for long-term restoration success including selection of site-adapted provenances.

With this study, we tried to rank tested beech provenances with respect to their adaptedness to specific habitat conditions at the Medvednica provenance trial. The aim was to establish a pattern of among-provenance variation, which may be used as a decision tool in selecting adapted forest reproductive material for artificial restoration of disturbed beech stands. To our knowledge, this is the first attempt to make such use of a provenance trial in this part of Europe.

MATERIALS AND METHODS

Field Trial

Beech provenance trial Medvednica is located at the homonymous mountain near Zagreb, the capital of Croatia (N 45°53'5.6"; E 15°55'13.8" - Figure 1).

The trial was established in spring 2007 with progeny from 21 provenances originating from 9 European countries (Figure 1, Table 1). One provenance was omitted from this study due to its high mortality. The saplings were grown and provided by the Thünen-Institute for Forest Genetics in Grosshansdorf, Germany. The trial was a part of the 2007 international series of beech provenance trials (Von Wuehlisch et al., 2010). Taxonomically, 19 provenances belong to European beech (*Fagus sylvatica* L.) and one provenance belongs to Oriental beech (*Fagus orientalis* Lipsky - provenance TR62, Table 1). The trial was set up according to a randomized complete block (RCB) experimental design with three replications (blocks), at a slope facing north-west approx. 740 m above sea level (729 – 750 m). Blocks were oriented perpendicular to the slope exposure. Each provenance was represented by 50 plants per block, planted in rectangular plots with 2.0 × 1.0 m spacing. The plants representing various provenances were not of the same age. Ten provenances were 2 years old, eight were 3 years old and two provenances were 4 years old in the spring of 2007 (Table 1).

Data Collection and Statistical Analyses

Heights of all plants were measured (with 1 cm precision) while plant survival was visually determined and scored in 2008 and 2015, after the 1st and the 8th growing seasons at the trial site. Height increments between 2008 and 2015 (HI_08_15)
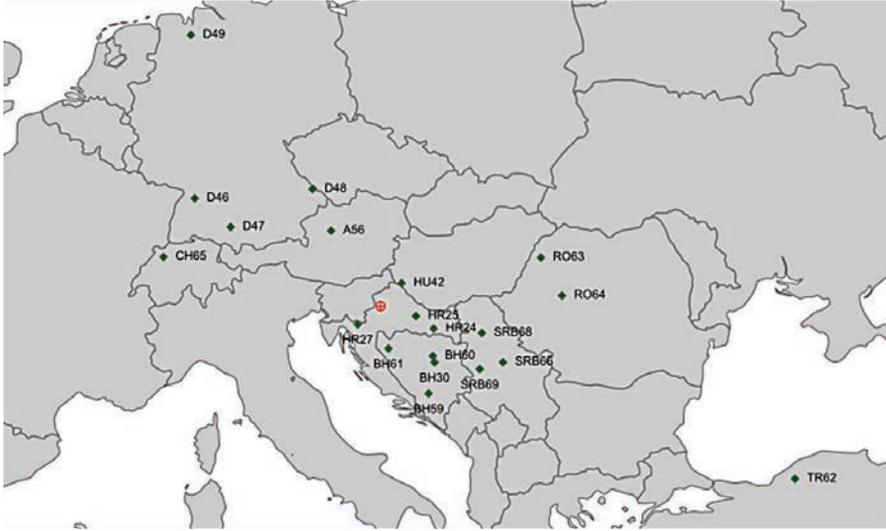
Figure 1. Geographic position of beech provenance stands of origin and provenance trial Medvednica (red label).

Table 1. Studied beech provenances and climate data of their stands of origin for the period 1981-2009. Climate data has been generated with the ClimateEU v4.63 software, available at http://tinyurl.com/ClimateEU.

Provenance label	Provenance name	Seed sampled	Country abbrev.	MAT (°C)	Elev (m)	MWMT (°C)	TD (°C)	MAP (mm)	MSP (mm)	SHM
A56	Scharnstein,Mitterndorf	2003	A	8.5	480	18.4	20.2	1323	709	26.0
BiH30	Tajan, Zepce	2003	BH	9.2	700	19.2	20.6	891	398	48.2
BH59	Vranica-Bistrica	2004	BH	10.0	750	19.5	18.6	1089	402	48.5
BH60	Crni vrh	2004	BH	10.1	500	20.1	20.7	882	396	50.7
BH61	Grmec,Bastra-Corkova	2004	BH	8.6	720	18.4	19.7	1102	433	42.5
CH65	Sihlwald, 101	2004	CH	6.6	1050	15.4	17.3	1559	651	23.7
D46	Pfalzgrafenweiler,XII Abt37	2003	D-BW	8.2	700	17.4	18.2	1076	458	38.0
D47	Schelklingen, IX 23-25 81023	2002	D-BW	8.1	650	17.6	18.8	1053	554	31.8
D48	Hoellerbach 81024	2003	D-BY	6.5	755	16.1	19.7	930	464	34.7
D49	Hasbruch 81001	2002	D-NI	9.2	35	17.3	15.7	767	354	48.8
HR24	Sjeverni Dilj Caglinski	2003	HR	10.1	350	20.2	20.8	811	391	51.7
HR25	Vrani kamen 12a, 15b	2003	HR	9.0	600	19.0	20.4	973	450	42.2
HR27	Vrbovsko-Meletka 36b	2003	HR	8.7	800	18.4	19.1	1689	652	28.2
HU42	Valkonya 19A	2003	HU	10.1	300	20.4	21.3	775	412	49.5
RO63	Alesd, U.P.II / 51A	2004	RO	8.7	490	18.9	21.7	607	309	61.1
RO64	Alba-Iulia, U.P.V/154A	2004	RO	7.4	860	17.1	19.9	707	404	42.3
SRB66	Avala	2004	SRB	10.1	475	19.8	20.6	677	320	61.9
SRB68	Fruska gora	2004	SRB	10.4	370	20.7	21.4	666	319	64.9
SRB69	Cer	2004	SRB	8.9	745	18.4	20.0	811	379	48.5
TR62	Devrek Tefen BA 3	2004	TR	10.0	950	19.3	18.6	779	256	75.5
Trial site	Medvednica			7.2	730	17.1	20.9	1205	575	29.8

Country abbreviations: A – Austria; BH – Bosnia and Herzegovina; CH – Switzerland; D – Germany; HR – Croatia; HU – Hungary; RO – Romania; SRB – Serbia; TR – Turkey.
Climate data: MAT – mean annual temperature; Elev – elevation; MWMT – mean warmest month (July) temperature; TD – continentality index; MAP – mean annual precipitation; MSP – mean summer (June-August) precipitation; SHM – summer heat to moisture index (MWMT/(MSP/1000)); Methodology of the climate data calculations described in Wang et al. (2011).

were calculated as difference between plant heights in 2015 and 2008. Correlation between initial plant heights and their HI_08_15 was calculated using Hmisc package in R (Frank E Harrell Jr, with contributions from Charles Dupont and many others. (2019). Hmisc: Harrell Miscellaneous. R package version 4.3-0. https://CRAN.R-project.org/package=Hmisc). The correlation was low (R=0.23) and non-significant (p<0.0001) and thus we assumed that HI_08_15 was not biased due to age differences among provenances. Therefore, data on individual height increments were used in further analyses.

Factorial analyses of variance (ANOVA) were performed on HI_08_15 data using the MIXED procedure (SAS/STAT software, a free version of SAS University Edition, by SAS Institute Inc., Cary, NC, USA) to determine the variance components due to effects of the blocks, provenances and provenance-by-block interactions according to the following linear model (Equation 1):

$$y_{ijk} = \mu + B_i + P_j + BP_{ij} + \varepsilon_{ijk}$$

where: y_{ijk} – individual value of a trait; μ – overall mean; B_i – random effect of the block i, i = 1,2,3; P_j – random effect of the provenance j, j = 1,2,...,20; BP_{ij} – random effect of the provenance-by-block interaction; ε_{ijk} – random error.

Since highly significant provenance-by-block interaction was revealed, Tukey-Kramer HSD test was used to determine statistical significance of differences among provenance-by-block least square means (LSmeans). Only two provenances (RO63 and TR62) had no significant differences among block LSmeans. Most provenances had one significantly different block LSmean and two provenances had all three block LSmeans significantly different among themselves (HR24 and A56). Therefore, provenances HR24 and A56 were excluded from further analyses, and data from significantly different blocks within provenances were omitted from further analyses, as well. Therefore, overall provenance mean height increments were recalculated with remaining data using proc MEANS in SAS. Above mentioned data corrections were done to reduce provenance-by-block interactions i.e. to reduce microsite effects on provenance mean estimates.

Provenance mean height increment multiplied with survival was used as a measure of a provenance's adaptedness (i.e. adaptedness index – AI, Ishizuka and Goto 2012) to the trial site conditions. Regression tree (RT) analysis was used to determine the pattern of among-provenance variation for AI, using the Orange Tree Widget (Demšar et al. 2013). A set of clusters was grown by repeated binary splits of the provenances. Splits were made using environmental predictor variables as criteria, so that the homogeneity of genetic response variable (i.e., the provenance mean AI) is maximized. No assumptions were made about the mathematical nature of the relationship between response and predictor variables. Various climatic variables related to the provenance stands of origin were used as environmental predictor variables. To characterize the long-term climate conditions at the provenances stands of origin, we used interpolated climate data for the 1981-2009 reference period that was generated with the ClimateEU software (Hamann A, Wang T, Spittlehouse DL, Murdock TQ, 2013; ClimateEU, unpublished software package for Europe freely available at http://www.ualberta.ca/~ahamann/ data/climateeu.html). Estimation of biologically relevant climate variables, lapse-rate elevation adjustments, and data extraction from grids for the sample locations were carried out with the ClimateEU software, as well. A detailed explanation of the estimation of all available climate variables given by the ClimateEU software can be found in Wang et al. (2011). Significance of difference among the clusters given by RT analyses was calculated with Tukey HSD test.

RESULTS

Average height increment (HI_08_15) of all plants at the trial was 135.7±56.4 cm (prior to data corrections). However, variations in height increment among provenances were evident (Figure 2). Generally, the lowest average height increment was achieved by provenance D47 (109.0 cm) and the highest by provenance HR24 (176.9 cm). Large HI_08_15 variations within all provenances could have been observed (Figure 2).
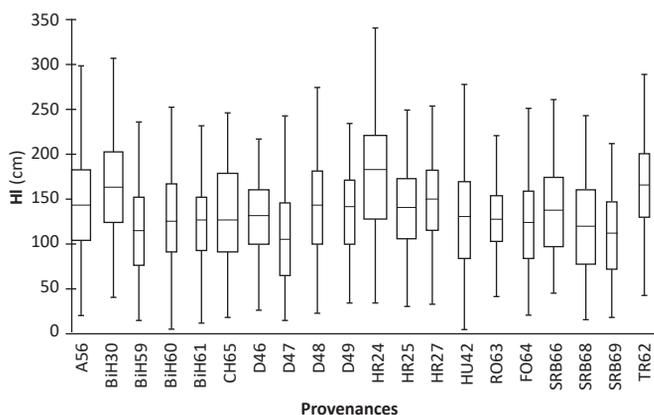
Figure 2. Boxplot of height increment (HI) data per provenance (HI between 2008 and 2015).

The coefficient of variation (CV) ranged from 31.6% (RO63) to 52.2% (D47). The analysis of variance revealed that all examined effects were statistically significant (provenance effect: F=2.07, p<0.05; block effect: F=5.07, p<0.05; provenance by block interaction: F=7.32, p<0.001). The highest proportion of total variance and the highest level of statistical significance had the provenances by block interaction effect. Generally, survival at the trial over the analyzed period (2008-2015) was rather high (95%). Of course, variations in survival among provenances were observed (Figure 3).

Provenance D46 had the lowest survival (0.83), while provenance RO64 exhibited the highest survival (1.0). However, significant differences in provenance survival were found regarding the blocks. For example, survival of provenance D46 was 0.73 in block 1 but 0.94 in block 2. The product of average provenance height increment and survival (adaptedness index - AI) was an indicator of its productivity, i.e. adaptedness to specific habitat conditions (Ishizuka and Goto 2012). Provenance AIs per block before data corrections are shown in Figure 4a. Significant provenance by block interactions is visualized by pronounced changes in provenance rankings across blocks. Four groups

of provenances can be distinguished from Figure 4a: i) provenances that showed the highest mean AI in the first block, while their mean AI decreased towards the 2nd and 3rd blocks (those are indicated by the solid red lines in Figure 4a); ii) provenances that had similar (lower) mean AI values at blocks 1 and 3, while highest mean AI at block 2 (those are indicated by dotted black lines - Figure 4a); iii) provenances that had similar (higher) mean AI values at blocks 1 and 3 while lowest mean AI at block 2 (those are indicated by dashed blue lines - Figure 4a); iv) stable provenances whose mean AI values were similar at all three blocks (indicated by the solid black lines - Figure 4a). Provenance BH61 stands out specific to all others because its mean AI value was lowest at blocks 1 and increased towards blocks 2 and 3 (indicated by a solid gray line - Figure 4a). We tried to reduce the effect of provenance by block interaction by excluding data from the blocks whose LSmeans (of HI08_15) were significantly different from the others. Two provenances were completely excluded from further analyses (A56 and HR24) because their LSmeans differed significantly among all three blocks. By this procedure, we significantly reduced the variation of a provenance AI at the trial (i.e. microsite effects on calculations of overall provenance mean AI were reduced) (Figure 4b).
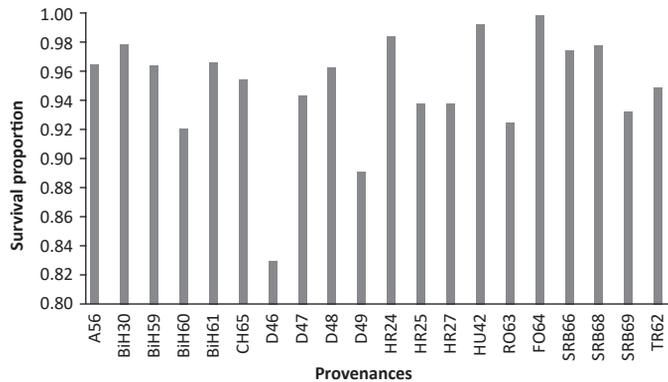
Figure 3. Proportion of living plants per provenance (survival between 2008 and 2015).
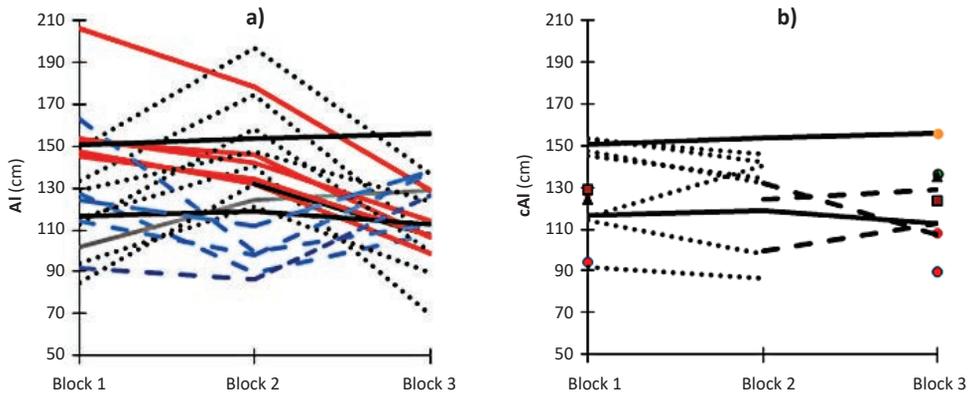
Figure 4. Provenance adaptedness index - AI (mean height increment × survival) per block. **a)** showing large provenance by block interaction before data corrections. **b)** Corrected provenance adaptedness index (cAI), after exclusion of data from blocks which HI_08_15 LSmean showed a significant difference from other blocks within the same provenance (showing reduction in provenance by block interaction).

Regression tree (RT) analysis was performed to determine the pattern of among-provenance variation regarding corrected adaptedness index (cAI). The RT analysis initially separated the provenances due to elevation - Elev (Figure 5).

Cluster 4 (Figure 5, 6) had the highest mean cAI (143.9±8.4 cm). It contained provenances (D48, HR27, CH65, RO64 and TR62) originating from highest altitude habitats (Elev>750 m). Other provenances (originating from lower altitude habitats) were divided by the variable July mean temperature (MWMT). The lowest mean cAI (106.7±14.8 cm) exhibited cluster 1 (Figure 5, 6). It contained provenances originating from habitats characterized by relatively lower average July temperatures (MWMT≤18.4°C). These were provenances D46, D47, D49, SRB69 and BH61. On the other hand, provenances originating from habitats characterized by relatively higher mean July temperatures (MWMT>18.4°C) were divided into two clusters due to the SHM variable (summer heat-to-moisture index). Cluster 2 (Figure 5, 6) contained provenances originating from habitats with relatively lower SHM values (SHM≤48.2). This cluster had the second highest mean cAI (141.2±1.5 cm). Cluster 2 contained provenances BH30 and HR25. Cluster 3 (Figure 5, 6) had a significantly lower mean cAI (116.8±8.6 cm), and contained provenances (BH59, BH60, HU42, RO63, SRB66, and SRB68) originating from habitats that were characterized with relatively higher SHM (>48.2). The Tukey HSD test separated clusters into two groups that were

significantly different between each other. Cluster 1 and Cluster 3 were in the first group, while Cluster 2 and Cluster 4 were in the second group (Figure 5). The results of the RT analysis indicated that among-provenance variation due to progeny mean cAI at the Medvednica trial can be primarily explained by differences in altitude among the provenance stands of origin. Provenances originating from higher (>750 m) altitudes showed, on average, the highest level of adaptedness to environmental conditions of the trial. Among the provenances originating from relatively lower altitudes (<750 m), the main discriminating climatic variables explaining the among-provenance variation were those associated with summer heat (MWMT) and with combination of heat and humidity of a habitat (SHM), also during summers. Relatively higher levels of adaptedness of the provenances originating below 750 m a.l.s. were those originating from habitats characterized with relatively higher mean July temperatures, but at the same time from habitats with relatively higher rainfall in the summer. It is interesting to note that the site of the Medvednica trial is at an average altitude of 740 m, its MWMT is 17.1°C, and the SHM is 29.8 (Table 1). Thus, the results showed that provenances originating from higher altitudes as well as provenances originating from slightly lower altitudes, but in this case from habitats that are on average characterized with warmer and drier summers compared to the trial site (Cluster 4 - Figure 6), performed better adaptedness.
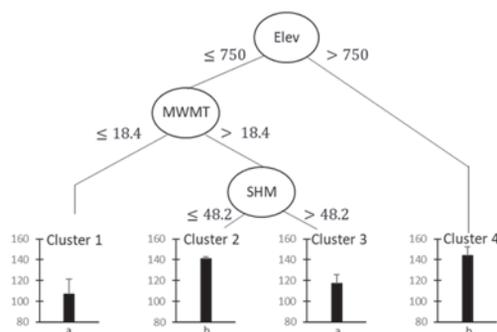
Figure 5. Regression tree analysis output showing clusters of provenances discriminated according to climate variables of their stands of origin. Elev – elevation; MWMT – mean warmest month temperature (July); SHM – summer heat to moisture index. Column charts represent mean (+standard deviation) of a cluster mean adaptedness index (cAI). Clusters labeled with the same letter were not statistically different.
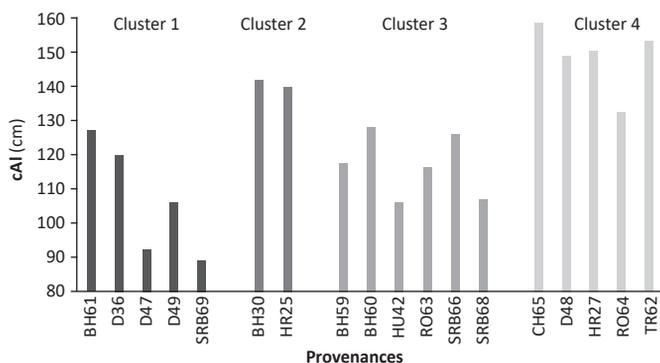
Figure 6. Corrected mean adaptedness index (cAI) of studied provenances at the trial Medvednica. Provenances grouped into four clusters due to regression tree analyses.

DISCUSSION

Extreme weather events have increased in frequency affecting forest ecosystem functioning and services at wide European scale. Croatia has also experienced an increase in extreme weather events during this decade that caused significant disturbances of forest stands (Vuletić et al. 2014). For example, two strong and several milder windstorms hit Medvednica mount damaging many trees, mostly of European beech (Balija 2015, HINA 2019). Therefore, it is important to select habitat-adapted forest reproductive material (FRM) for efficient long-term restoration of damaged stands, in which results of provenance trials may significantly assist (Mátyás 1996).

We used index (AI) calculated as the product of average height increment and survival as a measure of a provenance adaptedness. However, highly significant effect of provenance by block interaction on height increment was revealed. This indicates strong effects of micro-site variations on provenance performance at the trial, which is why environmental impact on among-provenance variation cannot be ruled out. Highly significant provenance-by-block interaction significantly reduces reliability of genetic values estimation, that is, reliability of a common garden experiment serving as a tool for selecting adapted FRM. The effect of micro-site variations on growth and adaptive traits in forest tree provenance trials is a common problem (Hamann et al. 2002, Zas 2006, Gömöry et al. 2011). Indeed, this is a problem because the basic principle of genetic testing (i.e. common garden experiment) is to homogenize environmental influence and thus to determine genetic differences among tested genetic entries (e.g. provenances). The problem of "masking" genetic differences because of micro-site variations can be mitigated by careful trial design, for example by using non-contiguous plots and by reducing a block surface (Libby and Cockerham 1980, Coterill and James 1984). However, it is almost impossible to predict all diversity and interactivity of a site variations and to design a perfect trial at which environmental conditions will be ultimately homogeneous (Gömöry et al. 2011). Despite these problems, common garden trials are still the best available source of information on provenance adaptedness to specific habitat conditions. Given the significant differences in rankings of provenance adaptedness indices across blocks (Figure 4a), we tried to reduce provenance-by-block interaction effect on AI calculation by excluding data from blocks that significantly differed from other blocks, per each provenance.

With this approach, we were able to reduce the effect of micro-site variations on provenance mean AI assessments (Figure 4b). Being aware that the effect of micro-site variations can be reduced by other statistical methods (see Zas 2006 and references therein), we used this simpler but effective approach, in this case.

Tested provenances performed different adaptedness to the habitat conditions at the trial, regarding height increment and survival during the analyzed period (Figure 2, 3). Differences in adaptedness may be explained, at least in part, by genetic differences among provenances, since the provenance effect for height increment was statistically significant. Genetic differentiation of common beech provenances for adaptive traits (drought resistance, frost tolerance, budburst, growth, survival) has been established through relatively many studies, both at wide-European (von Wuehlisch et al. 1995, Chmura and Rozkowski 2002, Ivankovic et al. 2011, Robson et al. 2012) and at regional levels (Müller and Finkeldey 2016, Pluess et al. 2016).

A regression tree analysis was used to determine the pattern of among-provenance variation. The provenances were separated into homogeneous clusters due to the corrected adaptedness index (cAI), based on various climate variables of their original habitats. Generally, all discriminatory climate variables were related to heat (MWMT) and to the combination of heat and humidity (Elev, SHM) of the provenance habitats of origin (Figure 5), while the correlation with their geographical location was not established. These results suggest an ecotypic pattern of among-provenance genetic variation that was shaped by natural selection in native habitats (at least partially), that is, the genetic structure of analyzed beech populations can be related to variations in their habitat's heat and humidity (Figure 5). The results corroborate other authors who reported an ecotypic pattern of among-provenance variation in common beech that was related to environmental variables of their native habitats (e.g., Ivanković et al. 2011, Dounavi et al. 2016, Horváth and Mátyás 2016, Pluess et al. 2016). Although ecotypic pattern of variation partly related to geographical location of provenances has also been reported by several authors (e.g. Chmura and Rozkowski 2002). Different conclusions about the spatial pattern of the European beech genetic structure indicate the need for further research on this topic.

The results of the study showed significantly better adaptedness of provenances originating from higher altitudes compared to the trial site, as well as of provenances from slightly lower (up to min. 600 m a.s.l.) altitudes, but warmer and drier during the summer season than the trial site (Figure 5, 6, Table 1). It is interesting to emphasize that provenances from higher altitudes mostly originated from warmer and drier habitats relative to the trial site, apart from the CH65 provenance. Given that there has been a trend of increasing air temperatures and decreasing rainfall throughout Croatia since 1990, especially during the growing season (DHMZ 2013), it is not so surprising that provenances originating from warmer and drier habitats have shown better adaptedness to the trial site. It should be emphasized that the trial was set up on a cleared site of northwestern exposure. Therefore, experimental plants were likely exposed to large fluctuations in air temperature and soil drying, which are conditions unlike in a naturally regenerating stand. From this, it can be assumed that local provenances would not show the best fitness and therefore would not be the best seed source for restoring damaged stands. It was often reported that common beech provenances show local adaptation i.e. better adaptedness of local provenances compared to foreign ones (Mátyás et al, 2009, Gömöry et al. 2015, Schueler and Liesebach 2015, Hajek et al. 2016). However, local provenances may also show maladaptation when habitat changes occur (Mátyás et al. 2009). Considering that climate conditions in the area of Medvednica have significantly changed (DHMZ 2013), which is most likely pronounced at cleared forest stands after windstorm devastation, it is a convincing assumption that local provenances might be less adapted to such habitat

conditions. Nonetheless, we do not suggest excluding FRM from local (closer to a specific site) provenances in artificial restoration but to consider supplementing them with FRM from provenances which performed better adaptedness to altered environments.

It is interesting to note that provenances originating from higher altitudes performed significantly better (cluster 4 - Figure 5, 6), although these provenances originated from quite different habitats (e.g. compare CH65 and HR27 - Table 1). Horváth (2016) pointed to complete absence of a correlation between growth and climatic parameters of common beech provenances from higher altitudes. Whether this means that such provenances are better adapted to a wider range of habitat conditions or exhibit a wider reaction norm remains an open question. We were particularly surprised by a high and stable performance of the TR62 provenance belonging to Oriental beech. This provenance showed the highest adaptedness (Figure 6), but also phenotypic stability at this trial (Figure 4a), although its original habitat is very different due to all climatic parameters (see Table 1). This is the only known provenance of Oriental beech in Croatia and its follow-up at the Medvednica trial deserves attention, especially in the context of climate change and analysis of its assisted migration.

The question arises whether the established pattern of among-provenance variation might be used as a kind of algorithm in selection of quality (i.e. adapted) FRM for artificial restoration of stands at Medvednica? In our opinion it might, if some assumptions were fulfilled, as well with caution when applying it. The first assumption is that tested provenances well represent genetic diversity of European beech in this part of Europe. The second assumption is that the progenies at the trial well represent genetic constitution of their original provenances. A third assumption is that we accurately determined provenance adaptedness indices, by correcting the data (cAIs). In addition, the call for caution in applying the results stems from the fact that the study clearly points to significant heterogeneity of habitat conditions at Medvednica mount. Altitude ranges, diverse exposures, and relief features (as well as other variables) result in large variations in environmental factors (Dobrović et al. 2006). Such conditions make it difficult to identify provenances that would perform a general adaptedness. The obtained "algorithm" (among-provenance variation pattern related to climate variables - Figure 5) can be more reliably applied in selecting FRM for artificial stand restoration where habitat conditions are more similar to those of the trial (altitude of about 750 m a.s.l., northwestern exposure). In such cases, it may be preferable to use FRM from provenances that, when compared to a particular stand, originate from higher altitudes or slightly lower (up to a minimum of 600 m a.s.l.), which are relatively warmer (average July temperatures >18.4°C) and drier during summer season (SHM = 30.0 to 48). The reliability of the study results, as well as reliability of consequent recommendations, surely decreases with increased difference between environmental conditions in restoration needed stands and habitat conditions prevailing at the trial during the analyzed test period.

CONCLUSIONS

The established among-provenance variation pattern can be used as a tool in selecting FRM for artificial restoration of devastated stands at Medvednica mount, at least for habitats featured with environmental conditions similar to the trial site (cleared sites facing northwest at altitudes between 700 and 800 m a.s.l.). For artificial restoration of such stands it is advisable to use FRM originating from higher altitude provenances, regarding the study results and with the foregoing assumptions. FRM originating from slightly lower altitudes (up to 150 m lower than a specific site) and relatively warmer and drier habitats (mean July temperatures >18.4°C; SHM from 30 to 48) may be recommended, as well. The heterogeneity of habitat conditions at Medvednica mount necessitates establishment of more provenance trials according to design options more suited to such conditions (e.g. smaller blocks, non-contiguous plots, etc.). Data processing should be carried out to minimize effect of micro-site variations (by addressing spatial autocorrelation with available tools), i.e. to accurately rank provenance performances at a trial site. Generally, this study showed that provenance trials may be utilized as a valuable decision tool in selecting adapted FRM for restoring disturbed forest stands but may also be misleading if not well designed and analyzed.

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Interaction Between the Effects of Provenance Genetic Structure and Habitat Conditions on Growth of Scots Pine in International Provenance Tests in Bosnia and Herzegovina

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ABSTRACT

Scots pine (*Pinus sylvestris* L.) is one of the most important tree species in European forests, characterized by high genetic variability and complex population structure. This research aimed to determine whether there is an interaction between the effects of provenance genetic structure and habitat conditions on the growth of Scots pine in two international provenance tests in Bosnia and Herzegovina, to chose the best provenances for both habitats. For this research, heights and root collar diameters of Scots pine plants on two provenance tests in Bosnia and Herzegovina were measured and the interaction between the effects of provenance genetic structure and habitat conditions for these two traits was estimated based on regression. Eleven provenances represented on both tests were researched. Provenance tests were established in 2012 on two locations with contrasted ecological conditions: Kupres and Žepče. By assessing the interaction between the effects of provenance genetic structure and habitat conditions on Kupres and Žepče provenance tests, two interactions for the height of plants (between Germany NJ2 and Italy I2 and between Austria A2 and Romania R1) and one interaction for the root collar diameter (between Austria A1 and Italy I2) were identified. The number of survived plants and their productivity is different for all provenances in both habitats, indicating that the adaptability of provenances is not only conditioned by their genetic constitution but also by habitat conditions. Considering the productivity of provenances and the number of survived plants in both habitats, Austria A1, Austria A2, Austria A3, and Poland P1 provenances showed the best adaptability. The results of this study can be used in the process of Scots pine breeding and for its conservation by *in situ* and *ex-situ* methods.

Keywords: *Pinus sylvestris*; morphological traits; genetic constitution; habitat conditions

INTRODUCTION

To preserve the gene pool of a particular tree species, knowledge of the existing variability of that species is required. Higher genetic variability makes the species more resilient and better adapted for survival in changing environmental conditions and in stress conditions (Villani and Eriksson 2006). Knowing whether a particular species has the flexibility and adaptability to survive in changing environmental conditions, as well as in stress conditions, is important for the functioning of forestry and the conservation of forest ecosystems.

Scots pine (*Pinus sylvestris* L.) is one of the most important tree species in European forests (Mátyás et al. 2004), characterized by high genetic variability and complex population structure (Omanović 2008, Stefanović et al. 1980). The territory of Bosnia and Herzegovina represents the southern border of the natural distribution of this forest

tree species. Provenance tests in Bosnia and Herzegovina, therefore, represent an opportunity to determine the adaptability and variability of different provenances of Scots pine from all over Europe, at the southern border of its natural distribution.

There have not been many studies on the interactions between effects of provenance genetic structure and habitat conditions on Scots pine. Some authors researched morphological traits of Scots pine plants in international provenance tests in Poland (Barzdajn et al. 2016), Spain (Alía et al. 2001), Turkey (Gülcü and Bilir 2017) and in Bosnia and Herzegovina (Ballian et al. 2009, Cvjetković et al. 2014). Taeger et al. (2013) researched the impact of climate and drought events on the growth of Scots pine (*Pinus sylvestris* L.) provenances in an international provenance test which also contained a provenance from Bosnia and Herzegovina. For researching the variability and adaptability of Scots pine, two international provenance tests of Scots pine were established in Bosnia and Herzegovina in 2012. Two experimental plots have contrasted ecological conditions, and they are suitable for studying interactions between provenances and habitats.

The study aims to determine whether there is an interaction between the effects of provenance genetic structure and habitat conditions on the growth of Scots pine in international provenance tests in Bosnia and Herzegovina on Kupres and Žepče test plots.

MATERIAL AND METHODS

For this research, the height and root collar diameter of eight-year-old Scots pine plants were measured in two international provenance tests in Bosnia and Herzegovina (Table 1). The height of the plants was measured in centimeters, with a precision of one centimeter, using a wooden lath, 2.50 m long. The root collar diameter was measured using a digital caliper, with the accuracy of 0.1 mm.

Based on the measured morphological traits, the values of regression coefficients were calculated, as well as the function y, which shows the interaction between the effects

of provenance genetic structure and habitat conditions. For calculation, the following formulas (Equations 1, 2, 3) were used:

$$b_1 = \frac{\sum xy - \dfrac{\sum x \sum y}{N}}{\sum x^2 - \dfrac{\sum x^2}{N}} \quad (1)$$

$$b_0 = \frac{\sum y - b_1 \sum x}{N} \quad (2)$$

$$y = b_0 + b_1 x \quad (3)$$

where in: b_1, b_0 – regression factors; $\sum x$ – the sum of 42 values for a particular trait (height of plants or root collar diameter) for the i-th provenance on Kupres plot; $\sum y$ – the sum of 42 values for a particular trait (height of plants or root collar diameter) for the i-th provenance on Žepče plot; $\sum x^2$ – the sum of 42 square values for a particular trait (height of plants or root collar diameter) for the i-th provenance on Kupres plot; $\sum xy$ – the sum of 42 values of multiplication of a particular trait (height of plants or root collar diameter) for the i-th provenance on Kupres plot and the i-th provenance on Žepče plot; N – the total number of values for a given property. For calculation 42 data per provenance were selected, while this was the smallest number of data in some of the studied provenances (Italy I2 and Italy I3 in Žepče plot). For calculation Microsoft Excel 2010 was used.

In graphical representations, the abscissa presents habitats, Kupres and Žepče, and the average values of a particular trait for all provenances. The ordinate contains the values of the function y for a given property. For plant height, habitats are presented as: Kupres =1.81 m, and Žepče =2.95 m. For root collar diameter, habitats are presented as: Kupres =5.16 cm, and Žepče =6.8 cm. On the abscissa, a randomly chosen third value was used, which is higher than the values representing the habitats, for better interpretation of the results.

On the graphical illustration of the values obtained, an interaction exists if a particular provenance (A) has a higher

Table 1. List of investigated provenances on Kupres and Žepče plots.

Country	Provenance label	Locality	Latitude	Longitude
Austria	A1	Kobersdorf/Lackenbach	47°53'12"	15°31'39"
Austria	A2	Pannholtz, Burgenland	47°07'14"	15°17'14"
Austria	A3	Hochwolkersdorf Stift Reicheresberg	47°13'49"	11°26'12"
Bosnia and Herzegovina	B1	Bugojno	44°03'00"	17°27'00"
Italy	I1	Ca Del Lupo	43°18'90"	13°27'89"
Italy	I2	Fenestrelle (TO)	45°01'47"	7°03'38"
Italy	I3	Piani - Valda (TN)	46°13'00"	11°16'00"
Germany	NJ2	Trippstadt	49°21'35"	7°46'29"
Poland	P1	Raciane – Nida	53°37'00"	21°29'00"
Romania	R1	Sacueni	47°21'9"	22°5'29"
Slovakia	SL1	Hanušovce	49°01'35"	21°30'01"

Norway (N1)	Italy (I3)	Romania (R1)						
Poland (P1)	Austria (A1)	Austria (A2)	Austria (A3)	Bosnia and Herzegovina (B1)	Ukraine (U1)	Slovakia (SL1)	Romania (R1)	Norway (N1)
Slovakia (SL1)	Norway (N2)	Germany (NJ1)	Germany (NJ2)	Poland (P1)	Italy (I1)	Italy (I2)	Italy (I3)	Austria (A3)
Norway (N1)	Austria (A3)	Bosnia and Herzegovina (B1)	Ukraine (U1)	Slovakia (SL1)	Romania (R1)	Norway (N1)	Norway (N2)	Germany (NJ1)
Austria (A1)	Germany (NJ2)	Poland (P1)	Italy (I1)	Italy (I2)	Italy (I3)	Italy (I1)	Austria (A1)	Austria (A2)
Norway (N2)	Ukraine (U1)	Slovakia (SL1)	Romania (R1)			Germany (NJ1)	Germany (NJ2)	Poland (P1)
Bosnia and Herzegovina (B1)	Italy (I1)	Italy (I2)	Italy (I3)			Austria (A2)	Austria (A3)	Bosnia and Herzegovina (B1)
Austria (A3)	Romania (R1)	Norway (N1)	Norway (N2)	Germany (NJ1)			Italy (I1)	Italy (I2)
Germany (NJ2)	Italy (I3)	Austria (A2)	Austria (A1)	Italy (I2)			Ukraine (U1)	Slovakia (SL1)
Ukraine (U1)	Norway (N2)	Germany (NJ1)	Germany (NJ2)	Poland (P1)				
Bosnia and Herzegovina (B1)	Austria (A1)	Austria (A2)						

Figure 1. Scheme of provenance arrangement on Kupres provenance test (provenances repeating in both localities are painted in grey).

value for an addressed trait than a provenance (B) in one habitat and lower in another habitat. Provenance tests were established in Kupres and Žepče, both in 2012 by planting two-year-old seedlings grown from seed material collected in nine different countries. Kupres provenance test contains 15 different provenances of Scots pine, and Žepče 14 different provenances. In this research, the plants of 11 provenances mutual for both provenance tests were measuered.

In Kupres provenance test each provenance was represented with 36 seedlings in five repetitions per randomized scheme (Figure 1). Kupres provenance test is located at 1140 m a.s.l., on terrain characterized by sinkholes and numerous hills and valleys. The geological substrate is mainly limestone and dolomite, and the type of soil is calcocambisol or brown soil on limestone and dolomite. According to the classification of climate types in Bosnia and Herzegovina (Milosavljević 1973), this area belongs to the mountain climate, with short summers and long, cold, and very snowy winters. The mean annual air temperature is 6.2°C. The minimum temperature was measured in January (-26.8°C) and the maximum in September (34.9°C). The annual average of registered frosts is 155 days. The average annual precipitation is 1221 mm, and its distribution by season varies slightly. Winds are numerous and often hard, therefore in winters they make big snowdrifts.

In Žepče provenance test each provenance was represented with 25 seedlings in four repetitions per randomized scheme (Figure 2). Žepče provenance test is located at 600 m in the belt of oak and beech forests. The soil type is deep acid brown soil (dystric cambisol). The climate in this area is temperate continental climate, with

Italy (I2)	Romania (R1)	Italy (I1)		
Norway (N2)	Germany (NJ1)	Germany (NJ2)	Poland (P1)	
Bosnia and Herzegovina (B1)	Ukraine (U1)	Slovakia (SL1)		Norway (N1)
Italy (I1)	Italy (I2)	Austria (A1)	Austria (A2)	Austria (A3)
Norway (N1)	Norway (N2)	Germany-(NJ1)	Germany (NJ2)	Poland (P1)
Austria (A3)	Bosnia and Herzegovina (B1)	Ukraine-(U1)	Slovakia (SL1)	Romania (R1)
Poland (P1)	Italy (I1)	Italy (I2)	Austria (A1)	Austria (A2)
Romania (R1)	Norway (N1)	Norway (N2)	Germany (NJ1)	Germany (NJ2)
Austria (A2)	Austria (A3)	Bosnia and Herzegovina (B1)	Ukraine (U1)	Slovakia (SL1)
Germany (NJ2)	Poland (P1)	Italy (I1)	Italy (I2)	Austria (A1)
Slovakia (SL1)	Romania (R1)	Norway (N1)	Norway (N2)	Germany (NJ1)
Austria (A1)	Austria (A2)	Austria (A3)	Bosnia and Herzegovina (B1)	Ukraine (U1)

Figure 2. Scheme of provenance arrangement on Žepče provenance test (provenances repeating in both localities are painted in grey).

warm summers and harsh winters. The average annual air temperature is 10.7°C. The minimum temperature was measured in January (-27.5°C) and the maximum in August (39.5°C). The annual average of registered frosts is 85 days. The average annual precipitation is 1040 mm. Winds are frequent and moderate with occasional higher intensity, which blows downstream in the Bosna River Valley (Strategija razvoja općine Žepče 2011-2018, 2011).

RESULTS

Assessment of the Interaction Between Provenance and Habitat for Plant Height

According to the above formulas, the regression coefficients and functions for the height trait were calculated. Based on the calculated coefficients and functions, graphical illustrations were made to evaluate the interaction between provenance and habitat. The values of regression coefficients and functions for the height trait are shown in Table 2.

Based on the graphical representation, there were two interactions for the plant height trait. The first interaction was present between Austria A2 and Romania R1 provenances, and the second between Italy I2 and Germany NJ2. These interactions are shown in Figures 3 and 4.

Figure 3 shows the presence of interaction between provenance and habitat for two provenances, Italy I2 and Germany NJ2. Germany NJ2 provenance had a lower average plant height at Kupres habitat at the younger age of plants and a higher average plant height at Žepče habitat at the older age of plants than Italy I2 provenance. The average plant height (at x=1.81) for Germany NJ2 provenance at Kupres was 3.14 meters and for Italy I2 3.17 meters. The average plant height (at x=5) for Germany NJ2 provenance in Žepče was 3.25 meters, and for Italy I2 provenance 3.22 meters. The difference in average heights for both habitats is small, but it tends to increase with the increase of the average plant height for habitat, in favor of Germany NJ2 provenance.

Figure 4 shows the presence of interaction between provenance and habitat for two provenances, Austria A2 and
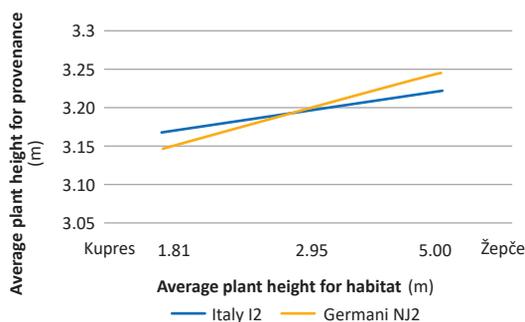
Figure 3. Graphic representation of the regression-based interaction between provenance and habitat for Italy I2 and Germany NJ2 provenances for plant height.
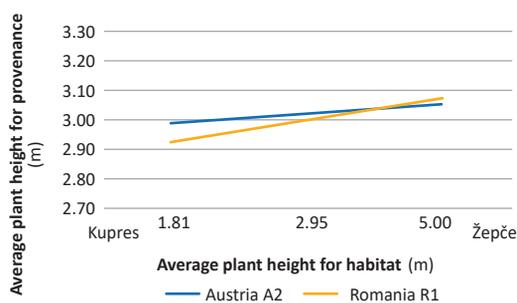
Figure 4. Graphic representation of the regression-based interaction between provenance and habitat for Austria A2 and Romania R1 provenances for plant height.

Romania R1. Romania R1 provenance had a lower average plant height at Kupres habitat at the younger age of plants and a higher average plant height at Žepče habitat at the older age of plants than Austria A2 provenance. According to this model, interaction occurs only after the value of the average plant height that defines the habitat in Žepče.

Table 2. Calculated values of regression coefficients and functions for plant height trait.

Provenance	b_1	b_0	y for x = 1.81	y for x = 2.95	y for x = 5
Austria (A1)	0.02776439	2.85068744	2.900940995	2.9325924	2.98950941
Austria (A2)	0.03012942	2.93694612	2.99148036	3.02582789	3.05746378
Austria (A3)	0.07066015	1.99509814	2.12299301	2.20354558	2.34839889
Bosnia and Herzegovina (B1)	0.04155722	2.58955596	2.664774525	2.71214975	2.79734205
Italy (I1)	0.02160781	3.26277421	3.301884355	3.32651726	3.37081328
Italy (I2)	0.0252712	3.12231243	3.168053293	3.19686246	3.22339722
Italy (I3)	0.03968585	2.73426401	2.806095409	2.85133728	2.93269328
Germany (NJ2)	0.04484098	3.06656431	3.147726472	3.19884518	3.24592821
Poland (P1)	0.02146884	3.6713284	3.710186997	3.73466147	3.7786726
Romania (R1)	0.0679466	2.80298103	2.92596438	3.00342351	3.07476744
Slovakia (SL1)	0.06385915	2.06632021	2.181905261	2.25470469	2.38561593

Assessment of the Interaction Between Provenance and Habitat for Root Collar Diameter

The values of regression coefficients and functions for the height trait are shown in Table 3.

Based on the graphical representation (Figure 5), there was one interaction for root collar diameter, between Austria A1 and Italy I2 provenances.

Figure 5 shows the interaction between provenance and habitat for two provenances, Austria A1 and Italy I2. Austria A1 provenance had a lower average root collar diameter at the younger age of trees at Kupres habitat than Italy I2 provenance, and a higher average root collar diameter at the older age at Žepče habitat. The average root collar diameter (at x̲=5.16) for Italy I2 provenance on Kupres was 7.37 cm, and for Austria A1 provenance was 7.42 cm. In Žepče, (at *x*=10) the average value of root collar diameter for Italy I2 provenance was 7.48 cm and for Austria A1 provenance 7.47 cm. The difference in average root-neck diameter for both habitats is small, but tends to increase sharply with the increase of average root collar diameter for habitat. According to this model, the difference in the future will be higher in favor of Italy I2 provenance.
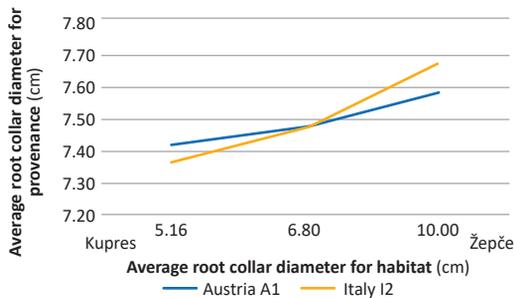

Figure 5. Graphic representation of the regression-based interaction between provenance and habitat for Austria A1 and Italy I2 provenances for root collar diameter.

DISCUSSION

Scots pine has been the subject of numerous morphological (Stefanović et al. 1980, Alía et al. 2001, Ballian et al. 2009, 2019, Cvjetković et al. 2014, Bardzajn et al. 2016, Ballian and Šito 2017, Gülcü and Bilir 2017) and genetic (Giertych and Mátyás 1991, Giertych and Oleksyn 1992) studies in Europe, and there is a need to continue research in the future.

Ballian et al. (2009) measured the diameter and height of the plants in 2007 and calculated basal area and volume of plants in one of the provenance tests of Scots pine in Bosnia and Herzegovina. The test was conducted in the Sokolac area in 1989 and contained 13 provenances from Bosnia and Herzegovina. The provenance geographically closest to the provenance test showed the best results of productivity. Cvjetković et al. (2014) measured and calculated the same characteristics at the same plot in 2011. The results confirmed the existence of significant differences within and among provenances, and the provenance geographically closest to the provenance test showed the best results.

Alía et al. (2001) researched 16 Spanish and 6 German provenances of Scots pine (*Pinus sylvestris* L.) in a provenance test in Spain. Based on data for total height, diameter, number of twigs at the fourth year whorl and survival, provenance by site interaction was very significant (*P*<0.01) for most traits.

Gülcü and Bilir (2017) researched tree height, diameter, and survival in a thirteen-year-old Scots pine provenance test established by 30 provenances at two sites in the southern part of Turkey. They found statistically significant differences within and among provenances for the researched traits. There were no statistically significant differences between the two sites, but provenance by site interaction was significant (◻<0.05).

In this research, the interaction between the effects of provenance genetic structure and habitat conditions for specific provenances was found for both of the reseached traits. The results of this research can be used to choose

Table 3. Calculated values of regression coefficients and functions for root collar diameter.

Provenance	b_1	b_0	y for *x* = 5,16	y for *x* = 6,8	y for *x* = 10
Austria (A1)	0.03496646	7.23568911	7.416116031	7.473461022	7.585353685
Austria (A2)	0.04148542	7.26240919	7.476473951	7.544510039	7.67726338
Austria (A3)	0.03767584	7.43688189	7.631289236	7.693077618	7.813640315
Bosnia and Herzegovina (B1)	0.06797794	5.85622663	6.206992804	6.318476628	6.536006041
Italy (I1)	0.06719365	6.65885583	7.005575041	7.115772621	7.330792288
Italy (I2)	0.06356439	7.04330153	7.371293798	7.475539402	7.678945458
Italy (I3)	0.06261167	6.30900923	6.632085461	6.734768605	6.88503662
Germany (NJ2)	0.04142208	7.29074702	7.504484967	7.572417183	7.704967849
Poland (P1)	0.02845056	8.41517287	8.561977741	8.608636654	8.699678435
Romania (R1)	0.05813623	6.31108847	6.6110714	6.706414812	6.892450738
Slovakia (SL1)	0.07538601	4.8690195	5.258011332	5.381644394	5.622879637

provenances for afforestation in observed habitats (Kupres and Žepče) in order to achieve best productivity.

The observed plants in provenances were only eight years old, so these are the first results and the research should be continued in the future. In comparison to the results of previous studies (Ballian and Šito 2017, Ballian et al. 2019), provenances overflowed each other in height and root collar diameter in both habitats. This confirms the rule that valid conclusions from provenance tests can only be made after 1/3 of the production period for the species tested.

CONCLUSIONS

The number of survived plants and the productivity of provenances is different for all provenances on both habitats, indicating that the adaptability of provenances is not only conditioned by their genetic constitution, but also by habitat conditions. Considering the productivity of provenances and the number of survived plants in both habitats, Austria A1, Austria A2, Austria A3, and Poland P1 provenances showed the best adaptability. Regarding the height of plants, two interactions between the effects of provenance genetic structure and habitat conditions were identified. Germany NJ2 provenance had a lower average plant height at Kupres habitat at a younger age and a higher average plant height at Žepče habitat at an older age than Italy I2 provenance. Romania R1 provenance had a lower average plant height at Kupres habitat at a younger age and a higher average plant height at Žepče habitat at an older age than Austria A2. For the root collar diameter, one interaction between the effects of provenance genetic structure and habitat conditions was identified. Austria A1 provenance had a lower average root collar diameter at a younger age at Kupres habitat than Italy I2, and a higher average root collar diameter at an older age at Žepče habitat. The results of this study can be used in the process of Scots pine breeding and for its conservation by *in situ* and *ex-situ* methods.

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Relationships Between Climatic Variables and Tree-Ring Width of Silver Fir (*Abies alba* Mill.) in Kozara National Park (Bosnia and Herzegovina)

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ABSTRACT

Silver fir (*Abies alba* Mill.) is the most common conifer species in Bosnia and Herzegovina. The paper describes the development of a silver fir master tree-ring chronology from Kozara National Park and analyzes the influence of the climate conditions on tree-ring widths. The knowledge concerning the effect of climate on cambium activity and, as a result, on tree growth is of great importance for silviculture, which is particularly complex in terms of deterioration of health condition. Four sites were selected for sampling at Kozara National Park. At each sampling site, 15 dominant trees were drilled with Pressler's increment borer at breast height. The development of chronology included three steps: i) cross-dating of a series of tree-ring width and checking of their quality using the COFECHA program, ii) data standardization in the ARSTAN program, and iii) master chronology development. Correlation analysis was performed in order to determine the links between tree-ring width and climate (primarily temperature and precipitation). In the 88-year-long master chronology, pointer years analysis identified five positive and nine negative years. Correlation analysis revealed that precipitation had stronger and more significant impact on silver fir radial growth, particularly at the beginning of the growing season, in April and May (r=0.175 and r=0.318, respectively), and in the crucial July–September period (r in the range of 0.143–0.222). The highest negative correlation coefficient with temperature was determined for September of the previous year (r=-0.282) and then for summer months (July and August) of the current year (r=-0.184 and r=-0.272, respectively). In addition, statistically significant correlation between radial growth and the Forestry Aridity Index was determined (r=-0.390). The results of tree-ring width climate analysis obtained in this study are in accordance with other studies carried out in Bosnia and Herzegovina and in the surrounding area.

Keywords: dendroecology; silver fir; temperature; precipitation; forestry aridity index

INTRODUCTION

Silver fir (*Abies alba* Mill.) grows naturally in central and southern Europe. In Bosnia and Herzegovina, fir is found in mixed beech and fir forests, beech, fir and spruce forests and very rarely in pure fir forests. In the mountain belt fir forms forests with spruce, sometimes with Scots pine, but also with beech. The most significant forests in Bosnia and Herzegovina are the community of beech-fir (*Abieti fagetum*) forests (Stefanović 1977, Stefanović et al. 1983,

Beus 1984). According to the National Forest Inventory, silver fir is the most common conifer species in Bosnia and Herzegovina. Beech and fir (with spruce) forests, i.e. forests with fir (pure and mixed), cover 599,000 ha or 28% of the total forest area (high and coppice forests). The highest stock of wood and the highest increment for high forests in Bosnia and Herzegovina were determined in these forests with fir (Čabaravdić et al. 2016).

According to Smith and Lewis (2007), dendrochronology is a scientific discipline that studies dating and annual

growth of rings in forest trees and shrubs. If all trees at one locality are influenced by common environmental factors such as climate, cross-dating provides a precise chronological record that can be used in dating events or describing differences in environmental conditions. Dendrochronology as a scientific discipline provides data on tree-ring growth, which can be used in a number of other scientific fields. By combining dendrochronology with these scientific disciplines, subdisciplines within dendrochronology have been differentiated, and one of the most important is dendroecology.

According to Amoroso et al. (2017), the term "dendroecology" is a relatively new term that was originally used in a much narrower sense. While dendroclimatology used past tree-growth patterns to reconstruct climate, dendroecology focused on how climate and other factors directly and indirectly influenced past tree-growth patterns. Dendroecology is a significant component of applied ecological research, providing important historical context for adapting the existing forest management strategies to mitigate and respond to current and future global environmental changes (Amoroso et al. 2017).

Silver fir has been the subject of research in a number of dendrochronological studies in Europe and in the Balkans (Rolland 1983, Levanič 1996, Smith et al. 1999, Toromani and Bojaxhi 2010, Bijak 2010, Carrer et al. 2010, Mazza 2013, Koprowski 2013, Jović et al. 2018).

Silver fir forests are endangered in some parts of Europe. The results of several studies suggested that fir is threatened with complete disappearance from the forests in central Europe (Elling et al. 1999, Badea et al. 2004, Filipiak 2005, Filipiak 2006, Filipiak and Napierała-Filipak 2009). The health status of silver fir forests in Bosnia and Herzegovina is deteriorating due to inadequate management. Silviculture does not take into account the ecological characteristics of common silver fir. This disrupts the stability of fir forests, which ultimately causes the spread of mistletoe *Viscum album* var. *abietis* Beck. as a primary parasite, followed by much more dangerous secondary pathogens (Uščuplić 1992, Uščuplić et al. 2007).

The knowledge about the effect of climate on cambium activity and, as a result, on tree growth is of great importance for silviculture, which is particularly complex in terms of the stated threats to firs in Bosnia and Herzegovina, i.e. their deterioration of health condition. The aim of the current study was to establish a silver fir tree-ring chronology from Kozara National Park and to analyze the effect of climatic variables on tree-ring widths.

MATERIALS AND METHODS

Study Area

The study area covers the area of Kozara National Park, which encompasses the central part of the eponymous mountain, located in the north-western part of Bosnia and Herzegovina (Figure 1). Kozara National Park is a member of the Federation of Nature and National Parks of Europe (EUROPARC Federation). The total area of the national park is 3,907.54 ha. The largest part of the national park is covered with high beech and fir forests (2,222.97 ha or 57% of the total area).
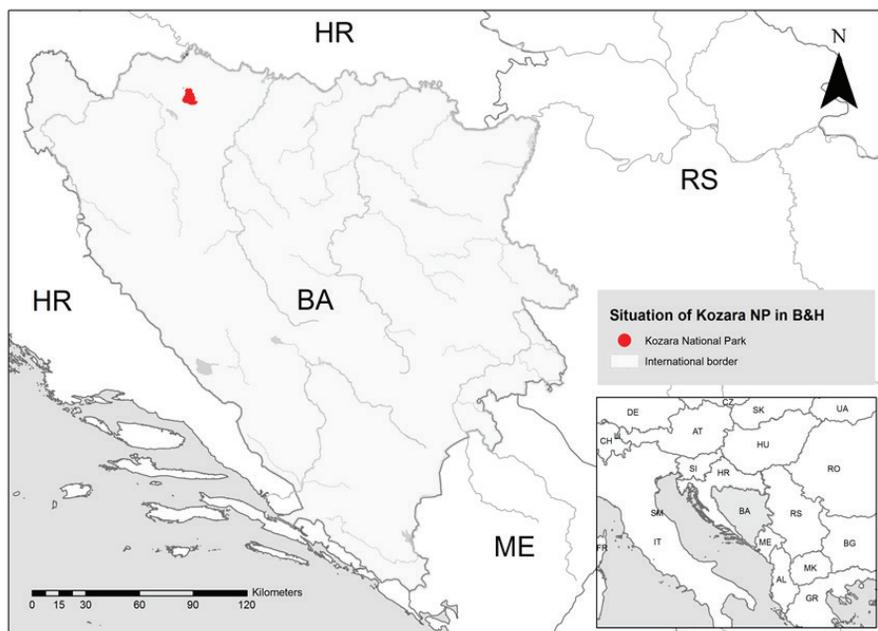

Figure 1. Geographical location of the study area.

Data Sampling

Based on the terrain reconnaissance, four sites (plots) were selected for sampling at Kozara National Park. In September 2015, core samples were taken from dominant silver fir trees in overstocked stands. All plots were located in the belt of Peripannonian beech and fir forests (*Festuco drymeiae-Abietetum* Vukelić et Baričević 2007), which are dominant in the central and northern, cooler part of the mountain. The dominant species in these forests is silver fir, which on the northern side of the mountain, atypically for such latitudes, extends to a very low altitude (to about 200 m above sea level). For all sample plots, the characteristic geological substrate was the Eocene flysch, with a very deep eutric cambisol. Samples were collected at different expositions (NNE, SW, NE and N) and in slopes ranging from 12 to 25° (Table 1).

At each sampling site, 15 dominant trees (larger than the average trees in the stand, with crowns extending above the general level of the crown cover and with well-developed crowns) were drilled with Pressler's increment borer (core diameters: 5.15 mm) at breast height (1.30 m). From each tree, two cores from opposite sides were extracted. Previous studies determined that minimum sample size for establishing a quality individual chronology of the site is 15 trees, if two increment cores are taken from each tree (Fritts 1976, Levanič 1996). Two out or three additional trees were drilled to serve as a substitute, in case that some of the sampled trees have atypical growth (overtopped trees, heart rot, missing or false tree-rings), so it could be difficult to cross-date them, or if some samples are damaged in the measurement preparation. The decay of wood at the center of the trunk was found in a considerable number of trees, which reflected in the length of the obtained chronology. The cores were scanned with a high-performance scanner: the Epson Perfection V30 Photo scanner. The tree-ring width was measured with the CooRecorder 7.6 and CDendro 7.6 software (available at http://www.cybis.se/forfun/dendro/).

Chronology Development

The procedure of chronology development consisted of three steps: cross-dating, data standardization and development of master chronology. Cross-dating technique ensures that each individual tree-ring is assigned to its exact year of formation. Cross-dating was done by using visual on-screen techniques (skeleton plotting) with CDendro software and statistical methods with the use of COFECHA specialized computer program (Holmes 1983, Grissino-Mayer 2001). The COFECHA program adds a high degree of confidence that tree-ring samples have been cross-dated correctly and measured accurately, ensuring that the environmental signal is maximized (Grissino-Mayer 2001). The first phase of processing in the COFECHA program was the detection of trees that significantly differ from the average value (i.e. trees which displayed a low correlation coefficient with the master chronology) and for which a larger number of "problematic segments" were identified. After this first phase, all trees that could not be synchronized (due to the appearance of overtopped trees which is very characteristic for firs, or the appearance of missing or false tree-rings), as well as those trees whose samples were damaged in transport or preparation for the analysis, were removed from further processing. Silver fir is a shade-tolerant species. Young fir trees can endure long periods in the deep shade, and continue their normal development when large quantities of light become available. Samples from additional trees replaced the eliminated samples.

The standardization (detrending) of tree-ring width time series is a traditional procedure in dendrochronology and essentially represents the elimination of the so-called age-related trend. The standardization of tree-ring width time series was performed using the ARSTAN program (Cook 1985, Cook and Holmes 1997). To remove the age-related trend cubic smoothing splines were used. Flexible cubic spline curves are very efficient at removing both the long-term trend and the effect of localized disturbance events, always present in the course of forest dynamics, which can lead to abrupt growth responses (reduction or release) that can persist in single trees even for many years after the disturbance event and conceal or fade the climatic signal retained in the tree-ring series (Cook and Peters 1981). Mean chronologies for each site were calculated as the biweight robust mean of detrended individual series (Cook 1985). For individual raw series, and for the mean chronologies, mean sensitivity was calculated to assess the high-frequency variations (Fritts 1976), and the first-order autocorrelation to measure the influence of the previous year's growth on the current ring. The results of the processing in the ARSTAN program are three curves, i.e. three versions of the chronology: standardized (std), residual (res) and arstan (ars). The ars chronology performed by the multivariate autoregressive analysis procedure has the best climate signal and was therefore used to study the influence of climatic variables on the tree-ring width.

Table 1. Characteristics of the sampling sites.

Site number	1	2	3	4
Latitude	45°0'51.8"N	45°0'49.7"N	45°0'48.5"N	45°0'12.5"N
Longitude	16°54'40.8"E	16°54'27.2"E	16°55'6.4"E	16°53'39.0"E
Geological substrate	Eocene flysch	Eocene flysch	Eocene flysch	Eocene flysch
Soil	Eutric cambisol	Eutric cambisol	Eutric cambisol	Eutric cambisol
Altitude (m a.s.l.)	780	790	735	620
Exposure	NNE	SW	NE	N
Slope (°)	15	22	12	25

Meteorological Data

For the calculation of the links between tree-ring width and climatic variables, primarily temperature and precipitation, data from the nearest meteorological station were used, that is, Prijedor meteorological station, located about 18 km southwest of the study area. Data on mean monthly temperatures and precipitation, in the 1961–2014 period (data available only for this period) were provided by the Republic Hydrometeorological Service of Republic of Srpska (Table 2).

In addition to temperature and precipitation, the forestry aridity index (FAI) (Führer et al. 2011) was used for the analysis of their combined effect on silver fir radial growth. The relationship between meteorological parameters and trees growth can be characterized by a simplified forestry aridity index: FAI = $100 \times T_{VII-VIII}$ / (P_{V-VII} + $P_{VII-VIII}$), where $T_{VII-VIII}$ is the average temperature in July and August (°C), P_{V-VII} is the precipitation sum (mm) in the May–July periods, and $P_{VII-VIII}$ is the precipitation sum (mm) of July and August (Führer et al. 2011). The higher index values suggest more arid climate.

RESULTS

Basic characteristics of trees and tree-ring width, including mean and maximum tree-ring width, standard deviation, autocorrelation coefficient and mean sensitivity, were calculated for empirical series of Sites 1–4. The mean diameter at breast height of analyzed trees was in the range

of 57.6–73.2 cm, whereas the height was in the range of 32.5–36.4 m. The total number of tree segments (1925–1974, 1950–1999, 1975–2024) at the sites ranged from 31 to 39. At Sites 1, 2 and 4, two problematic segments appeared, and at Site 3 only one, i.e. the share of problematic segments was in the range of 2.94–5.13%, which can be considered as acceptable deviation. The serial intercorrelation of the empirical tree-ring widths ranged from 0.376 to 0.605. The arithmetic mean of all the individual series of empirical tree-ring width increments ranged from 3.45 mm at Site 1 to 3.77 mm at Site 3, i.e. the values were about the same. Filtered (indexed) data, as expected, are characterized by significantly different values of analyzed parameters. Furthermore, the effect of autocorrelation is practically eliminated, which is confirmed by autocorrelation coefficients close to zero. The best results were obtained by analyzing the tree-ring widths from Site 3, whereas somewhat weaker results were obtained from Site 2 (Table 3).

Height in relation to diameter (in relation to the local site quality classification - uneven-aged stand (Drinić et al. 1990), and average tree-ring widths per sites show that the studied sites are highly productive habitats for silver fir, which was to be expected given the habitat conditions described, primarily soil characteristics (very deep eutric cambisol).

Figure 2 shows the average empirical tree-ring series. The age trend was clearly manifested, i.e. there was a decrease in the mean tree-ring widths at sites with increasing age. It is also noticeable that the tree-ring widths were more variable over the last 20 years than in the previous period.

Table 2. Mean monthly and annual (y) temperature and precipitation in Prijedor during the 1961–2014 period.

Variable	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	y
t (°C)	-0.3	2.0	6.5	11.4	16.0	19.6	21.3	20.7	16.4	11.4	5.9	1.2	11
p (mm)	62	55	65	81	85	89	80	77	93	80	89	77	931

Table 3. The basic characteristics and dendrochronological-statistical parameters of empirical series of tree-ring width.

Variable	Site 1	Site 2	Site 3	Site 4
dbh (cm)	60.4	73.2	57.6	58.5
h (m)	32.5	36.4	34.2	32.6
No. Years	1057	900	931	993
No. Segments	39	31	34	36
No. Problematic segments	2	2	1	2
Series intercorrelation	0.574	0.376	0.605	0.554
Unfiltered				
Mean (mm)	3.45 (0.97-5.07)	3.76 (2.28-5.76)	3.77 (1.88-5.42)	3.66 (2.78-6.86)
Standard deviation (mm)	1.51 (0.51-2.08)	1.84 (0.72-2.68)	1.77 (1.09-4.71)	1.70 (1.08-3.06)
Autocorrelation	0.752 (0.429–0.912)	0.813 (0.640–0.912)	0.685 (0.403–0.912)	0.795 (0.669–0.920)
Mean sensitivity	0.237 (0.169–0.306)	0.220 (0.136–0.312)	0.248 (0.193–0.311)	0.224 (0.108–0.305)
Filtered				
Standard deviation	0.28 (0.21-0.40)	0.30 (0.17-0.47)	0.29 (0.16-0.37)	0.28 (0.16-0.42)
Autocorrelation	- 0.002	0.002	- 0.008	0.007
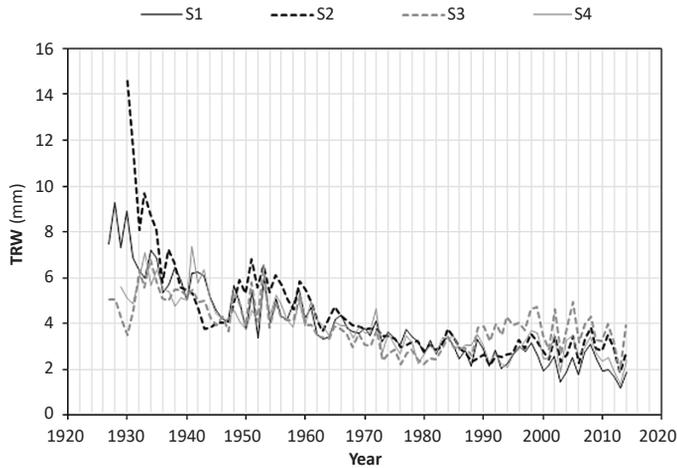
Figure 2. Average empirical tree-ring series per sampling sites.

In order to remove the age related-trends in tree diameter increment, standardization of the original (empirical) data series was performed in the ARSTAN program. During the standardization, each tree-ring width series was fitted with the cubic smoothing spline. After data smoothing, a dimensionless tree-rings width index (I_t) was calculated. The results of the chronology analysis with the ARSTAN program are three curves, i.e. three versions of the chronology and a comprehensive statistical analysis. The obtained arstan (ars) version of chronologies for the four sampling sites are shown in Figure 3.

The adequacy of merging these four sites' chronologies into one master chronology was determined based on the degree of correlation between chronologies measured by the product moment correlation coefficient and its corresponding t-value, as defined by Baillie and Pilcher (Baillie and Pilcher 1973). Statistically significant positive correlation coefficients were determined between all four sites. The calculated correlation coefficients ranged

from 0.647 (between Site 2 and Site 3) to 0.841 (between Site 3 and Site 4). The Gleichläufigkeit (glk) (Huber 1943, Eckstein and Bauch 1969), i.e. the percentage of common signs of year-to-year growth change between two series, was high and statistically significant, ranging from 0.657 to 0.738. These results showed that there is a significant harmonization and a high similarity between the obtained chronologies (i.e. similar patterns of tree-ring increment) at different sites. This indicates that these chronologies were suitable and a master chronology was developed for the Kozara Mountain area (Table 4).

An 88-year-long master chronology was built (Figure 4). The first year of chronology was 1927 and the last was 2014. The average I_t of the master chronology is 1.003, the standard deviation is 0.107, the skewness -0.006, and kurtosis 3.189. The mean sensitivity is 0.138, and the serial correlation is -0.379. The obtained version of the ars chronology was further used for determining the influence of climate parameters on tree-ring formation.
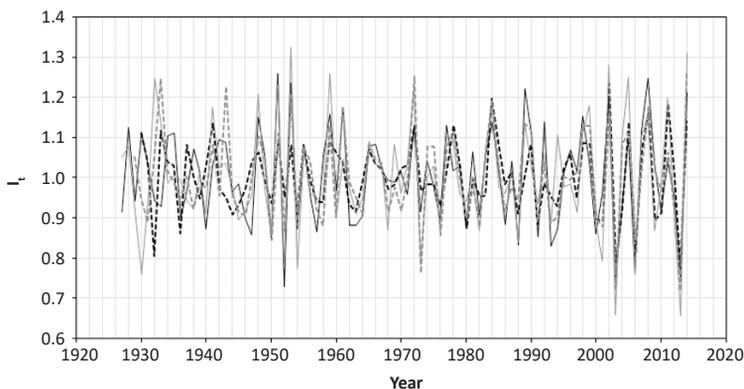
Figure 3. Site chronologies of silver fir – arstan version.

Table 4. Correlation coefficients (r) (upper triangular of the matrix) and Gleichläufigkeit (glk) (lower triangular of the matrix) between site chronologies.

Site	Site 1	Site 2	Site 3	Site4
Site 1	*	r=0.693 (p=0.00)	r=0.713 (p=0.00)	r=0.742 (p=0.00)
Site 2	glk=0.699	*	r=0.647 (p=0.00)	r=0.688 (p=0.00)
Site 3	glk=0.698	glk=0.657	*	r=0.841 (p=0.00)
Site 4	glk=0.720	glk=0.693	glk=0.738	*
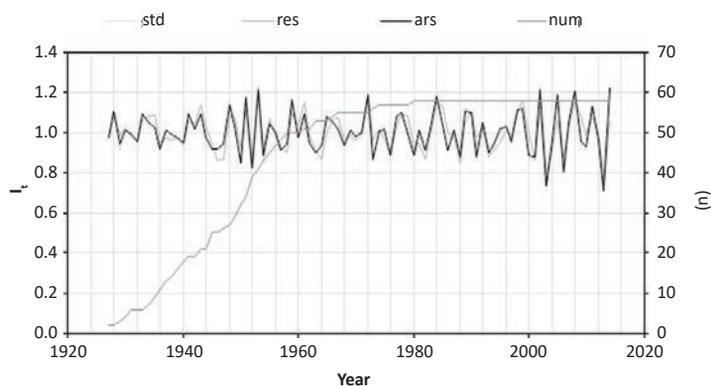
Figure 4. Master chronology of silver fir for Kozara Mountain – std (standardized), res (residual) and ars (arstan) version (num displays the number of tree-ring series in master chronology).

Pointer year was defined as the year when in a sample of 10 or more individual tree-ring series more than 90% of the series respond with the same increasing or decreasing trend in tree-ring width compared to the previous year (Schweingruber 1983, Schweingruber et al. 1990). The years 1951, 1953, 1955, 2007 and 2014 were determined as strong positive pointer years. On the other hand, the negative pointer years were 1936, 1954, 1973, 2000, 2003, 2006, 2009 and 2013 (Figure 5). It is indicative that the same number of negative pointer years was recorded since the beginning of the 21st century (in only 15 years) as in the previous 64 years (in the 1936–1999 period).

Correlation analysis was performed to examine the relationship between annual tree-ring width and temperature and precipitation variability. The master chronology was correlated with the climate data on mean monthly temperature and precipitation. Given that the climate conditions in the period prior to the tree-ring formation affect its development in great measure (Fritts 1976), the correlation analysis covered thermal and pluvial conditions in the August–October of the previous growth year periods and in April–October of the current growth year periods.

Climatic description of the study area is given based on climatic data on mean monthly and annual temperature and precipitation from Prijedor meteorological station in the 1961–2014 period. The mean annual temperature in Prijedor is 11.0°C. The warmest month is July with an average temperature of 21.3°C, whereas the coldest month is January (-0.3°C). The mean temperature of the growing season (April-October) is 17.5°C. The average annual precipitation

is 931 mm. The maximum precipitation occurs in September (93 mm), whereas the minimum is recorded in February (55 mm). During the 1961–2014 period, temperature displayed a significant upward trend in the summer season in the range of 0.58°C per decade (p<0.01). In that period, the most prominent downward trend in precipitation was also determined in summer (-13.6 mm per decade, p<0.07).

The relationship between the silver fir tree-ring width and the air temperature is mostly negative (Figure 6). The highest negative correlation coefficient was determined for September of the previous year (r=-0.282) and then for summer months – July and August of the current growth year (r=-0.184 and r=-0.272, respectively). High summer temperatures negatively affect the trees' radial growth. The obtained negative relation in the July–August period is in accordance with the results from previous studies suggesting that high summer temperatures limit tree growth, i.e. forest trees exposed to high temperatures and reduced humidity (water supply) produce narrower annual tree rings (Fritts 1976). A weak positive correlation was determined for April of the current growth year. Due to higher temperatures in April, tree-ring formation begins earlier, i.e. the growing season is prolonged, which positively affects the annual tree-ring width.

In contrast to temperature, the correlation between the silver fir tree-ring width and precipitation is stronger, more significant and predominantly positive, particularly at the beginning of the growing season, in April and May (r=0.175 and r=0.318, respectively), and in the crucial July–September period (r in the range of 0.143–0.222). Frequent lack of available moisture in this period of the year (July–September)
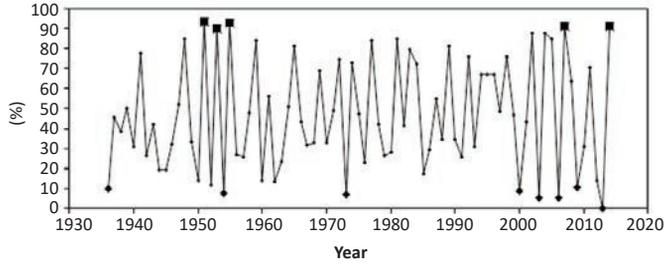
Figure 5. Positive (marked with a square) and negative (marked with a rhombus) pointer years in the master chronology of silver fir for Kozara Mountain.
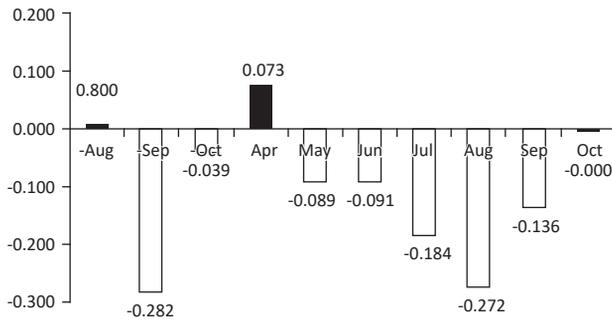
Figure 6. Correlation coefficients between silver fir master chronology tree-ring index (It) and mean monthly temperature from Prijedor meteorological station.

negatively affects the tree increment, i.e. slows down the formation of tree rings (Figure 7).

Based on the correlation analysis it could be concluded that precipitation has a greater impact on the tree-ring formation than temperature. When it comes to precipitation, correlation coefficient greater than 0.200 was determined for four months, whereas for temperature only for two months.

The forestry aridity index (FAI) represents the ratio of average temperatures in July and August, and rainfall from May to August. In this research, FAI was used in order to eliminate the consequences mutual inter-correlation between temperature and precipitation (higher temperatures also cause less precipitation and vice versa). The determined relationship between I_t and FAI was significant and negative: with an increase in FAI (i.e. with increasing aridity), the tree ring width decreased. The determined correlation coefficient of -0.390 was statistically significant at the 99% level. This is in accordance with the previously stated fact that adequate water supply plays a crucial role in silver fir increment. During the 1961–2014 periods, six negative pointer years were
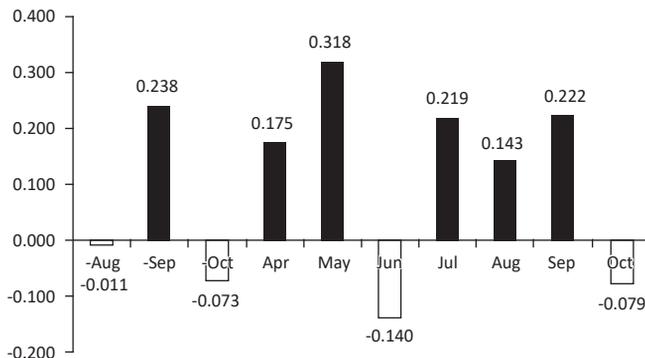
Figure 7. Correlation coefficients between silver fir master chronology tree-ring index (It) and monthly precipitation from Prijedor meteorological station.

determined. For these negative pointer years, the average value of FAI was 8.1, whereas for the entire observed period it was 5.7. For the two positive pointer years, the average value of the FAI was 5.2.

The matching of years with minimum value of tree-ring width index (I_t) and minimum precipitation in July (2013) confirms that the standardization process was reasonable and well-performed. The lowest I_t values were recorded in 2003 and 2013, in the years of secondary and tertiary minimum of FAI.

DISCUSSION

Pointer years detected in silver fir chronology for Kozara Mountain match relatively well with the chronology of silver fir from the Dinaric region in Slovenia, in which 11 negative and 9 positive pointer years during the 1900–1993 period have been determined (Levanič 1996). At several sites in Slovenia 1976, 1967, 1962, 1960, 1947 and 1942 were detected as negative pointer years, whereas 1989, 1961, 1959, 1951 and 1943 were detected as positive (Levanič 1996). According to Toromani et al. (2011), the most prominent negative pointer years in the silver fir chronology from the Koritnik Mountain (southern Kosovo) were 1985, 1997 and 2003. In the 64-year-long silver fir chronology from Kosovo, 1909, 1953, 1980 and 1995 were detected as strong negative pointer years, whereas 1947, 1955, 1971 and 1994 as positive (Toromani and Bojaxhi 2010). The authors in the study stated that the pointer years 1953 and 1955 appeared to be the most geographically extended pointer years for silver fir throughout Europe. The 9 negative (1874, 1880, 1891, 1931, 1943, 1963, 1971, 1987 and 2000) and 5 positive (1876, 1930, 1941, 1959 and 1969) pointer years were identified for Austrian pine (*Pinus nigra*) in Bosnia and Herzegovina (Poljanšek et al. 2012). The number of negative and positive years in the observed surveys is approximately the same as the determined number for the Kozara National Park area considering the chronology length, but the same characteristic years in different chronologies have not been observed.

Ducić et al. (2015) found that precipitation, i.e. drought in the summer months of July and August, is crucial for radial increment of the silver fir from Bokšanica Mountain (eastern Bosnia and Herzegovina). Statistically significant positive correlation coefficients between the residual ring-width chronology and precipitation were determined for July (0.384) and August (0.367), whereas significant links of the opposite sign were found for January (-0.313). Although the annual data displayed weak connections, for the growing season (April–October) a statistically significant correlation coefficient was determined (0.327). The significant relationship with air temperature was obtained only for July (-0.288). The observed effect of precipitation on fir radial increment at Bokšanica Mountain is in accordance with the results obtained for the effect of precipitation at Kozara Mountain in summer (the effect of precipitation is also more prominent than the effect of air temperature).

Toromani and Bojaxhi (2010) determined that radial growth of silver fir in Kosovo depends strongly on spring temperature and precipitation (i.e. climate at the beginning of the growing season), which play a significant role particularly for early wood production. High spring temperatures and ample rainfall during the first part of the growing season had positive influence on silver fir growth (i.e. the formation of wide tree-rings) in southern Kosovo, whereas lower spring temperatures and scarce precipitation had an inverse effect (Toromani et al. 2011). Temperatures in April and May of the current growth year positively influenced silver fir growth at the low-elevation sites, whereas the July precipitation of the previous growth year had a negative effect (Toromani et al. 2011). More prominent effect of air temperature in comparison to the results obtained for Kozara Mountain, especially in spring, can be explained by the significantly higher altitudes of the investigated sites (1200 m and 1500 m).

Bronisz et al. (2010) found that silver fir trees in Poland showed a significant negative relationship to thermal conditions in March and July-September periods. On the other hand, a positive correlation with water supply was observed in February and July. Additionally, silver fir trees at some sites showed a significant negative relation with precipitation in August of the year prior to the ring formation and in May of the current year.

Climate–growth relationship analysis determined that silver fir forests' growth at the south-western distribution limit in Europe is severely constrained by low spring–summer water availability, whereas in non-Mediterranean areas it is limited by cold conditions in the late winter–early spring periods (Gazol et al. 2015). The same study also found that most populations in the Mediterranean area have displayed a marked decline in growth since the 1980s (Gazol et al. 2015). Silver fir in marginal populations in central Italy showed a positive influence of late-spring and summer precipitations and a negative effect of summer temperatures, during the last century (Mazza et al. 2013). Moreover, the results suggested that the shifting influence of summer precipitation on tree-rings growth from July to August of the previous year is a possible response of silver fir to the significant reduction in spring precipitation and the general warming trend present throughout the 20th century (Mazza et al. 2013).

Similar patterns of climate-growth relationship were found for Scots pine (*Pinus sylvestris* L.) and Austrian pine (*Pinus nigra* Arn.) in Bosnia and Herzegovina and in the surrounding area. The significant positive effects of above-average temperatures in January–March were determined, whereas the above-average temperatures in May–August had a negative impact on Austrian pine increment (Poljanšek et al. 2012). Furthermore, a positive relationship between tree-ring width and above-average precipitation in May–August was found at several sites (Poljanšek et al. 2012). The fact that sufficient precipitation with a good distribution throughout the year has a strong positive influence on the radial growth of Austrian pine (*Pinus nigra* Arn.) was also observed in Serbia (Koprivica et al. 2009, Ćirković-Mitrović et al. 2013). According to Koprivica et al. (2009), in middle-aged Austrian pine plantations, a statistically significant dependence of the diameter increment on the precipitation was determined, whereas the dependence on the air temperature and thinning is random. Ćirković-Mitrović

et al. (2013) found a strong positive correlation between the trends in the diameter increment of Austrian pine and annual precipitation, but also with precipitation during the growing season (Ćirković-Mitrović et al. 2013).

Different patterns of climate dependence have been identified for silver fir trees in the northern part of the European continent. A dendroclimatological study of silver fir growing in the Kaszubskie Lakeland in northern Poland showed that winter temperature and precipitation have the most important effect on fir radial growth (Bijak 2010). During the observed 1914–2006 period, tree-ring width was strongly dictated by winter, early spring and summer temperatures, whereas a significant relation with moisture availability was determined only for January. Given the results, it seems that in colder Poland silver fir prefers warm and dry winters and, to some extent warmth in early spring and in the middle part of the growing season.

Climate change (lack of precipitation, increase in temperature, increase in intensity or frequency of extreme weather events, etc.) is expected to have a strong impact on silver fir radial growth (Dobrowolska et al. 2017). Stojanović et al. (2012) used the Forestry Aridity Index to evaluate the impact of climate change on beech forests in Serbia. The obtained results showed that the 1981–2010 period was drier compared to the 1961–1990 period. Matović (2013) investigated the impact of climate change by the end of the 21st century on the future growth and distribution of the main tree species in Montenegro, including the silver fir. Based on the FAI, it was determined that, according to the EBU-POM regional climate model (Djurdjević and Rajković 2010), in the 2071–2100 period, about 19.1% of the primary areas where the presence of silver fir was identified would be outside the existing ecological niches (that is, the area in which the silver firs can successfully survive based on their internal and physiological constraints). Compared to other species such as spruce or beech, the percentage of decrease is significantly higher (Matović 2013).

The results of radial growth-climate analysis obtained in this study are similar to the results of other studies in Bosnia and Herzegovina and in the surrounding area, which addressed the issues of the silver fir's (and other conifer species') growth dependence on climate. It can be assumed that the dependence of radial growth on climatic variables is less pronounced (relatively small values of correlation coefficients), due to the fact that they are localities with good habitat conditions for silver fir (soil characteristics, altitude, etc.). The structure of a forest stand also has effect on the dependence of radial growth on climatic variables, i.e. the values of correlation coefficients. Trees in overstocked stands are crowded and less vigorous.

CONCLUSIONS

The results of the current study contribute to a better understanding of silver fir radial tree growth at Kozara Mountain. Based on core samples extracted from 58 trees at four sites in the broader Mrakovica area, the first silver fir master chronology for this area was built. In the 88-year-long master chronology developed, five positive and eight negative pointer years were identified. Climate is an important site factor, which has a direct impact on silver fir radial growth. Analysis of tree-rings growth dependence on climate variables revealed that precipitation has a stronger impact on tree-ring formation than temperature. The negative impact of high summer temperatures was also determined. A significant positive trend in the FAI, which indicates an increase in aridity, negatively affects the tree-ring growth. Considering the detected tree-ring formation dependence on precipitation and temperature, especially during the summer season, it is particularly important to highlight that climate change in the study area is most prominent during this part of the year. During the 1961–2014 period, temperature displayed a significant upward trend in the summer season in the range of 0.58°C per decade. In that period, the most prominent downward trend in precipitation was also determined in summer (-13.6 mm per decade).

Studies on the impact of high temperatures and low precipitation (i.e. drought) on the radial tree growth of silver fir are certainly significant. Knowledge on these impacts is of great importance in the making and implementation of management plans (including silvicultural treatment) for the conservation of sensitive ecosystems and tree species in protected areas.

Conflicts of Interest

The authors declare no conflict of interest.

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Estimating Potential Woody Biomass Supply under Sustainable Timber Production from Plantation Forests in a Snowy Mountainous Region of Japan

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ABSTRACT

Numerous woody biomass-powered stations for energy generation have been constructed in Japan since the Feed-in Tariff Scheme was introduced. However, a stable, long-term woody biomass supply from plantation forests is necessary for the construction and operation of such power stations. The logs that are used to produce fuel chips are harvested from roundwood. Thus, the main objective of this study was to estimate the potential supply of woody biomass resources under the condition of maximum sustainable harvesting from privately-owned plantation forests. Another aim was to examine the stability of sustainable harvest volumes from periodical changes. The study focused on privately-owned forests in the Sampoku district of Murakami City in the northernmost part of Niigata Prefecture, Japan. Trees in the studied region are commonly bent during periods of high snowfall, and the current bucking strategy – influenced by the construction of a biomass-powered generation facility – was integrated into the model. The revenues and costs of plantation management and timber production were calculated based on stands' geographical attributes using a geographic information system. A mixed integer programming model was used to predict the maximum sustainable harvest levels that would provide stable profits. The simulation showed that even though the trees had a unimodal age class distribution, sustainable harvest volumes ensured a sustainable supply of woody biomass over various rotation ages. Extending the range of rotation ages by 20 years dramatically increased the potential supply of woody biomass resources. Fluctuations in each woody biomass resources were mostly less than 20% over the planning horizon. The presented research could be useful to regional forest resource managers and stakeholders involved in biomass-powered energy generation or the purchasing of woody biomass.

Keywords: bucking strategy; maximum sustainable harvest; profitability; sustainability; woody biomass-powered station

INTRODUCTION

A Feed-in Tariff (FIT) Scheme for renewable energy use has been in effect since July 2012 in Japan. This FIT scheme has stabilized the return from investment expected for Japanese renewable energy projects, since the scheme guarantees that the electricity generated from renewable energy sources will be purchased at a fixed price over a long-term period. As a result, the Japanese renewable energy market has rapidly expanded following the entry of companies which were previously not linked to electricity generation (NEDO 2014). The tax exclusive tariffs for electricity generated from woody biomass – that is, biomass from thinning and final cutting in areas covered by 'forest management plans' - are 32 yen·kWh^{-1} (0.256 €·kWh^{-1}; currency exchange rate: yen/€, January 2020) for facilities with a generation capacity over 2,000 kw and 40 yen·kWh^{-1} (0.32 €·kWh^{-1}) for facilities with a generation capacity under 2,000 kw. These tariffs are higher than what is provided for other feedstocks, such as sewage residues and building material waste. For this reason, many power stations which use woody biomass as a feedstock have been

constructed after the induction of the FIT Scheme, which has considerably burdened the supply of woody biomass (Ando 2013). Thus, a stable, long-term supply of woody biomass from plantation forests is necessary for the construction and operation of woody biomass-powered generation facilities.

In the northern part of Niigata Prefecture, Japan, the bucking strategy for felled trees with butts that have been bent by snow cover has changed after one biomass-powered generation facility started to purchase fuel wood chips. In the case of regular roundwood, the top part of felled trees used to become residue after bucking, while bent trees were cut to avoid the bent part of the tree. More specifically, the butt log used to be cut about 1 m above ground level to obtain the best possible log. In the current situation, which includes a buyer of fuel wood chips, small-diameter logs are cut from the top part of the felled tree. When bucking bent trees, a 2-meter-long butt log is cut from the regular stump height (this includes the bent part, which can be used to produce fuel wood chips). The logs that will be used to produce wood chips are harvested from roundwood because the profitability of plantation forest management in Japan is generally low and, as such, it makes sense from an economic perspective to utilize as much of a felled tree for value-added products as possible.

Researchers have previously attempted to estimate the potential supply of woody biomass by using geographic information system (GIS) data and forest growth models. Nord-Larsen and Talbot (2004) estimated economically feasible annual fuel wood resources in Denmark by applying a model that optimized allocation and transportation costs. Ranta (2005) applied a similar approach by creating a model that used availability comparisons between power generation facilities and resource allocation to estimate the technically and economically viable supplies of biomass from final cuttings in Finland. Yamaguchi et al. (2014) estimated the annual supply of timber and logging residues at a regional scale in Japan based on known cutting and extraction rates. In another Japanese study, Aruga et al. (2014) calculated the potential annual economic supply of forest biomass resources at a regional level under a rotation age of 60 years. Moreover, the potential supply of woody biomass was estimated by incorporating the long-term harvesting schedules of plantation forests. Aruga et al. (2006) investigated the potential of timber and logging residue volumes in a specific Japanese county by applying a random search technique to a model that included a harvesting cost constraint. In a Norwegian study, Rørstad et al. (2010) used linear programming that maximized profitability under non-declining harvest constraints to estimate the supply of harvest residues' supply at a regional scale. However, the sustainable supply of woody biomass, i.e. the supply that will remain stable over a long-term period, in Japan has not yet been clarified.

The presented research aimed to estimate the potential supply of woody biomass in a specific district under the condition of maximum sustainable harvest of timber from plantation forests, as well as to determine how changes in the rotation age would influence this supply. An additional objective was to clarify how resistant the supply is to changes by the ten-year planning period, since the district under study is located in a snowy mountainous region of

Japan. Bucking was simulated based on the current strategy in the district under study. The focal district includes plantation forests with unimodal age class distributions. However, it is important to note that some of the plantation forests are not suitable for profitable timber production. Furthermore, sustainable harvest levels cannot be easily predicted based on the growing stock, and its growth, in the district. In this research, sustainable harvest was defined as the stable production and profits over a planning horizon. The geographical attributes of stands were used to calculate the revenues and costs that are relevant to decisions regarding final cutting age. The supply of biomass resources was then predicted for several scenarios based on maximum sustainable harvest levels.

MATERIALS AND METHODS

Study Area

The study focused on numerous small-scale, privately-owned forests in the Sampoku district of Murakami City in the northernmost part of Niigata Prefecture, Japan (Figure 1). The district is one of the prefecture's most active forestry regions. The studied forests included 13,792 sugi (*Cryptomeria japonica* D. Don) stands covering at least 0.1 ha, representing an area of 8,701.5 ha. A clear majority (86%) of these sugi plantations are covered by the 'forest management plans' from 2015. The stands currently show a unimodal age class distribution, which is a common characteristic of Japanese plantation forests (Figure 2). Timber production in Sampoku district amounts to 15,000–20,000 $m^3 \cdot year^{-1}$. The Northern Niigata Wood Processing Cooperative produces sawn and laminated wood from domestic medium-diameter logs, and has a log processing capacity of 24,000 $m^3 \cdot year^{-1}$. Niigata Prefecture Forest Owners' Co-operative Association mediates arranged transactions of logs - with agreed volumes at fixed prices - between log producers and customers such as the Northern Niigata Wood Processing Cooperative, sawmills, plywood manufacturers, and suppliers of fuel wood chips to a woody biomass-powered facility in Niigata City with a generation capacity of 5,700 kW. The regeneration treatments and prescribed silvicultural regimes (hereinafter prescriptions) applied in the sugi plantations are summarized in Tables 1 and 2, respectively.

Data

The presented research used forest GIS data provided by the Niigata prefectural government, while contour and elevation point data were provided by the Geospatial Information Authority of Japan (2012), and road data were digitized from 1:5,000 scale forest maps (Tatsuhara and Dobashi 2006). The research is also based on information from interviews with representatives of the forest owners' cooperative in Murakami City about the current status of forests and silvicultural operations in the study site. Information about the current subsidies provided in the prefecture was obtained from the Niigata prefectural government, while the relevant quality standards and prices for logs were obtained from the Niigata timber market and interviews with customers of sugi logs.
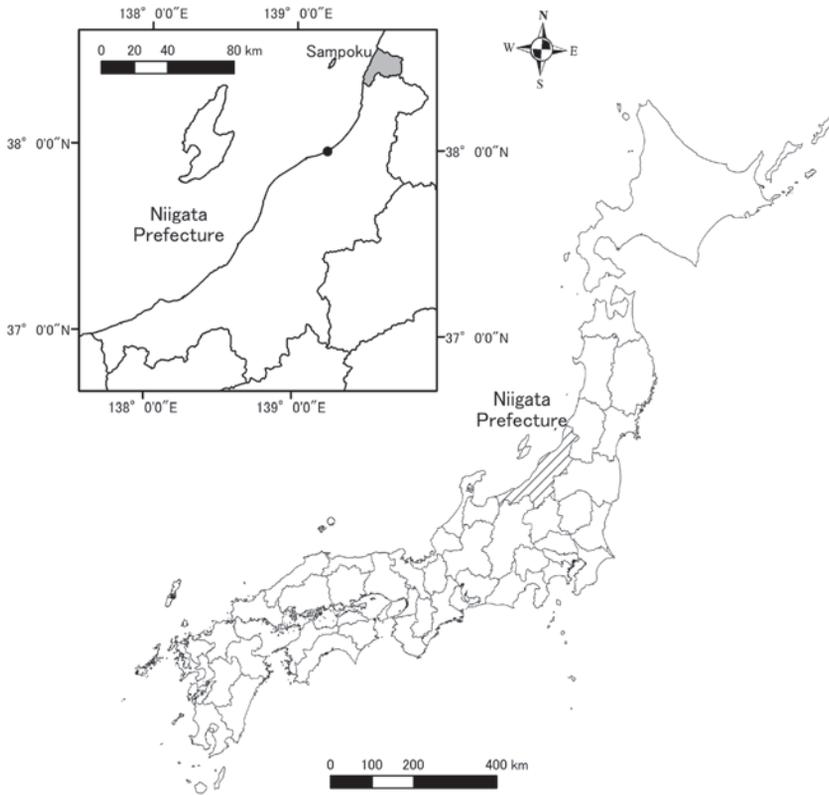
Figure 1. Study site. Black circle depicts the location of a woody biomass-powered station which purchases logs.
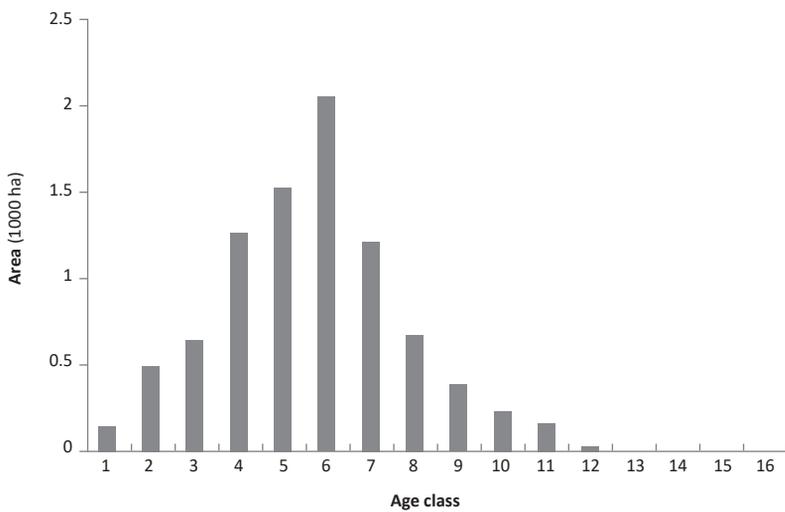
Figure 2. Age class distribution, in 10-year classes, of the studied stands at the beginning of the planning horizon.

Predicting Yield and Calculating Profit

Yields and profits were calculated for stands under each prescription according to the methodology presented by Moriya and Tatsuhara (2014, 2017), as summarized below. ArcGIS 10.3 (Esri, Redlands, CA, USA) was used as the GIS software to obtain spatial information.

The average heights of dominant and codominant trees in each stand were calculated from stand age and site class entries in the forest inventory data according to height/age curves published by the Niigata Prefectural Government (1980). The yield from each stand, which took into consideration the applied prescription, was predicted using the stand density control diagram for sugi plantations along the Sea of Japan in the Tohoku and Hokuriku regions published by the Forestry Agency of Japan (1981). The height of trees in each diameter class was estimated using

a previously presented height-diameter curve (Nagumo et al. 1981). The stem form of trees in each diameter class was estimated using the following formula, with the values of certain parameters based on the stand density control diagram:

$$y^2 = px^r \qquad (1)$$

where p and r are parameters describing size and form, respectively (Prodan 1965). The simulations included a specific bucking strategy to predict the number and volume of logs that would be obtained from stands under each prescription. More specifically, the 2-meter-long butt log was cut from regular stump height. Then, as many four-meter large-diameter logs with a minimum top-end diameter of 30 cm as possible were cut from each harvested tree at 10 cm intervals. When no more large-diameter logs could be cut, the process was next repeated for four-meter medium-diameter logs (minimum top-end diameter of 16 cm), and then for two-meter small-diameter logs (minimum top-end diameter of 6 cm). A third of the large- and medium-diameter logs were assumed to be of A-grade (with the remaining two-thirds of B-grade), while all of the small-diameter logs were treated as C-grade.

Eight prescriptions were applied in the simulations, more specifically, seven of the prescriptions were variants of clearcutting systems with rotation ages between 60 and 120 years, while one prescription represented a non-clearcutting system without any operations (Table 2). All of the thinning

Table 1. Regeneration treatments and the corresponding person-day (person-day·ha^{-1}) requirements.

Stand age (year)	Treatment	Site class	
		1 and 2	3 and 4
1	Land preparation	26.68	26.68
	Planting	20.25	20.25
2	Weeding	8.85	8.85
3	Weeding	8.85	8.85
4	Weeding	8.85	8.85
5	Weeding	8.85	8.85
6	Weeding	8.85	8.85
7	Weeding	–	8.85
12	Pre-commercial thinning	16.24	16.24
15	Pre-commercial thinning	16.24	16.24
20	Pre-commercial thinning	16.24	16.24

Person-days obtained from Toyama et al. (2009)

Table 2. Assumed prescriptions, from Moriya and Tatsuhara (2017).

Prescription No.	Rotation age (year)	Thinning ages (year)
1	60	20, 40
2	70	20, 40
3	80	20, 40, 70
4	90	20, 40, 70
5	100	20, 40, 70
6	110	20, 40, 70
7	120	20, 40, 70
8	No operations	

Table 3. Assumed bucking priority and log prices (currency exchange rate: yen/€, January 2020).

Order of priority	Log length (m)	Top end diameter (cm)	Grade	Customer	Price
1 (Butt log only)	2.0		C	Supplier of fuel wood chips to a biomass-powered station	4,572 yen·t^{-1} (36.576 €·t^{-1})
2	4.0	30+	A	Niigata timber market	18,000 yen·m^{-3} (144 €·t^{-1})
			B	Plywood manufacturer	8,800 yen·m^{-3} (70.4 €·t^{-1})
3	4.0	16 - 28	A	Sawmill and laminated wood manufacturing complex	10,500 yen·m^{-3} (84 €·t^{-1})
			B	Plywood manufacturer	8,600 yen·m^{-3} (68.8 €·t^{-1})
4	2.0	6+	C	Supplier of fuel wood chips to a biomass-powered station	4,572 yen·t^{-1}* (36.576 €·t^{-1})

* Transportation cost is subtracted.

prescriptions were assumed to be low thinning in which the thinning ratio was 40% in terms of stem numbers. The number and volume of logs of each length and top diameter class for stands under each prescription were predicted for both final cutting and thinning harvests.

The revenue from the sale of logs was estimated based on the predicted log production and log prices. Log prices were set at the median of the daily middle prices on the timber market and purchase prices of main customers (Table 3). Logs for fuel wood chips are traded at landings as well as at the power station. These prices were averaged and weighted for the current trading amounts, after considering transportation cost to the power station. The green wood density of sugi was set as 0.81 t·m^{-3}, while the moisture content was 130%. Subsidies for regeneration treatments from Niigata prefectural government were considered when calculating profits.

The cost of managing the stands was estimated by summing the regeneration and logging (thinning and final cutting) costs. The estimations assumed that the logging system for both final cutting and commercial thinning includes felling and limbing with a chain saw, full tree cable yarding, and bucking with a chainsaw at landings on forest roads. In each simulation, the thinning operation was specified as either commercial or thinning-to-waste based on the potential profitability of commercial thinning, i.e. sales revenue minus the cost of commercial thinning plus the subsidy provided by the prefectural government for commercial thinning. The area, initial age, and site class of stands were obtained from the inventory data, while slope and distance to a road were calculated using GIS. Maximum and average yarding distances were also calculated in GIS by measuring the distance from each 10 m × 10 m cell to the road, and then obtaining the maximum and average distances for cells in each stand. The regeneration cost was based on the person-days required for the treatment, which are listed in Toyama et al. (2009) according to stand slope and distance to road (Takahashi et al. 1996). The logging cost was estimated by summing the felling, bucking, yarding, and log transportation costs (Suzuki and Tatsuhara 2016).

The profit over a rotation period for stands under each prescription was calculated based on the corresponding revenue, subsidy and cost estimates. Only prescriptions with a rotation age that did not exceed the initial age and that yielded positive annual profit per ha were considered as possible prescriptions for individual stands. These prescriptions were treated as candidate prescriptions in which they could be assigned to the stand in harvest scheduling.

Predicting Production of Woody Biomass Resources

Harvesting over the planning horizon was simulated according to four scenarios presented by Moriya and Tatsuhara (2014), namely:
- Scenario 1a, maximizing sustainable level of total harvest volume with a rotation age range of 6–10 age class or 60–100 years;
- Scenario 1b, maximizing sustainable level of total harvest volume with a rotation age range of 6–12 age class or 60–120 years;

- Scenario 2a, maximizing sustainable level of medium-diameter log harvest volume with a rotation age range of 6–10 age class or 60–100 years;
- Scenario 2b, maximizing sustainable level of medium-diameter log harvest volume with a rotation age range of 6–12 age class or 60–120 years.

The 'sustainable levels' indicate the middle of the maximum and minimum of periodic harvest volumes over a planning horizon. Scenarios 1a and 1b aimed for sustainable general timber production, whereas Scenarios 2a and 2b aimed for sustainable timber production based on the type of wood sold to the Northern Niigata Wood Processing Cooperative (medium-diameter logs). Scenarios 1a and 2a reflected the current rotation age range, while Scenarios 1b and 2b expanded the current rotation age range by 20 years.

The following harvest scheduling model was formulated using mixed integer programming (Greenberg 1971). Both the length of a planning period and width of an age class were set at 10 years, and the length of the planning horizon was set at 150 years (15 planning periods). The predicted volumes, revenues and profits could only fluctuate by up to ±10% over the 150-year planning horizon, which reflects the threshold for deviation applied by Nelson et al. (1991) and Suzuki et al. (2018) in previous simulations of harvesting and net revenue.

$$\text{Maximize } z = V \tag{2}$$

subject to

$$\sum_{i=1}^{I}\sum_{j=1}^{J} v_{i,j,t}x_{i,j} \geq (1-r)V \quad \forall t \in \{1,\cdots,T\} \tag{3}$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J} v_{i,j,t}x_{i,j} \leq (1+r)V \quad \forall t \in \{1,\cdots,T\} \tag{4}$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J} p_{i,j,t}x_{i,j} \geq (1-r)P \quad \forall t \in \{1,\cdots,T\} \tag{5}$$

$$\sum_{i=1}^{I}\sum_{j=1}^{J} p_{i,j,t}x_{i,j} \leq (1+r)P \quad \forall t \in \{1,\cdots,T\} \tag{6}$$

$$\sum_{i=1}^{I} S0_i \leq \sum_{i=1}^{I}\sum_{j=1}^{J} ST_{i,j}x_{i,j} \tag{7}$$

$$\sum_{j=1}^{J} x_{i,j} = 1 \quad \forall i \tag{8}$$

$$y_{i,j} \geq x_{i,j} \quad \forall i,j \tag{9}$$

$$x_{i,j}, y_{i,j} \in \{0,1\} \quad \forall i,j \tag{10}$$

where z is the objective function, V is the sustainable harvest volume level during the planning horizon, P is the profit level during the planning horizon, I is the number of stands (1,372), J is the number of alternative prescriptions (8), T is the number of planning periods (15), $x_{i,j}$ is 1 when stand i is assigned to prescription j, and otherwise 0, $v_{i,j,t}$ is the harvest

volume in the t-th planning period when stand i is assigned to prescription j, $p_{i,j,t}$ is the profit in the t-th planning period when stand i is assigned to prescription j, $S0_i$ is the stand volume of stand i at the beginning of the planning horizon, $ST_{i,j}$ is the stand volume of stand i at the end of the planning horizon when stand i is assigned to prescription j, r is the threshold for deviation in the periodic harvest volume and periodic profit (0.1), $y_{i,j}$ is 1 when prescription j is a candidate for stand i, and otherwise 0.

Equations (3)–(4) and (5)–(6) include even-flow constraints for harvesting and profit, respectively, while Equation (7) includes a non-declining constraint for growing stock. Equations (8), (9), and (10) include constraints for the assignment of a single prescription, possible prescriptions, and binary integers, respectively. The model was solved using IBM ILOG CPLEX Optimization Studio V12.5.1 (IBM, Armonk, NY, USA) on a personal computer. Approximate solutions were obtained by applying the branch-and-cut method with a relative gap tolerance of 0.5%.

The estimations found that 40% of the A-grade log volume comprised sawing residues. Total woody biomass resources encompassed sawing residues along with the harvested small-diameter logs and butt logs. The sustainability of woody biomass supply was tested by calculating fluctuations in small-diameter log volumes, butt log volumes, sawing residues, and total woody biomass resources as follows:

$$f = (max - (max + min)/2) / ((max + min)/2)$$
$$= (max - min) / (max + min) \qquad (11)$$

where f is fluctuation, max is the maximum value over the planning horizon, and min is the minimum value over the planning horizon.

RESULTS

Harvest Levels

Scenarios 1a and 1b produced total harvest levels of 245.8 thousand $m^3 \cdot decade^{-1}$ and 467.4 thousand $m^3 \cdot decade^{-1}$, respectively, whereas Scenarios 2a and 2b yielded medium-diameter log harvest levels of 32.3 thousand $m^3 \cdot decade^{-1}$ and 71.2 thousand $m^3 \cdot decade^{-1}$, respectively. Both the levels of total harvest (Scenarios 1a and 1b) and the levels of medium-diameter log harvest (Scenarios 2a and 2b) volume increased approximately two-fold when the maximum rotation age grew by 20 years.
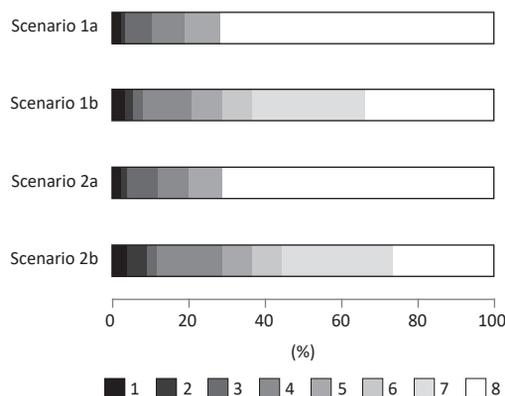
Figure 3. Proportions of the total area of stands assigned to each prescription.

The predicted harvest volumes of total logs and woody biomass resources also increased about two-fold when the maximum rotation age grew by 20 years. However, the growth in volume differed among the various classes of logs. For example, the volume of C-grade logs, especially small-diameter logs, increased less than the volume of total logs when the rotation age was extended by 20 years (Table 4).

Proportions of Stands Assigned to the Prescriptions

The two scenarios that included the current range of rotation ages (Scenarios 1a and 2a) showed clear similarities in the proportions of stands that were assigned to various prescriptions (Figure 3). In these scenarios, 70% of the total stands were assigned to Prescription 8 (no operations), while less than 4% of the total stands were assigned to Prescriptions 1 and 2 (rotation ages of 60 and 70, respectively). The other two scenarios – both of which included a 20-year extension of the range of rotation ages (Scenarios 1b and 2b) - also showed similarities in terms of the proportion of stands that were assigned to specific prescriptions. In these scenarios, 37% of the total stands were assigned to prescriptions with extended rotation ages (i.e., Prescriptions 6 and 7), while far less stands (less than half the proportion observed in the first two scenarios) were assigned to Prescription 8.

Table 4. Comparison of average annual total log harvest and woody biomass resources between scenarios including different ranges of rotation ages.

	Harvest of total logs ($m^3 \cdot year^{-1}$)	Harvest of small-diameter logs ($t \cdot year^{-1}$)	Harvest of butt logs ($t \cdot year^{-1}$)	Sawing residues ($t \cdot year^{-1}$)
Scenario 1a	22,989	1,464	2,686	1,930
Scenario 1b	47,783	2,736	5,463	4,067
Ratio	2.08	1.87	2.03	2.11
Scenario 2a	22,921	1,514	2,751	1,907
Scenario 2b	53,552	3,187	6,229	4,528
Ratio	2.34	2.10	2.26	2.37

Periodic Harvest and Production of Woody Biomass Resources

There were little difference in the various studied harvest volumes (total volume, butt logs, and small-diameter logs) between the tested scenarios that applied the current range of rotation ages (Scenarios 1a and 2a) (Figure 4). In contrast, large differences arose when the tested scenarios included an extended range of rotation lengths (Scenarios 1b and 2b). Scenario 2b showed greater periodic harvested volumes (total volume, butt logs, and small-diameter logs) than Scenario 2a in all the time periods except planning period 1 (Figure 5). The proportion of woody biomass resources in
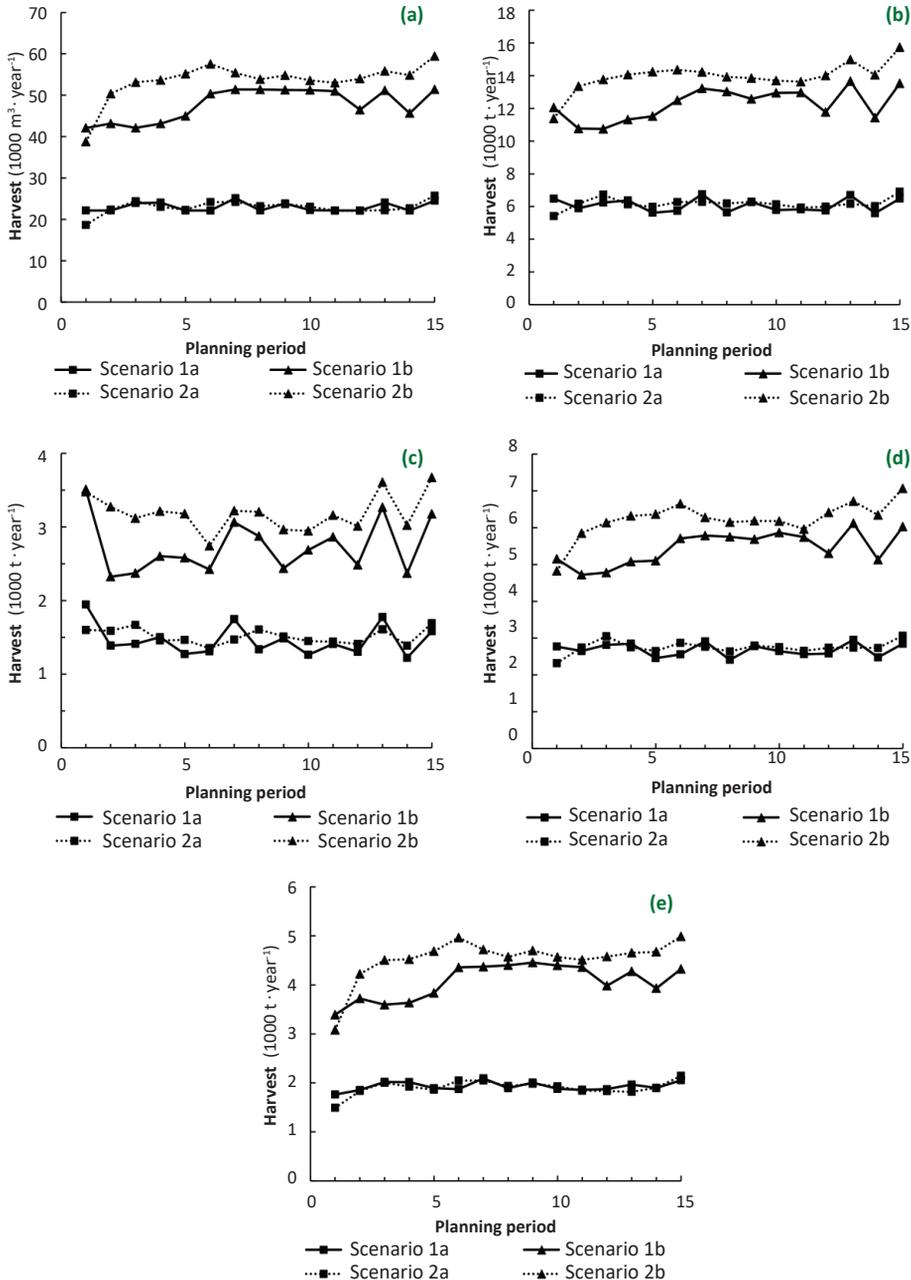
Figure 4. Changes in the periodic production of **(a)** total logs, **(b)** woody biomass resources, **(c)** small-diameter logs, **(d)** butt logs, and **(e)** sawing residues.

Table 5. Fluctuation of woody biomass resources over the planning horizon.

Scenario	Harvest of small-diameter logs	Harvest of butt logs	Harvest of C-grade logs	Sawing residues	Total woody biomass resources
1a	0.228	0.101	0.122	0.085	0.094
1b	0.203	0.129	0.143	0.136	0.119
2a	0.112	0.138	0.096	0.180	0.121
2b	0.145	0.189	0.128	0.237	0.160

Table 6. Fluctuation of woody biomass resources over the planning horizon (with planning period 1 eliminated).

Scenario	Harvest of small-diameter logs	Harvest of butt logs	Harvest of C-grade logs	Sawing residues	Total woody biomass resources
1a	0.184	0.101	0.122	0.060	0.094
1b	0.169	0.129	0.143	0.107	0.119
2a	0.112	0.075	0.075	0.082	0.075
2b	0.145	0.094	0.082	0.083	0.082

the total harvest volume was at its maximum during planning period 1 (35%–36%), after which it decreased to 30%–34%. The proportion of small-diameter logs in woody biomass resources varied between 19%–26% in the periods other than planning period 1, which showed a maximum proportion of 29%–30%. The proportion of butt logs in woody biomass resources varied between 42%–46%. The proportion of sawing residues in woody biomass resources was lowest in planning period 1 (27%–28%), while this proportion ranged from 29%–36% during the other periods.

Fluctuations in total woody biomass resources were mostly less than 20%, with the exception of the harvested volume of small-diameter logs in Scenarios 1a and 1b (Table 5). However, if planning period 1 – which showed large fluctuation – is eliminated, all of the other observed fluctuations remained under 20%, with most under 15% (Table 6). Scenarios 1a and 2a showed less fluctuation than Scenarios 1b and 2b.

DISCUSSION

This paper showed the harvest of woody biomass resources over 150 years in a snowy mountainous region in Japan under the constraint of sustainable timber production. The developed model, which included a deviation threshold of 10%, demonstrated that the harvested timber volumes fluctuated by 15% over the simulation period, which can be considered to represent a sustainable supply. Sacchelli et al. (2013) described forest energy supply chains using a model that included several parameters, and classified the biomass potential of the studied supply chains as either ecological, technical, economic, or sustainable. In this study, the maximal supply potential of woody biomass was estimated in the context of sustainability.

Aruga et al. (2014) only considered a rotation age of 60 years, which was used to calculate revenues and costs and then describe profitable stands. Kinoshita et al. (2010) earlier highlighted that extending the rotation age from

40 to 80 years could increase the biomass chip supply in Japan because it increases the profitability of plantation management. This study included a wide range of rotation ages, and certain scenarios extended the current maximal rotation age by a further 20 years. Expanding the range of rotation ages by 20 years dramatically increased the harvested volumes of various woody biomass resources, as well as the proportion of stands that would be harvested, and reduced the proportion of stands classified as Prescription 8. Stands were assigned to Prescription 8 when the profit over a rotation period was negative, or when the stand could not be harvested during the rotation period because of a constraint to stable harvests. The changes from a 20-year expansion can be explained by two reasons (Moriya and Tatsuhara 2014). First, a longer rotation age would lead to an increase in the harvested volume of large logs, which have an advantage in price when compared to smaller-diameter logs. Hence, an expansion of the rotation age would improve the profitability of plantations. The second reason – which is based on the unimodal age class distribution of trees (Figure 1) – is that some stands could not be harvested in order to stay within the 10% threshold for deviation between periods. An expansion in rotation age would decrease the proportion of such stands. The two reasons showed that revenues from harvest increased by expanding the range of rotation ages and ecological potential of woody biomass resources in the study site were much larger that the estimates in this study. The second reason showed that economical potential of woody biomass resources in the study site were also larger that the estimates in this study.

Extending the range of rotation ages also increased fluctuation in all of the tested types of woody biomass resources. The total harvested volume during each planning period was constrained to a 10% threshold for deviation by the harvest scheduling model. The variation observed for the harvested volumes of each grade and size of logs was higher than what was observed for the total harvested volume. Furthermore, changes in the objective functions did not noticeably affect the fluctuation of woody biomass
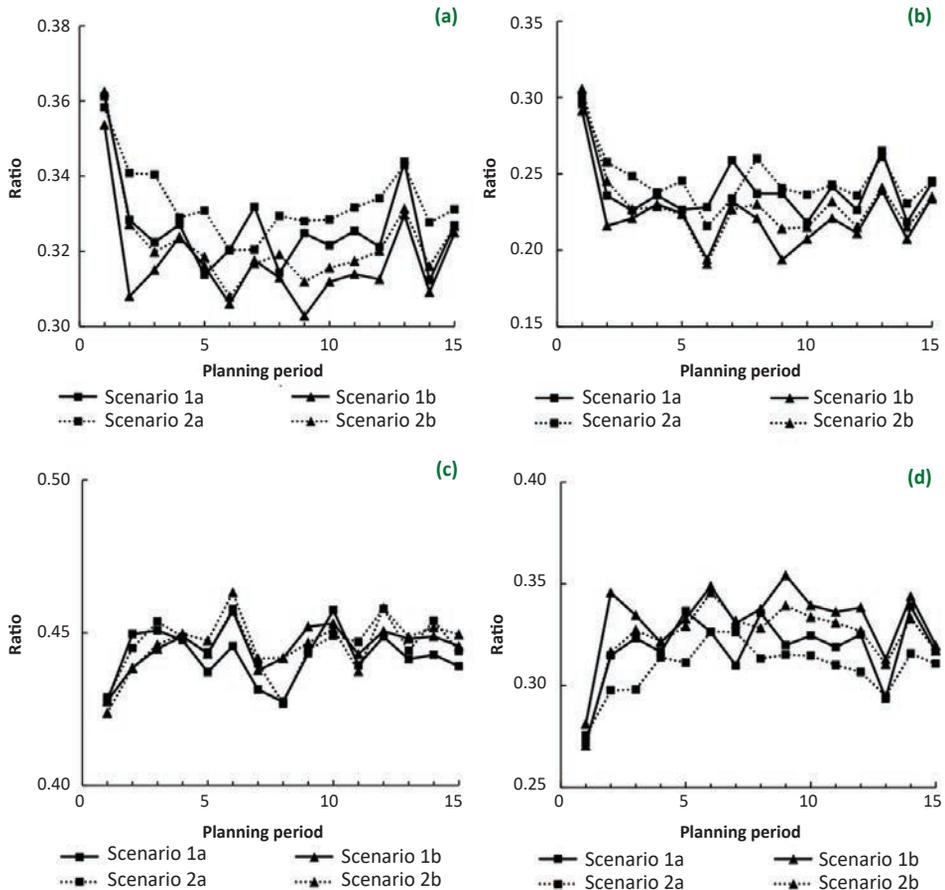
Figure 5. Changes in ratios of **(a)** periodic production of total woody biomass resources to periodic harvest of total logs, along with changes in the ratios of **(b)** periodic production of small-diameter logs, **(c)** periodic production of butt logs, **(d)** periodic production of sawing residues to periodic production of total woody biomass resources.

resources. However, Scenario 2b produced more woody biomass resources than Scenario 1b, with the exception of planning period 1, because of the increased harvesting of wood. This difference could be explained by an increase in the area of profitable stands in the current age class 6 (which represents the peak of the current age class distribution) after planning period 2. This dramatically increased the area of harvestable stands, and also explained the instability in production of woody biomass resources noticed in planning period 1.

The generation of electricity by woody biomass-powered stations in the snowy regions of Japan has led to changes in the bucking strategy and increased the merchantable volume ratio. In terms of volume, butt logs comprise two-thirds of C-grade logs. These logs were previously not utilized whenever the butt log was bent due to a tree being bent by snowfall, but can now be used to produce fuel wood chips. Even though the price of woody

biomass fuel chips is low compared to the price of regular roundwood, and only provides small additional income, this fraction nevertheless plays an important role in making sugi plantation management profitable (Suzuki and Tatsuhara (2016). Butt logs and small-diameter logs are cut to produce fuel chips, while sawing residues can either be used to produce fuel chips for woody biomass-powered generation facilities and boilers that dry lumber inside large sawmills, or material for mushroom cultivation beds. The estimated potential sustainable supply of biomass indicated that the C-grade logs and part of the sawing residues would be used to generate electricity at biomass-powered stations.

Trømborg and Solberg (2010) showed that an increase in energy price will reduce the production of particleboard and pulp in Norway. The change in the bucking strategy at the study site (i.e., bent butt logs are cut to produce fuel chips) could be expected to reduce the supply of logs for sawn wood, laminated wood, and plywood. Regarding

sawing residues, fuel chips for woody biomass-powered stations compete with material for mushroom cultivation beds. Thus, this competition should be modelled based on real-world prices, or, as an alternative, future predictions of potential biomass supply should establish which proportion of sawing residues will be used to produce fuel chips for woody biomass-powered generation facilities.

CONCLUSIONS

The presented model estimated the potential supply of woody biomass from plantation forests in a snowy district in Japan under a constraint of maximum sustainable harvest. The simulation showed that even though the age class distribution of trees was unimodal, the sustainable harvest of timber led to a sustainable supply of woody biomass over various rotation ages. Furthermore, extending the range of rotation ages by 20 years dramatically increased the potential supply of woody biomass resources. The methodology presented in this paper could be useful to individuals or organizations that are planning new woody biomass-powered facilities for energy generation and/or purchase biomass fuel chips. The presented research is also relevant to regional forest resource managers who mediate arranged transactions of logs between log producers and biomass purchasers. The potential supply estimated in this paper can be considered to represent the actual maximum supply for the study site because the value is based on maximum sustainable harvesting and assumes that all of the

sawing residues will be used to produce fuel chips, which may not hold in the actual market for sawing residues. Most of the plantation forests in the study site were covered by 'forest management plans'. Other estimates of the potential supply of fuel chips for biomass-powered generation facilities should take into account which stands are included in 'forest management plans', as this is a prerequisite for obtaining higher tariffs from the current FIT scheme.

Conflicts of Interest

The author declares no conflict of interest.

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ORIGINAL SCIENTIFIC PAPER

Predicting Diameter at Breast Height from Stump Measurements of Removed Trees to Estimate Cuttings, Illegal Loggings and Natural Disturbances

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ABSTRACT

Predicting diameter at breast height (DBH) of trees from stump information may be necessary to reconstruct silvicultural practices, to assess harvested timber and wood, or to estimate forest products' losses caused by illegal cuttings or natural disasters (disturbances). A model to predict DBH of felled trees was developed by the first Italian National Forest Inventory in 1985 (IFNI85). The model distinguished between the two broad groups of conifers and broadleaves and used stump diameter as the sole quantitative variable. Using an original dataset containing data from about 1200 trees of sixteen species recorded throughout Italy, we assessed the performance of that model. To improve the prediction of the DBH of removed trees over large areas and for multiple species, we developed new models using the same dataset. Performance of the new models was tested through indices computed on cross-validated data obtained through the leave-one-out method. A new model that performs better than the old one was finally selected. Compared to the old NFI model, the selected model improved DBH prediction for fourteen species up to 31.28%. This study proved that species specification and stump height are variables needed to improve the models' performance and suggested that data collection should be continued to get enhanced models, accurate for different ecological and stand conditions.

Keywords: tree diameter prediction; DBH; stump diameter; stump height; tree species

INTRODUCTION

Diameter at breast height (DBH) is one of the most important tree variables measured in forestry (Corral-Rivas et al. 2007, Bueno-López and Bevilacqua 2013). Its frequency distribution is used to describe stand structure (Pond and Froese 2015), but above all, it allows the computing of basal area, a variable highly correlated with tree volume, biomass and increment; it is also an attribute easy to measure with sufficient accuracy. In some cases, DBH of removed trees may also be of interest. Estimating DBH of trees from stump dimension may help to reconstruct silvicultural practices and to assess harvested timber and wood, when pre-operation data are not available. Pre-operation data are surely not available in case of illegal cuttings, and estimating DBH is the only way to assess the loss, through a two-step procedure

(Corral-Rivas et al. 2007, Özçelík et al. 2010), unless volume functions based on stump information are available. Another application of diameter prediction occurs when a harvest crosses ownership boundary (Chhetri et al. 1996, Pond and Froese 2014). The need to estimate DBH may also arise after natural disasters. In case of forest fires, stumps might be the only remaining part and consequently losses can be estimated based on their dimension. Other disturbances may lead to a variety of conditions that depend on type and intensity. For example, depending on the species, local windstorms might uproot trees still valuable for timber supply, which hold economic value and are for this reason still worth to be measured individually. On the contrary, hurricanes over wide regions may result in a mixture of a huge number of trees uprooted and broken, of different sizes and values that are too difficult and expensive to be measured individually,

also considering that exploitation might be urgent to free the roads and the watercourses as well as to avoid pests and wood deterioration. In such cases, estimates of losses are conducted with less accuracy and using stump dimension to DBH relationships after the removal is a possible method. The example of an event that did not allow the measuring of all downed trees is the recent Vaia storm, which occurred on 29th October 2018 and caused considerable damages in the southern side of the central and eastern Alps (Italy, Austria, Switzerland, Slovenia). In the North-East of Italy (Trentino Alto-Adige, Veneto, Friuli Venezia Giulia) it downed about eight million cubic meters of wood and left hundreds of kilometres of forest roads to be restored. In forest inventory surveys, stump measurements are carried out because they are connected to net increment calculation (Gschwantner et al. 2016).

The Italian National Forest Inventory (NFI) measures stump diameter of the trees cut in the last 12 months to estimate the current annual removals (Gasparini et al. 2010, Gasparini and Di Cosmo 2016). Whether the felling was carried out in the 12 months before the survey or earlier is generally assessed visually, based on stump conditions, and the volume and biomass of each felled tree are estimated through a two-step procedure. In the first two Italian NFIs the DBH of felled trees was predicted using a model developed by the first Italian NFI in 1985 (MAF-ISAFA 1988). The model is a linear equation, using stump diameter (SD) as the only predictive quantitative variable and with distinct regression parameters for the conifers and the broadleaves. To our knowledge, it is the only model developed in Italy for predicting DBH from stump information over wide areas, but, unfortunately, regression metadata was only partially reported. The model was built based on about 650 standing sample trees on which two right angle diameters were measured at stump height (SH) and at breast height. MAF-ISAFA (1988) did not report which species were measured, the regression goodness of fit and the reason why equations were developed separately for the two groups of species, e.g. if after an in-depth analysis, based on expert assessment or because of the limited number of observations by species. Concern about suitable use in any condition arises especially from the fact that sample trees were selected only in high forests, when stumps were found in the NFI plots; the sample might be unbalanced towards high trees and, given the longer rotation, also towards large diameters, potentially resulting in biased estimates, particularly in coppice stands.

The aim of this work was to assess the performance of the model developed by the first Italian NFI using a new dataset containing data from about 1200 trees collected throughout Italy and possibly provide a new, enhanced model for predicting DBH from stump measurements of removed trees. Especially, the new dataset contained information about the species and stump height, two variables not available or not used by the first Italian NFI.

MATERIALS AND METHODS

Data

The data used for this study came from the database created by Tabacchi et al. (2011) to construct tree volume and above-ground biomass models. Data collection started in 2001 thanks to a project launched to support the estimation procedure within the second Italian National Forest Inventory (INFC 2005). The project aimed to construct national models, applicable country-wide, and covering a relevant number of forest tree species. Based on 1289 sample trees measured over large areas of the country, prediction equations were finally made available for 26 species or groups of species (Tabacchi et al. 2011). Collection of sample trees has slowed down in recent years due to financial constrains; nevertheless, the overall goal of measuring 2800 trees in total has not been shelved and since 2011 the database has been enriched with about 150 additional sample trees. The data recorded include measurements of DBH, SD and SH. Details about the selection and number of sample trees, as well as about the measurements protocol, may be found in Tabacchi et al. (2011). Relevant to this article, the dataset includes trees from 11 out of 21 administrative regions of Italy and 58 species. A subset of the data came from the existing forest harvesting operations and consequently recorded on trees subjectively selected following the criterion of each operation. Other subsets came from the felling of trees undertaken for development operations (e.g. road construction, natural disasters) with no subjective selection due to silvicultural criteria. Finally, felling was commissioned specifically for the aim of the project, to fill the gaps in sampling for some species or diameter and/or height classes.

For this article, we decided to consider the species with at least forty sample trees. The available data are still limited and do not allow the analysis of the possible benefits from stratifying the sample by variables other than the species (e.g. management system, high trees vs coppice shoots). In this respect, while the analysis of the first NFI model's performance conducted in this study may be considered deep and complete, other models developed and tested should be better considered as preliminary results within a long-term research. Table 1 shows the number of sample trees used in this study, by species and species group, their DBH, SD and SH range and variation.

The DBH of each tree was measured before felling, with the first diameter measured from the uphill side in case of sloping terrain. Once the tree was felled, the diameter of the cut section and the stump height were measured. Diameters were measured with reference to two right-angled directions and expressed in centimetres with a millimetre approximation, while SH was measured in centimetres.

Analysis

Firstly, the dataset described in Table 1 was used to assess the performance of the model developed by the first Italian NFI (MAF-ISAFA 1988) (here named Model 0) and to re-calibrate it with the available data to obtain Model 1. Secondly, the data in Table 1 were used to study the relationship between DBH and stump dimensions through OLS regression analysis. A set of potential predictors was tested: SD, SH, 130/SH, 130-SH, and their transformed values (squares and/or natural logarithm). Both the species and the species group (conifers or broadleaves) were tested as dummy variables. During the analysis, results showed the existence of heteroscedasticity that could not be solved through feasible weighted least squares estimation. Therefore, we followed two alternative

Table 1. Number of sample trees (n.obs), diameter at breast height (DBH), stump diameter (SD) and stump height (SH) used in the study. For each quantitative variable, the table shows the minimum (Min), mean and maximum (Max) value recorded by species, as well as the standard deviation (StDev).

No.	Species Name	n.obs	DBH (cm) Min	Mean	Max	StDev	SD (cm) Min	Mean	Max	StDev	SH (cm) Min	Mean	Max	StDev
1	*Abies alba* Mill.	70	6	30.5	68	16.61	8	39.4	86	22.22	2	13.5	75	11.79
2	*Cupressus* spp.	44	5	17.7	38	8.45	8	22.7	46	9.60	1	10.1	29	6.31
3	*Larix decidua* Mill.	62	6	31.4	90	19.94	11	41.3	98	21.89	2	10.6	30	5.84
4	*Picea abies* K.	157	8	33.7	82	16.77	9	43.1	95	20.49	1	16.4	86	12.90
5	*Pinus cembra* L.	49	8	28.3	64	15.15	10	37.1	78	19.57	2	10.9	79	12.13
6	*Pinus halepensis* Mill.	43	5	22.8	56	12.03	8	28.7	59	13.21	1	7.6	15	3.67
7	*Pinus nigra* Arn.	70	9	27.6	64	12.34	11	33.4	74	13.58	1	9.5	22	4.66
8	*Pinus pinea* L.	43	13	31.3	61	10.89	18	39.9	73	11.59	4	10.0	33	4.57
9	*Pinus sylvestris* L.	53	8	24.4	59	12.51	11	30.0	64	13.87	3	9.6	28	6.12
	Total conifers	591	5	29.9	90	15.61	8	36.8	98	18.87	1	12.0	86	9.79
10	*Castanea sativa* Mill.	98	7	27.5	57	12.46	11	42.3	98	20.97	1	13.1	57	8.46
11	*Fagus sylvatica* L.	110	7	26.6	61	11.60	11	40.3	108	16.92	1	14.7	70	10.98
12	*Ostrya carpinifolia* Scop	64	6	17.6	32	6.60	7	26.9	51	10.27	1	10.4	64	8.26
13	*Quercus cerris* L.	68	8	28.5	50	10.38	15	42.6	72	15.38	3	11.5	43	7.94
14	*Quercus ilex* L.	83	5	20.7	55	12.02	7	29.6	84	18.65	1	8.3	22	3.96
15	*Quercus pubescens* Willd.	123	6	24.0	56	9.91	8	36.2	74	15.14	2	7.8	30	4.45
16	*Robinia pseudoacacia* L.	50	8	22.9	40	8.79	10	31.1	55	12.37	5	9.5	21	3.68
	Total broadleaves	596	5	24.3	61	11.16	7	36.3	108	17.35	1	10.9	70	7.88

approaches: i) evaluating the ongoing results also based on the heteroscedastic-robust standard errors (Models 2 and 3), and ii) running a logarithmic regression assuming the natural logarithm of DBH as the variable to regress (Models 4 and 5). In all cases, the quantitative explanatory variables were selected through a backward stepwise approach with tolerance ≤0.2 (Variance Inflation Factor ≤5). The best model within each approach was selected based on the following model parameters: adjusted coefficient of determination (R^2_{adj}), root mean squared error (RMSEmod), Akaike's information criterion (AIC), and Bayesian information criterion (BIC) computed on the fitting data.

The performance of Model 0 to predict the DBH of the trees in the dataset and that of the five newly developed models (Table 2) was compared by assessing their predictive accuracy through the following indices of fit: the mean error (ME), the mean absolute error (MAE), root mean squared error (RMSE), the mean percent deviation (MPD) and the mean percent standard error (MPSE) (Zeng, 2015). The indices were calculated as follows:

$$ME = \frac{1}{n}\sum_{i=1}^{n} \hat{e}_i \qquad (1)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n} |\hat{e}_i| \qquad (2)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{e}_i)^2}{n}} \qquad (3)$$

$$MPD = 100\left(\sum_{i=1}^{n} \hat{y}_{(i)} - \sum_{i=1}^{n} \hat{y}_i\right) \Big/ \sum_{i=1}^{n} \hat{y}_i \qquad (4)$$

$$MPSE = \sum_{i=1}^{n} \left(100 |\hat{e}_i| \Big/ \hat{y}_i\right) \Big/ n \qquad (5)$$

where $\hat{e}_i = y_i - \hat{y}_i$; y_i are observed values, $\hat{y}_i$ are values estimated by the model.

For models 1 to 5, the indices were calculated using the leave-one-out cross-validation method. Performance assessment included the evaluation of the relationship between the observed values plotted vs the predicted ones. Specifically, we tested whether the intercept of the regression line equalled zero (i.e. the line passed through the axes origin) and whether its slope was 45° (the 1:1 slope condition), that is whether the predictions were unbiased.

In order to assess the models' performance on real scale values (centimetres) the Log-normal DBHs predicted by Models 3 and 4 in Table 2 were back-transformed following Sprugel (1983) before the cross validation.

The analyses were carried out using R version 3.5.3 (R Core Team 2019).

RESULTS

Table 2 shows the models developed, their residual standard error, Adjusted R-squared, the Akaike and Bayesian information criteria.

All the models provided a good level of fit as the adjusted *R*-squared value ranged between 0.93 and 0.95. The models that account for the species showed lower RSE compared to those that used the species group as a dummy variable. Table 3 shows the calculated performance indices of all the models tested and reports about the existence of bias when applying the model to independent data. Model 0 showed bias for both conifers and broadleaves. Model 1 showed that the re-calibration of Model 0 with the available data improved its performance consistently and the predictions for the two groups are unbiased; however, bias is still present in eight species. In general, model performance was improved by considering the species among the predictors. This is shown comparing the pairs of models with the same structure, that is, Model 2 vs Model 3 and Model 4 vs Model 5. For both pairs, RMSE was lower in eleven species and MPSE in nine species, out of sixteen. The importance of accounting for the species is also shown by the gains in the ME, the MAE and the MPD.

In Figure 1, ME and MPD for Models 2 and 3 are compared.

Based on the data in Table 3, similar patterns exist between Models 4 and 5. This shows that adopting the species as dummy variable instead of their two broad groups allowed the obtaining of more accurate estimates. MPD computed for Model 2 never exceeded 0.3 in absolute terms, while for Model 3 it exceeded 2% in nine species over sixteen. Based on the MPD, estimates of Model 3 were particularly poor for *Robinia pseudoacacia* (-7.8%), *Ostrya carpinifolia* (+6%), *Pinus nigra* (-5.4%), *Quercus ilex* (-5.3%), *Pinus sylvestris* (-4.1%). MPD of Model 2 is lower for all the species except *Pinus halepensis*, compared to the MPD of Model 3. Accounting for the species also reduced the presence of bias. Estimates of Model 5 are biased for ten species, while those obtained from Model 4 are biased only for three species. However, Model 2 is the only one that allowed unbiased estimates for all the species; its correspondent Model 3 was biased in six cases. Figure 2 shows the observed values plotted versus the estimated ones by Model 2, for four selected species, two conifers (*Abies alba* and *Larix decidua*) and two broadleaves, one deciduous (*Fagus sylvatica*) and one evergreen (*Quercus ilex*).

Table 2. The models developed and tested in the study. Variables: diameter at breast height (DBH), stump diameter (SD), stump height (SH); dummy variables: species (SP) or species group (SPgroup).

| Model	Formula	RSE	R^2_{adj}	AIC	BIC
1	$DBH=(a_0+ b_1 \times SD) \times SPgroup + u_j$	3.723	0.93	6494.9	6520.3
2	$DBH=(a_0+ b_1 \times SD + b_2 \times SD^2 + b_3 \times SH) \times SPgroup + u_j$	3.306	0.94	6271.5	6601.7
3	$DBH=(a_0+ b_1 \times SD + b_2 \times SD^2 + b_3 \times SH) \times SPgroup + u_j$	3.563	0.93	6394.7	6440.4
4	$\ln(DBH)=a_0+b_1 \times \ln(SD)+b_2 \times (130-SH) \times SP+u_j$	0.122	0.95	-1586.2	-1337.3
5	$\ln(DBH)=a_0+b_1 \times \ln(SD)+b_2 \times (130-SH) \times SP+u_j$	0.129	0.94	-1480.6	-1445.0
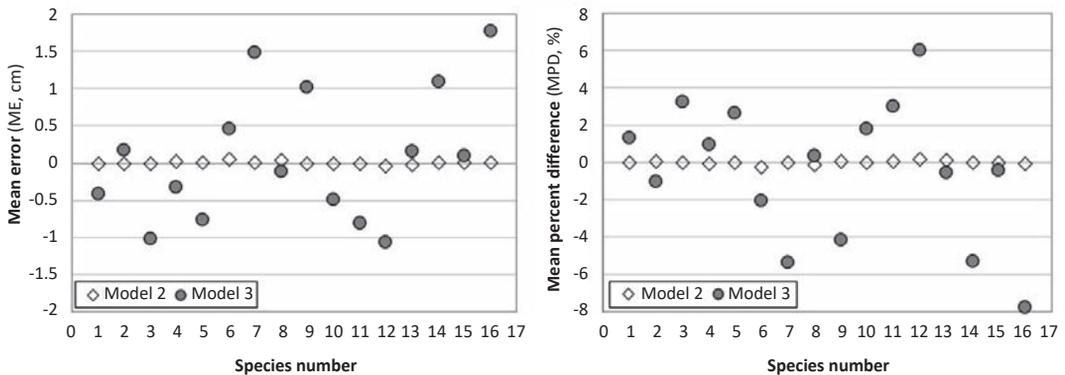
Figure 1. Mean error (ME) and mean percent difference (MPD) computed on cross validated data for Model 2 and Model 3. The species number is shown in Table 1.

Table 3. Indices of fit computed on the complete dataset for Model 0 and on cross-validated data for the Models 1 to 5, by species. Crosses in the bias columns show cases when the line regressing the observed values plotted vs the predicted ones does not source form the axes origin, or its slope differs from 45°.

Species (N., name) \ Model	Mean error (ME, cm)						Mean absolute error (MAE, cm)						Root mean square error (RMSE, cm)					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
1 Abies alba Mill.	-1.455	-0.418	-0.001	-0.407	0.190	-0.690	3.434	2.856	2.762	2.825	2.741	2.985	5.415	4.465	4.035	4.368	4.134	4.626
2 Cupressus spp.	0.274	0.158	-0.007	0.181	0.083	0.228	1.455	1.596	1.533	1.503	1.612	1.591	1.905	1.955	1.931	1.883	2.050	1.949
3 Larix decidua Mill.	-2.331	-1.145	-0.006	-1.020	-0.145	-1.207	3.806	3.551	2.791	3.652	2.759	3.424	4.670	4.629	3.729	4.831	3.772	4.365
4 Picea abies K	-1.541	-0.239	0.028	-0.317	0.074	-0.623	3.147	2.802	2.889	2.842	2.856	2.909	4.431	3.874	3.993	3.947	3.942	4.078
5 Pinus cembra L	-1.685	-0.803	0.004	-0.755	0.098	-0.846	3.184	2.798	2.543	2.726	2.606	2.765	4.847	4.086	3.910	3.981	3.911	4.095
6 Pinus halepensis Mill	0.083	0.389	0.057	0.463	0.019	0.535	1.835	2.039	1.973	2.001	1.957	2.027	2.491	2.758	2.756	2.759	2.683	2.769
7 Pinus nigra Arn.	0.850	1.482	0.003	1.483	0.195	1.603	1.991	2.426	1.544	2.365	1.646	2.422	2.342	2.896	1.987	2.835	2.172	2.901
8 Pinus pinea L.	-1.162	-0.078	0.042	-0.115	0.093	0.032	2.424	2.342	2.151	2.342	2.052	2.256	3.038	3.031	2.758	3.019	2.664	2.914
9 Pinus sylvestris L.	0.596	0.995	-0.012	1.011	-0.025	1.083	1.938	2.036	1.874	2.051	1.966	2.032	2.552	2.891	2.536	2.882	2.616	2.835
10 Castanea sativa Mill.	-4.001	-0.455	-0.001	-0.490	0.159	-0.651	5.093	3.045	2.493	2.495	2.552	2.610	7.258	4.184	3.284	3.311	3.522	3.631
11 Fagus sylvatica L.	-3.352	-0.151	-0.014	-0.800	-0.052	-0.668	4.695	3.858	3.558	3.454	3.475	3.451	6.602	5.040	4.746	4.591	4.764	4.622
12 Ostrya carpinifolia Scop	-1.870	-1.000	-0.030	-1.063	0.013	-0.821	2.477	1.823	1.820	2.002	1.808	1.779	3.409	2.445	2.352	2.838	2.334	2.451
13 Quercus cerris L.	-3.214	0.389	-0.026	0.152	0.147	0.217	4.203	3.012	2.814	2.736	2.972	2.799	5.577	3.951	3.557	3.465	3.912	3.545
14 Quercus ilex L.	-0.935	0.416	0.006	1.096	0.095	0.795	3.132	2.523	2.388	2.501	2.448	2.441	4.689	3.525	3.353	3.388	3.619	3.467
15 Quercus pubescens Willd.	-2.694	-0.208	0.007	0.098	-0.031	0.067	3.622	2.365	2.392	2.361	2.384	2.333	4.835	3.242	3.358	3.318	3.358	3.271
16 Robinia pseudoacacia L.	0.163	1.768	0.015	1.776	0.068	1.954	2.100	2.120	1.914	2.171	1.925	2.178	2.582	3.074	2.475	2.976	2.462	3.113
All conifers	-0.870	0.000	0.013	0.003	0.070	-0.099	2.734	2.595	2.364	2.591	2.372	2.614	3.944	3.637	3.364	3.652	3.382	-0.099
All broadleaves	-2.517	-0.002	-0.005	-0.003	0.047	-0.005	3.809	2.769	2.570	2.593	2.588	2.581	5.473	3.832	3.518	3.541	3.636	-0.005
All dataset	-1.697	-0.001	0.004	0.000	0.059	-0.052	3.274	2.682	2.467	2.592	2.481	2.597	4.773	3.736	3.442	3.597	3.512	-0.052

Species (N., name) \ Model	Mean percent difference (MPD, %)						Mean percent standard error (MPSE, %)						Bias					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
1 Abies alba Mill.	4.765	1.369	0.005	1.332	-0.618	2.259	11.594	9.491	9.418	9.850	9.182	9.684	x	x	-	x	x	x
2 Cupressus spp.	-1.553	-0.893	0.040	-1.024	-0.469	-1.293	9.893	10.310	9.822	9.714	10.467	10.315	-	-	-	-	-	-
3 Larix decidua Mill.	7.430	3.649	0.018	3.251	0.465	3.849	12.437	12.117	10.736	12.250	10.139	11.862	x	x	-	x	-	x
4 Picea abies K	4.573	0.708	-0.083	0.940	-0.220	1.848	9.268	8.319	8.773	8.462	8.715	8.426	x	-	-	-	-	x
5 Pinus cembra L	5.949	2.833	-0.013	2.667	-0.346	2.986	10.842	9.480	9.673	9.452	9.687	9.217	x	-	-	-	-	x
6 Pinus halepensis Mill	-0.364	-1.709	-0.252	-2.032	-0.082	-2.350	8.563	9.102	9.135	8.830	8.946	9.084	-	x	-	-	-	x
7 Pinus nigra Arn.	-3.079	-5.369	-0.011	-5.374	-0.701	-5.809	8.457	9.426	6.907	9.236	6.827	9.447	-	x	-	x	x	x
8 Pinus pinea L.	3.711	0.249	-0.134	0.369	-0.297	-0.102	7.939	7.711	7.657	7.694	7.485	7.468	-	x	-	x	-	x
9 Pinus sylvestris L.	-2.441	-4.074	0.048	-4.140	0.101	-4.436	9.614	8.689	7.633	8.980	8.288	8.709	-	x	-	x	-	x
10 Castanea sativa Mill.	14.555	1.655	0.004	1.781	-0.574	2.368	14.207	10.950	9.498	8.945	9.300	9.247	x	x	-	-	-	x
11 Fagus sylvatica L.	12.618	0.570	0.054	3.011	0.197	2.516	14.543	14.039	14.001	12.525	13.845	12.512	x	-	-	-	-	-
12 Ostrya carpinifolia Scop	10.620	5.676	0.170	6.037	-0.071	4.661	12.504	10.303	10.794	11.066	11.385	9.965	x	-	-	-	-	-
13 Quercus cerris L.	11.283	-1.367	0.091	-0.535	-0.514	-0.762	12.702	10.686	9.910	9.610	10.518	9.922	x	-	-	-	-	-
14 Quercus ilex L.	4.518	-2.012	-0.028	-5.297	-0.459	-3.842	14.643	12.485	11.567	13.975	11.253	11.769	x	-	-	-	x	-
15 Quercus pubescens Willd.	11.223	0.868	-0.030	-0.408	0.130	-0.279	13.458	10.310	10.414	10.442	10.749	10.184	x	-	-	-	-	-
16 Robinia pseudoacacia L.	-0.711	-7.719	-0.066	-7.758	-0.295	-8.531	9.855	9.732	8.463	10.960	8.253	10.400	x	x	-	-	-	x
All conifers	3.008	0.001	-0.045	-0.011	-0.243	0.341	9.845	9.405	8.8615	9.385	8.860	9.357	x	-	-	-	x	x
All broadleaves	10.451	0.110	0.101	0.120	-0.284	0.128	13.130	11.215	10.664	11.075	10.757	10.571	x	-	-	-	x	-
All dataset	6.377	0.005	-0.014	0.000	-0.220	0.195	11.655	10.343	9.872	10.202	9.895	9.959	x	-	-	-	-	x
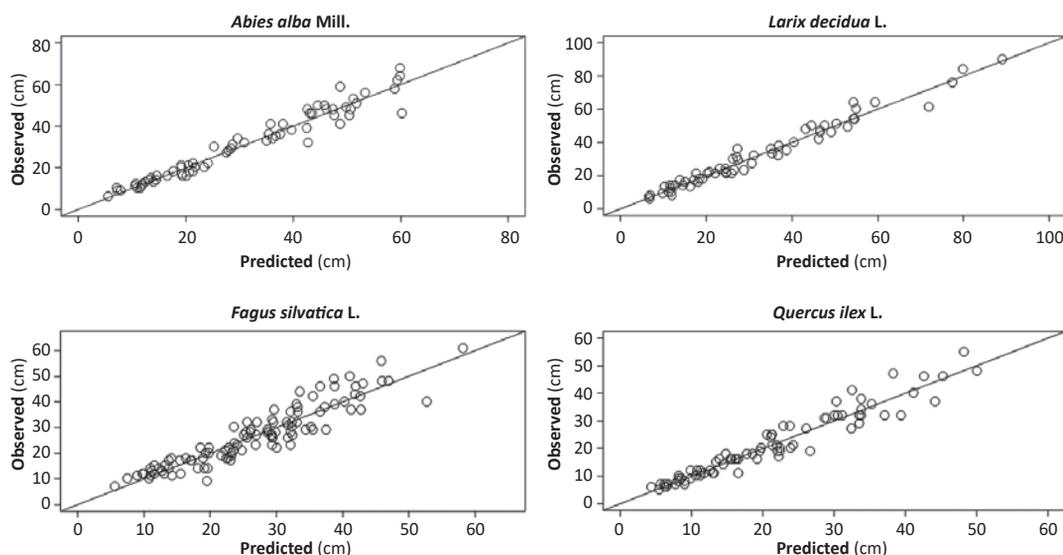
Figure 2. Examples of observed DBH values plotted against cross-validated values predicted by Model 1. Regression lines intercept does not differ from 0 and the slope does not differ from line 1:1.

RMSEs of Model 2 are equal or lower than those of Model 4 in eleven cases out of sixteen. Improvement ranged between 0% (*Quercus pubescens*) and 9.08% (*Quercus cerris*); in the remaining five cases, Model 4 showed RMSE reduced by from 0.51% (*Robinia pseudoacacia*) to 3.51% (*Pinus pinea*).

Table 4 provides the estimated parameters for Model 2 with their heteroscedastic-robust standard errors. For the

Table 4. Model 2: explanatory variables, estimated coefficients (Estimate), heteroscedastic-robust standard error (St. Error), *t*-value and *P*-value of the *t*-test.

Variable	Estimate	St.Error	*t*-value	Pr(>|t|)
(Intercept)	-2.16952	1.71786	-1.26292	0.206881
SD	0.93400	0.12200	7.65607	0.000000
SD^2	-0.00258	0.00150	-1.71868	0.085948
SH	0.08761	0.03796	2.30786	0.021188
SD x *Larix decidua*	-0.38716	0.14286	-2.71009	0.006829
SD x *Pinus silvestris*	-0.38447	0.17495	-2.19759	0.028182
SD x *Quercus pubescens*	-0.30830	0.15629	-1.97262	0.048784
SD^2 x *Larix decidua*	0.00608	0.00172	3.53088	0.000431
SD^2 x *Pinus halepensis*	0.00669	0.00288	2.31904	0.020572
SD^2 x *Pinus pinea*	0.00466	0.00217	2.14821	0.031911
SD^2 x *Pinus silvestris*	0.00739	0.00252	2.93199	0.003436
SH x *Picea abies*	-0.10348	0.04914	-2.10587	0.035437
SH x *Ostrya carpinifolia*	-0.09178	0.04332	-2.11838	0.034362
SH x *Castanea sativa*	0.16570	0.05426	3.05365	0.002314
SH x *Quercus cerris*	0.17576	0.06493	2.70670	0.006899
SH x *Pinus pinea*	0.17293	0.08152	2.12125	0.034119
SH x *Pinus nigra*	0.13766	0.06720	2.04851	0.040742

dummy variables only the coefficients significantly different from zero are showed. Complete coefficient estimates are given in Supplementary File 1.

The estimated parameters in Table 4 show that SD is the main explanatory variable. For example, applying the model with the parameters in the table first to the mean SD and SH values reported in Table 1 and then to SD and SH values increased in turn by 1 cm revealed that the higher relative importance of SH to SD is for *Quercus cerris*. Nevertheless, the 1 cm increase in SD enlarged the DBH estimate by 0.71 cm, while the 1 cm increase in SH enlarged the DBH estimate by 0.26 cm.

Figure 3 shows the residuals of Model 2 plotted against the stump diameters. Heteroscedasticity was confirmed by the Breush-Pagan test (BP = 267.29, df = 63, *p*-value < 2.2e-16).

Compared to Model 1, Model 2 RMSE is lower in fourteen species; the RMSE is reduced consistently for most species, up to 21.49% for *Castanea sativa* and 31.38% for *Pinus nigra*. In two cases (*Picea abies* and *Quercus pubescens*) Model 1 allowed estimates with reduced RMSE by from 3.07% to 3.58%.

In Table 5, the estimated parameters for Model 3 are given.

Figure 3. Residuals (cm) of Model 2 plotted against stump diameter (SD, cm).

Table 5. Model 3: explanatory variables, estimated coefficients (Estimate), heteroscedastic-robust standard errors (St. Error), t-value and P-value of the t-test.

Variable	Estimate	St.Error	t-value	Pr(>|t|)
(Intercept)	-1.51407	0.74383	-2.03550	0.042024
SD	0.83716	0.04916	17.03073	0.000000
SD²	-0.00044	0.00067	-0.65952	0.509692
SH	0.02876	0.01651	1.74223	0.081730
Broadleaves	-0.49377	0.90772	-0.54397	0.586568
SD x Broadleaves	-0.05889	0.05932	-0.99275	0.321038
SD² x Broadleaves	-0.00176	0.00079	-2.23831	0.025387
SH x Broadleaves	0.12160	0.03475	3.49952	0.000483

DISCUSSION

DBH prediction is rarely the ultimate goal of an estimation procedure. Particularly when the economic value of the loss of forest products is under estimation, caused by illegal logging or natural hazards, estimates are addressed to assess tree volume or biomass weight. In this respect, estimating accurately the DBH from stump information is a matter of no secondary importance because the relationship between DBH and those two variables is multiplicative (Pond and Froese 2014), and errors in predicting DBH result in amplified errors in volume and weight predictions. Westfall and McRoberts (2017) found that, within a sampling approach, a two-step procedure based first on reconstructing DBH and then on applying a tree-volume model based on it gave better estimates than a model estimating merchantable volume directly from stumps information, suggesting that DBH prediction is unavoidable also when stump information to tree volume models is available. Despite its importance, Westfall (2010) noticed that little work on the prediction of DBH from stump dimensions has been done since the 1990s; our impression is that since that period such kind of works have become even less frequent. One reason might be the availability of such models that were developed in the past, but are now used without caring about their characteristics. Based on the new data available we could assess the performance of a model developed in the 1980s, based on data collected across wide areas but poorly described. The analysis was important because it showed the accuracy one might expect using that model. Its parametrization with the new data improved the accuracy on cross-validated data and provided overall unbiased predictions, as well as unbiased predictions for the conifers and the broadleaves groups, but also showed that predictions might be biased if used for particular species. In this respect, although the new Model 2 developed in the study is a preliminary result within a long-term research, it allows improved predictions compared to the old model. The main gain from using Model 2 is to obtain unbiased predictions, a result of the flexibility given to the model structure by the increased number of dummies to take into account the species; however, in terms of accuracy the improvement was limited, as indicated by the RMSE values in Table 3. The accuracy of Model 2

predictions is low, compared to other studies (e.g. Pond and Froese 2014), suggesting that dataset enrichment is necessary to identify the strata in order to reduce variability and optimize the sample size. In fact, almost twice the data available for Model 0 were used, but the number of samples by species is still limited and exceeds one-hundred for only three of them (*Picea abies, Fagus sylvatica, Quercus pubescens*). Furthermore, our data introduced additional variability because they come from trees felled following the criteria that differed between the operations and, for the broadleaves, include coppice shoots too. Like in the old dataset, our data capture a great variety of environmental and silvicultural conditions in Italy. The country spans over 10° latitude, two biogeographic regions (Pignatti 1979), and trees grow from the Mediterranean vegetation at the sea level to the tree line both on the Apennines and in the Alps. Also, the measuring of individual stumps instead of groups in sampling plots is a source of variability because sampling stumps close to each other in sample plots could capture at least the effects of homogeneous growing conditions at the very local scale. Ercanli et al. (2014) found that even selecting a subset of the available dataset may improve calibration to get better estimates of DBHs of independent data, highlighting the importance of the sampling scenario and of having enough data to run a deep analysis. Besides the variability in the data, accuracy performance might have been affected by the modelling approach. An OLS regression analysis was performed because it was functional to investigate the importance of considering the species and, in such case, whether individually or grouped through nested models; modelling by species or species groups is common in this kind of analysis. Either non-linear models or species-specific models might have improved accuracy. For practical uses, there are some other reasons for recommending using Model 2 (or Model 3) instead of Model 0. Metadata have been clearly described and this provides awareness of its limits and qualities. The dataset used in the study contained data on stumps from 7 cm diameter, also recorded in coppice stands; within the NFI computations, that threshold is consistent with the reference used for the current NFI measurements, that is, a minimum DBH of 4.5 cm for callipered trees (Bosela et al. 2016). The model presented in this paper was developed for stumps with bark, so its use is recommended only for intact or weakly/medium decayed stumps.

In addition to providing a new model useful for forest practice, this study provided some directions about the continuation of the research. Model 2 allows the estimation of DBH for sixteen species which are among the most important forest species in Italy. Those species mark the Italian forest categories or sub-categories, as adopted by the second Italian NFI, and amount to a relevant percentage of the country forest area (INFC 2007). Nevertheless, there are other important tree species not covered by the model, and this is an additional reason for underlying the importance of pursing the continuation of data collection. This is a matter of no secondary importance for practical implications. Corral-Rivas et al. (2007) demonstrated that when a species-based model is incorrectly used for estimating the DBH of another species, errors may be severe and the use of more general models, i.e. fitted with multi-species data, is recommended

instead. Although the good performance for one species that was not in the dataset used for calibration should be proved, Model 3 might be used to predict DBH of species other than those used in our analysis. In fact, choosing a species-specific model developed for species other than the one in hand might result as even more unjustified, because it requires the particularly hard judging of similarities to disclose for relationships like those implicated with stem taper. More precisely, Model 3 might be preferred in case of doubts about the stump species in mixed forests composed by the species in our dataset and for this reason it may be considered an additional result of this study. SH is a variable not recorded during the first Italian NFI but recorded from the second Italian NFI onwards (Gasparini and Di Cosmo 2015). Although SD remained the main explanatory variable, our analysis showed that, besides the species, SH improves the predictive accuracy of models. This confirms the finding of Pond and Froese (2014), who compared models with and without SH and stressed about the importance of including such a variable among the predictors.

CONCLUSIONS

Based on the analysis carried out, the following conclusions can be drawn:
- Prediction of tree diameter at breast height (DBH) is improved when the stump height (SH) variable is considered among the quantitative explanatory variables, although stump diameter (SD) remains the most important predictive variable.
- Introducing the species among the explanatory variables allowed the model to perform unbiased estimates.
- Model accuracy was limited by the sample size, suggesting importance to collect additional data to accomplish the study.
- Future steps of the study should be addressed to the identification of statistical strata to direct new data acquiring and inclusion in the modelling.
- Comparison of model performance consistently benefitted from the assessment of possible bias in predictions.

Author Contributions
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Conflicts of Interest
The authors declare no conflict of interest.

Supplementary Materials
Supplementary File 1. Estimated parameters for Model 2.

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Growth of *Tilia cordata* Mill. in Urban Forests

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ABSTRACT

Tilia cordata Mill. is considered to be the main tree species resistant to urban conditions and it is widely used in the greening of cities. The aim of the study is to assess the patterns of growth and development of small-leaved linden plantations in the urban environment. The research is based on the method of sample areas where continuous enumeration survey has been carried out. The study of urban greening objects was conducted using the methods of landscape assessment and complete enumeration of trees in alley plantings. It was found that *Tilia cordata* Mill. grows in all districts and zones of the city of Ufa, occupying 34.4% of green areas. During this research it was found that artificially created alley plantings of *Tilia cordata* Mill. are more well-kept and have well-developed crowns which create a tree shade sometimes 8-10 meters wide (the average crown projection being 7 m). Since alley plantings have repeatedly been pruned, trees in this area are characterized by a large increase in diameter and strong height growth stagnation. Thus, when studying linden plantings of the same age, a sharp difference in the size and shape of trees was observed.

Keywords: *Tilia cordata* Mill.; urban forests; soil; planting; silvicultural and taxation indicators

INTRODUCTION

Today, large cities are artificial systems created and managed by man (Strohbach and HaasE 2012, Zölch et al. 2017). Ecological balance support, as well as the search for the improvement of the technogenic habitat, is an urgent problem. Not every living plant, whether shrubs or tree plantations, can adapt to the urban environment and survive in it, not to mention their positive impact on the environment (Moser et al. 2017, Sultanova et al. 2018, Rahman et al. 2019), which is further considered in the 'Results and Discussion' section.

For several decades, scientists have been actively studying the role of trees and shrubs in improving the quality of man-made and urban habitats, as well as their impact on the environment. Long-term studies have revealed an important positive environmental role of green plantings in the regulation of atmospheric air, city micro-climate, and in the protection of the urban environment from negative anthropogenic factors (Konashova et al. 2018, Sultanova et al. 2018, Rahman et al. 2019, Urošević et al. 2019). Tree plantations are a powerful natural factor in countering negative environmental effects of urbanization and man-made pollution (Glibovitskaia 2014).

Ecological, silvicultural and social significance of green plantings cannot be overestimated (Kabisch and Haase 2014, Konashova et al. 2018, Sultanova et al. 2018). Green plantings affect the city micro-climate, reducing summer temperature by 4-6°C (Rahman et al. 2017, 2019). Having large transpiring ability, they transpire moisture 20 times more than the area they occupy, significantly increasing air humidity (Margaritis and Kang 2017, Zölch et al. 2017). In some cases, tree species in urban conditions have a greater influence on radiation than on changes in temperature and humidity (Takács et al. 2016). Tree plantations significantly reduce the speed of atmospheric circulation (Strohbach and Haase 2012). Their leaves have high resonance ability, reducing the noise level by 10-15 dB (Margaritis and Kang 2017). Green plantings play an important role in the purification of urban air from dust: dust content of air in urban parks in winter is 37% and in spring and summer 42% lower than in open spaces (Strohbach and Haase 2012, Margaritis and Kang 2017). The aspects of the social functions of plantations are as follows: provision of recreational services (Konashova et al. 2018, Sultanova et al. 2018), improvement of physical, spiritual and moral conditions of humans (Xu et al. 2018), and the creation of

population vacation destinations (Bijker and Sijtsma 2017).

The urban environment is usually characterized by various types of stress, creating problems for tree species (Moser et al. 2017, Moser-Reischl et al. 2019). Atmospheric pollution has both direct and indirect impact on the formation of the chemical composition of assimilating plant organs through the soil, resulting in failure to supply plants with nutrients. Plant damage begins with the penetration of toxicants into the leaf and their impact on biochemical cellular processes. From this point of view, the research of assimilation organs, which absorb pollutants as a result of intensive gas exchange, is of scientific interest (Ziiatdinova et al. 2012, Moser et al. 2015).

In this context, linden is one of the main tree species that are resistant to urban conditions and are widely used in different climatic zones of Russia for the greening of cities. Plants of this species are used as street plantings in public gardens, cities and forest parks (Chopikashvili et al. 2014).

Almost all linden trees have the advantages such as compact leafy crown; they are shade-tolerant, easy-going about soil conditions, resistant to wind, non-allergenic, and quite highly resistant to aggressive urban environment characterized by smoke, mud, dust, and air pollution (Teplaya 2013 Moser et al. 2015, Rahman et al. 2017, Sultanova et al. 2018). In addition, when transplanted even in an adult state, *Tilia cordata* Mill. successfully adapts to growing conditions. It is not much exposed to diseases and can resist pests. Moreover, linden has won its great popularity thanks to its decorative features which it possesses throughout the year (Zölch et al. 2017, Sultanova et al. 2018, Zhang et al. 2019).

Natural areas of *Tilia cordata* are Europe and adjacent areas of Asia (Moser et al. 2017, Konashova et al. 2018, Urošević et al. 2019). It is widespread in the middle and southern forest zone and in the European forest-steppe zone of Russia. Some area fragments of this species are represented in Western Siberia (e.g. linden forest outliers in Kuznetsk Alatau Reserve and in other areas). Linden trees form pure forests (Tilientums), but they also grow as mixed plantings in broad-leaved and mixed forests where the basis of the stand are other species, such as English oak, for example. Linden trees often grow in grove forest understory and in mixed coniferous broad-leaved forests (Glibovitskaia 2014).

These trees are widely grown as urban plantings along the streets, in parks and squares, as well as a type of hedgerow. They respond well to crown cutting. They grow in Moscow and other cities of the European part of Russia. Large-leaved linden (*Tilia platyphyllos*) which originates from Central Europe is also found in plantings together with *Tilia cordata*. It differs from the domestic linden and has larger leaves and flowers, and its flowering time begins earlier (about 2 weeks earlier) (Chopikashvili et al. 2014, Sultanova et al. 2018).

Small-leaved linden (*Tilia cordata* Mill.) can be used as a bioindicator for bioindication studies. The prospect of its use to assess environmental health under anthropogenic load is quite justified (Chopikashvili et al. 2014, Kabisch et al. 2016, Konashova et al. 2018, Sultanova et al. 2018). Leaves of linden trees in urban environments accumulate nitrogen dioxide, sulfur dioxide, heavy metals, salts and dust. As a result of leaf fall, all harmful substances get into the soil, thereby salting it. However, due to a large amount of calcium in the leaves of linden trees (in conditions of the city of Ufa ranging from 22.5 to 37.5 mg.eq·100g.soil^{-1}), leaf fall during decomposition reduces soil acidity and enriches it with humus.

Small-leaved linden is rather widespread in the Republic of Bashkortostan and makes more than 30% of lime woods of Russia. However, despite natural occurrence of small-leaved linden, its biological and environmental characteristics under man-made conditions have been recently studied quite poorly. At the same time, there is some fragmentary information on the impact of polluted environment on the above-ground organs of plants, but there is no such information on root system formation under the same conditions.

The aim of the study is to assess the patterns of growth and development of small-leaved linden plantations in the urban environment on the example of the city of Ufa in the Republic of Bashkortostan.

MATERIALS AND METHODS

Study Area

The research was carried out in the city of Ufa, the Republic of Bashkortostan, on the areas where the plantings of pure *Tilia cordata* Mill. grow on the territory of the forest-steppe region of the European part of Russia (Figure 1).

Long-term experimental studies (2007-2018) of silvicultural and taxation indicators of natural and artificial plantations of small-leaved linden were carried out on permanent study areas (PSA), and laid out using well-known forest estimation methods. Study areas were laid out according to regulatory requirements in compliance with the "Forest management study areas" All-Union Standard 56-60-83. Plantation establishment method" (Gost 2018). Taxation of the selected sample areas was performed by ocular estimation method. The average taxonomic indicators were determined in accordance with Forest Management Instructions (Forest Management Instruction 2018). In alleys with the area of 0.96 ha the sample coverage of linden trees was 680 trunks, out of which 612 trunks were of *Tilia cordata* Mill. (0.2 ha). The methods of landscape assessment and plantation trees' complete enumeration were applied to study urban greening objects. The state of the trees was determined in conjunction with their biomorphological features such as the crown color and its density; the color and damage of leaves; the presence of pests; relative shoots and wood growth; leaves' size; the presence of dry branches; bark and phloem state. Height and crown length were defined using a hypsometer. The crown projected diameter was determined by sighting crown projection in the direction of North-South and West-East, and by measuring the distance between the points of sighting.

In accordance with the requirements of the State All-Union Standard (State All-Union Standard 12536 of 2014) aggregation analysis of soils was made in the forest research experimental production laboratory (FSREPL) (Gost 2016). Soil subsample was selected by applying the double quartering method. Then the soil was weighed and bolted through a standard sieve. From the sample mass quantity 100% of the mass fractions were calculated. The calculations helped to establish the content of units of different size in

Figure 1. Research objects: The G. M. Rutto Park; alley alongside Prospect Oktyabrya Avenue.

the soil. The results were interpreted using the classification of mechanical elements. The amount of P2O5 in milligrams per 100 g of dry soil was determined in accordance with the calibration curve.

Statistical Analysis

The statistical and mathematical data processing was conducted with the use of Statistica 12 application program package and Microsoft Excel. The estimate reliability of potential correlations was determined with respect to the student's criterion (t≤0.05). The equation significance was estimated by the correlation coefficient (r, %). The obtained values were processed by standard variation statistics methods. Dependencies were found between: 1) diameters and heights of linden trunks; 2) diameter and length of the crown.

RESULTS AND DISCUSSION

The analysis of urban plantations in Ufa showed that they currently occupy the area of 227.4 ha. Soft-wooded broadleaved species predominate and account for 63.3% of the forest area. Hard-wooded broadleaved species make up 27.3% and coniferous species make up 4.2%. Other species and shrubs occupy 5.2% of the area (Figure 2).

Soft-wooded broadleaved tree plantation

Hard-wooded broadleaved tree plantation

Conifers

Others
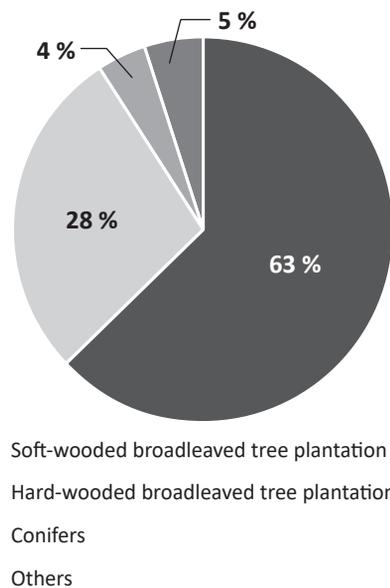
Figure 2. Grouping of urban green plantations in accordance with tree species they belong to.

Soft-wooded broadleaved tree plantations are represented by the following species: linden – 54.4%, black alder – 17.8%, birch – 8.1%, sedge – 7.1%, black poplar – 6.0%, poplar – 3.6%, aspen – 2.6%, gray alder – 0.4%. Low oak trees predominate in hard-wooded broadleaved plantations (42.0%), elm family trees come second (39.5%), ash trees make 9.8%, maple trees 5.3% and high oaks 3.4%. Pine dominates among conifers making 51.3%, spruce 33.7%, larch 14.7% and cedar 0.3% (Figure 3).

Silvicultural and taxation assessment of urban plantations showed uneven-aged grouping of tree species: 5.4% are small pole, 42.1% are middle-aged trees, 20.1% are stands approaching maturity, and 32.4% are mature and overmature trees. The share of close stands accounts for 15.4%, medium-stocked make 46%, and the number of low-stocked amounts to 38.6%. The average stocking is 0.6. In terms of productivity, plantations are classified as middle class bonitet (II, 6). Urban forests are characterized by the predominance of nettle and meadowsweet trees (57%). Mixed herb forests make 32.6%, sedge and *Sphagnum* forests amount to 8.8%, and grass forests account for 1.6%.

Tilia cordata Mill. is a favorite tree for urban landscaping. Ufa is not an exception. This tree species is also widely used in landscaping of large cities in Russia such as Moscow, Voronezh, Tula, and Ekaterinburg. Small-leaved linden possesses high aesthetic qualities such as shade tolerance and frost resistance. It is able to stand temperature changes against the background of moisture lack in the soil (Rahman et al. 2019).

However, it responds well to artificial crown formation.

Tilia cordata Mill. is grown in all districts and zones of the city of Ufa, occupying 34.4% of its green areas. To study the dynamics of woody vegetation development in urban conditions, long-term observation on permanent sample areas (PSA) is required. Small-leaved linden trees' complete enumeration was conducted during the research on PSA. Taxational characters of 1303 trunks were measured.

The studied areas of small-leaved linden plantations differ from each other in structure, soil and hydrological conditions, light regime, intraspecific competition, recreational loading and in other indicators. The studied plantations of *Tilia cordata* Mill. are included in the group of urban green areas of general purpose.

It should be noted that the quantity and morphology of urban woody species are still poorly studied, especially with regard to changes caused by the urban environment. However, forest valuation indicators and tree functions are strongly intertwined. Thus, Rahman et al. (2017) analyzed the growth and development of *Tilia cordata* Mill. growing in Munich (Germany) and found out that growing conditions influence significantly the ratio of tree parameters such as height and diameter.

During this research, the dynamics of growth and development of small-leaved linden (Table 1) growing either in natural conditions (G. M. Rutto Forest Park) or in artificially created groves (e.g. tree alley alongside Prospekt Oktyabrya Avenue) were studied.
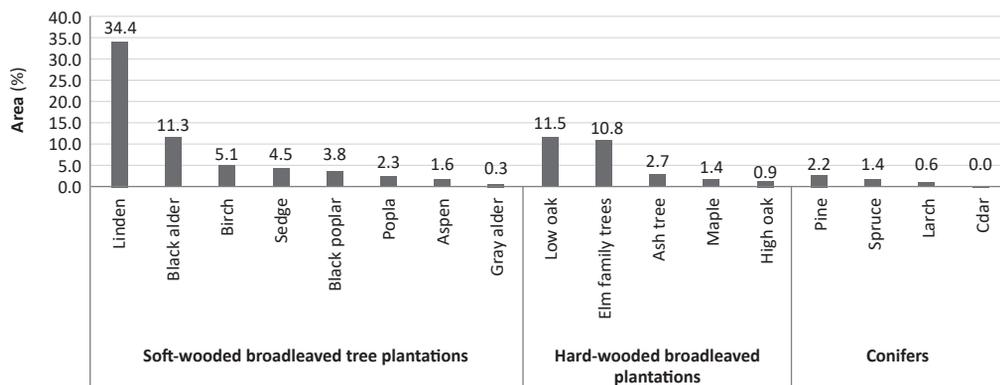

Figure 3. Species representation of urban plantations.

Table 1. Average silvicultural and taxation indicators of sample areas grouped by year.

| Year of measurement	Quantity (pcs)	Height (m)	Diameter (cm)	Crown length (m)	Crown diameter (m)
Comparison of average growth and development indicators of trees growing in the G. M. Rutto Park					
2007	626	18.2±0.03	18.4±0.03	8.5±0.01	6±0.01
2018	623	20.8±0.03	19.7±0.03	3.67±0.01	9.4±0.01
Comparison of average growth and development indicators of trees growing on the alley alongside Prospekt Oktyabrya Avenue					
2007	727	13.1±0.04	30.1±0.02	5±0.01	9±0.01
2018	688	14±0.04	34.1±0.02	7.5±0.01	10.2±0.01

The average diameter of trees growing in the G. M. Rutto Park is 19.7±0.03 cm, and their average height is 20.8±0.03 m. The tables contain data on trees' distribution according to bonitet classes they belong to. Based on the tables date it can be assumed that plantings are of bonitet class II. Taking into account the data of Matveev-Motin tables for linden high forest growth, it can be seen that park tree parameters differ in minimum average values from the parameters of naturally growing plants. However, there is a fallback in diameter due to high forest density (1.0).

The alley alongside Prospekt Oktyabrya Avenue is managed better and differs in strongly developed crowns creating a shadow which is sometimes 8-10 m wide (the average crown projection is 7 m). Since alley plantings have repeatedly been pruned, trees in this area are characterized by a large increase in diameter and strong height growth stagnation. The average diameter of a plant is 34.1±0.02 cm, and its average height is 14±0.04 m. Diameter growth indicators of *Tilia cordata* Mill. growing in the conditions of the city of Munich (Germany) range from 11 cm to 102.2 cm. The average annual increase in diameter is 0.39-0.40 cm·year⁻¹ (Moser et al. 2015).

The average height of alley trees alongside Prospekt Oktyabrya Avenue for 11 years increased by 0.6±0.01 m (0.05±0.01 m·year⁻¹), and the average diameter increased by 4±0.01 cm (0.36±0.01 m·year⁻¹). The increase of average diameter of trees growing in the G. M. Rutto Park for 11 years was 1.3±0.01 cm (0.12±0.01 m·year⁻¹), and their height increased by 2.6±0.01 m (0.26±0.01 m·year⁻¹). The data prove that conditions in which trees in the park grow and develop are closer to natural habitat than those in the alley. Diameter increase in alley trees is higher due to tree pruning and because of lower tree competition, as the distance between individual trees is bigger.

Figure 4 shows the curve of dependence of trunk diameter of small-leaved linden growing in the alley on its height. The variability is approximated by the equation $y=-0.004x^2+0.3999x+5.1391$, when the correlation coefficient $r=0.3310$. Regression equation has the form $y=0.1281x+9.6192$.

The variability of crown diameters and crown length of small-leaved linden trunks is characterized by the equation $y=0.0284x^2+0.0792x+7.9006$, when the value of the correlation coefficient $r=0.4189$. Regression equation has the form $y=0.1281x+9.6192$.

Weak and insignificant correlation of trunk diameter, crown diameter and height of trees growing in the Rutto Forest Park can be explained by the presence of a small sampling size.

On the basis of measurements, the analysis of linden silvicultural indicators was made. Sampling helped to make quantitative and percentage diameter distribution table (Figure 5, 6).

The number of trees growing in the park (Figure 6) whose diameter is thinner than the average diameter makes 36.6% of their total number. The trees with thicker diameter amount to 63.4 % (according to A.V. Tiurin they make 57.25% and 42.5%
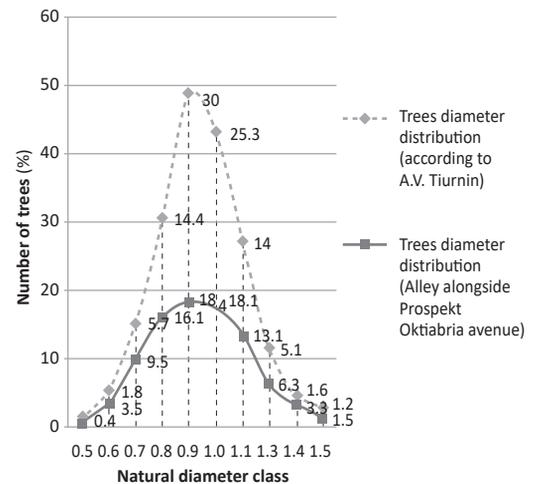
Figure 5. Trees' diameter distribution table (Alley alongside Prospekt Oktyabrya Avenue).

Figure 4. Dependence of the diameter of small-leaved linden trunks on height.
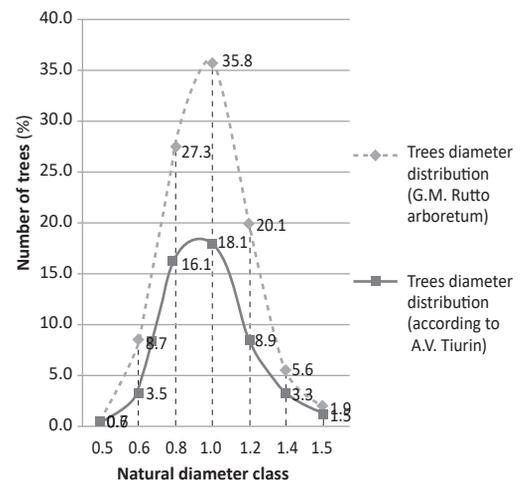
Figure 6. Stand trees' diameter distribution table (G. M. Rutto arboretum).

respectively). This results in low plantation thinning and mortality of weakened trees provoked by their mechanical damage.

Trees of central diameter class (28, 32, 36, 40) growing in the alley amount to 83.7% of the total number of trees. Maximum number of normal stands are of 24, 28, and 32 diameter classes making up 64.7% of the total number of trunks. The series of trees' diameter distribution is characterized by an asymmetric single-humped curve.

Currently 680 linden trees grow in the alley, but according to research data in 2007 724.44 linden trees were cut down due to the construction of new bus stops, pedestrian crossings and the clearing of dead trees. The average taxation indicators of trees growing in the alley and in the forest park increased on the whole.

Plants growing in the city require all necessary nutrients for their growth and development, which can be provided by the soil as their primary supplier. However, in the urban environment soils accumulate not only various forms of waste, but also pollutants that can have a negative impact on the rhizosphere, which kills trees (Glibovitskaia 2014, Sultanova et al. 2018).

During the research soil pits were made. Soil structural composition and its characteristics were obtained.

A0-A1 soil layer in the linden forest in G. M. Rutto Park contains only 33.17% - 37.30% of small granular structure aggregates. This suggests that the soils there are waterlogged and their water and heat balance is broken (Field studies of the soil 1984 ,Nature protection 2008, Ziiatdinova et al. 2012, Mudarisov et al. 2019). There is also excessive compaction provoked by people's recreational activities.

This indicator in the topsoil of the alley made up 2.45-3.3. In the park topsoil it was only 0.5-0.6. The alley soil structure rating was 5 times higher in comparison with the same indicator in the park soils. There are some factors that suppress small-leaved linden trees, but despite them a positive increase in trees' diameter can be observed, which amounts to 4.4±0.01 cm for an eleven-year period (0.4±0.01 m·year^{-1}).

Top soil layers (A0-A1-A2-AB soil horizons) of the analyzed sample areas are characterized mainly by neutral and weak acid medium (pH medium=Of 5.84-of 6.98 U). In the lower horizons the soil becomes more acidic (pH medium=4.17-of 7.02 U). Though pH medium indicators in the alley soils are within the limits characterizing a neutral medium, in some layers they provide alkaline reaction, and the indicators are then on the border. Medium reactions that take place in the soils of the forest park indicate that the soil there is more acidic.

Park soils are more saturated with calcium cations (23.8-31.3 mg.eq·100g.soil[-1]) and magnesium (4.8-5.5 mg.eq·100g.soil[-1]) than those in the alley, in which the content of calcium is 22.5-26.3 mg.eq·100g.soil[-1], while magnesium content is 3.5-6.5 mg.eq·100g.soil[-1]. Calcium prevails in all cases. ·

Soil saturation with ammonium (NH4) in the sample areas throughout the soil profile is to some extent similarly low. The content of nitrate (NO3) in the alley soils is slightly higher than in the forest park, but in both cases its provision rate is low. Throughout the soil profile in the

alley, the indicators range from 1.35-28.2 mg.eq·100g[-1] (NO3), 3.5-15.1 mg.eq·100g[-1] (NH4) and within 0.96-11.6 mg.eq·100g[-1] (NO3), 1.5-19 mg.eq·100g[-1] (NH4).

The analysis of the soils in the areas where linden plantations are grown showed that there are large silt particles in the soil mantle. So, soils in that areas are dark gray forest soils. Such soils are characterized by a small quantity of sand particles, and thus can be named heavy loamy clay-silt soils. This process does not greatly affect the development of the root system of trees and plantations growing in such soils. Based on their exploitable use for linden tree planting, it can be concluded that these areas have proper soil conditions.

At the same time, the soil under small-leaved linden canopy accumulates various substances. Studies conducted in Louisville (USA) showed that the movement of salts, heavy metals and other elements in the soil are of great importance in determining the state of urban plantations (Trammell et al. 2011). As for the city of Ufa, soil root occupation here increased as well as the proportion of semiskeletal and skeletal roots. During the growing period there was a migration of lead, copper and cobalt from the leaves to the roots. In Germany, the study of soils in urban areas where *Tilia cordata* Mill. grows revealed that this tree species contributes to the soil humidity decrease and to the soil temperature rise (Moser et al. 2015, 2017, Rahman et al. 2017, Moser-Reischl et al. 2019), increasing at the same time the latent current (Moser et al. 2017). This research has shown that the soils on which *Tilia cordata* Mill. grows are waterlogged and that their air-heat balance is disturbed.

The study of the structural response of 52 *Tilia cordata* Mill. trees conducted in Munich (Germany) showed that growing conditions had a significant impact on the height-diameter ratio in the presence of pronounced crown asymmetry (Bayer et al. 2018). Moser et al. (2015, 2017) and Moser-Reischl et al. (2019) in their studies analyzed the growth of *Tilia cordata* Mill. in urban conditions and revealed a close relationship (r[2]>0.7) between crown diameter, trunk diameter, crown projection area and height of *Tilia cordata* Mill. The results of our studies have shown that *Tilia cordata* Mill. growing in parks has a better developed crown than in street alleys. However, it has to be noted that in the park growing and development conditions for trees are closer to the natural habitat, rather than the conditions in the alley. Diameter increase in alley trees is higher due to tree pruning and because of lower tree competition, as the distance between individual trees is bigger.

It should be noted that the analysis of growth and development of urban plantations is limited due to the lack of empirical data (Rahman et al. 2017, 2019). Methodological problems become more urgent since there is a need for comprehensive studies to reveal the factors affecting woody species growing in urban environments (Rahman et al. 2017). The assessment of the regularities of growth and development of small-leaved linden plantations in the urban environment allows to estimate the plantations' silvicultural and taxation parameters to a greater extent. The sample size is not representative for the whole set of features.

Table 2. Results of chemical analysis of separate and composite soil samples.

Soil pit number, sample depth (cm)	Humus (%)	pH		Hydrologic acidity	Ca	Mg	Ca+Mg	P$_2$O	K$_2$O	N-NO$_3$	N-NH$_4$	Degree of saturation
		Water	Salt	(mg.eq·100g.soil^{-1})				(mg.100g. soil^{-1})				
Soil pit No.1 Prospect Oktyabrya avenue (Halle District bus stop - Ufa department store bus stop)												
0-30	4.4	4	6.70	0.76	22.5	3.5	26.0	25.8	21.0	3.5	15.1	97.15
30-40	4.0	4	6.12	1.53	24.3	3.8	28.1	5.5	9.0	2.75	11.0	94.83
40-60	1.9	C	5.77	1.56	28.3	3.3	31.6	5.3	11.6	1.51	5.1	95.29
60-80	1.5	C	5.68	1.56	27.8	6.5	34.3	8.3	11.6	1.35	8.0	95.64
Soil pit No.2 Prospect Oktyabrya Avenue (Ufa Department store bus stop - Sportivnaia bus stop)												
0-20	3.8	4	6.45	1.18	24.5	6.5	31.0	11.0	16.0	28.2	15.5	96.33
20-40	3.7	4	6.11	1.6	25.2	5.0	30.0	7.1	9.5	8.5	7.5	94.93
40-65	3.5	C	5.98	1.78	23.3	4.5	27.8	13.1	12.2	8.5	11.3	93.98
66-80	3.3	C	5.61	2.62	22.8	4.8	27.6	9.8	11.3	5.6	7.5	91.33
81-100	1.9	C	5.50	2.31	27.8	4.3	29.1	6.1	12.5	3.5	4.5	92.6
Soil pit No.3 Prospect Oktyabrya Avenue (Circus bus stop - Railway Hospital bus stop)												
0-20	3.9	4	6.93	0.73	26.3	5.5	31.8	5.8	13.5	20.0	11.5	97.77
31-40	2.6	4	6.99	0.54	27.3	4.8	32.1	3.4	9.0	9.8	4.5	98.34
41-65	1.8	4	6.92	0.6	35.0	5.0	40.0	13.0	10.0	4.6	3.5	98.55
66-102	1.7	4	7.02	0.47	31.0	3.5	34.5	2.2	8.5	3.5	3.5	97.22
Soil pit No.4 G. M. Rutto Forest Park (Test 1)												
0-37	3.2	4	6.22	1.46	31.3	4.8	36.1	4.6	16.5	11.2	10.0	96.11
37-45	2.7	C	5.69	2.02	35.8	5.5	41.3	3.6	15.6	7.4	5.5	95.33
54-64	2.5	C	5.84	1.6	37.5	4.8	42.3	16.7	17.2	5.8	8.0	96.35
72-82	1.5	4	6.98	0.63	33.3	3.8	37.1	2.7	11.0	8.0	3.5	98.33
87-90	0.9	4	7.15	0.44	23.8	2.8	26.6	1.3	9.0	7.1	3.5	98.37
90-100	0.6	4	7.25	0.36	16.8	2.3	19.1	1.9	6.5	4.1	1.5	98.15
Soil pit No.5. G. M. Rutto Forest Park (Test 1)												
0-25	5.0	C	6.02	2.11	23.8	5.5	29.3	2.7	28.0	6.3	19.0	95.87
25-40	3.0	4	5.41	2.92	23.0	6.0	29.0	1.2	16.8	4.0	8.5	94.21
40-60	3.2	4	5.12	3.4	24.8	5.8	30.6	0.8	16.2	2.24	9.5	98.12
60-70	2.0	N	4.55	5.37	26.3	6.8	33.1	0.4	15.0	1.05	9.0	93.4
81-100	1.9	C	4.17	5.48	29.0	7.3	36.3	0.4	14.6	0.96	5.5	94.79

CONCLUSIONS

In this study the growth and development of small-leaved linden plantations in the urban environment were assessed at the example of the city of Ufa in the Republic of Bashkortostan. Based on the measurements, the analysis of linden silvicultural indicators was conducted. Sampling helped to make quantitative and percentage diameter distribution table. Statistical stand indicators were calculated. Based on this analysis, the following conclusions were made:

– artificially created alleys where *Tilia cordata* Mill. trees grow are better looked after, tree crowns there are more strongly developed, creating a shadow sometimes 8-10 meters wide (the average crown projection is 7 m). Since alley plantings have repeatedly been pruned, trees in this area are characterized by a large increase in diameter and strong height growth stagnation. The average plant diameter is 34.1±0.02 cm, and the average height is 14±0.04 m. Differences in diameter growth (t_{fact}=-0.07≤t_{theor}=1.96) and height (t_{fact}=0.02≤t_{theor}=1.96) were statistically insignificant;

– the studied even-aged linden trees showed a sharp difference in size and shape in comparison with other plantings. A tree growing in an open area (e.g. in the alley alongside Prospekt Oktyabrya Avenue) is of

small height, has a spherical crown, thick and fissured bark, and thick boughs all over its tapered trunk. A forest tree (e.g. growing in the G. M. Rutto Park) has a full-boled, non-tapered trunk and a pyramid shaped crown;

– in the studied urbanized soils, the content of a fine granular structure in 0-30 cm of the soil layer under the canopy of linden alley plantings of 70 years of age is 71.27%-76.47%, which determines their safety during repeated treatments and after artificial and natural moisture. Soils in the G. M. Rutto Park within A_0-A_1 horizon contain only 33.17%-37.30% of aggregates of fine granular structure. This suggests that the soils there are waterlogged, and that their air-heat balance is broken.

The results of this research allow to offer the following recommendations: the application of 100-120 kg·ha^{-1} of phosphoric and potassium fertilizers, since supply with these components is low, which will reduce the negative impact of pollution and strengthen the activity of microorganisms; creating alley plantings of small-leaved linden along the streets of the city of Ufa, increasing the number of rows to 2-3 and leaving a bigger distance between trees (at least 4 m); care for the existing green plantings, including topiary, sanitary and renovation pruning.

Author Contributions

MM, RS1 conceived and designed the research; GO and RS2 carried out the field measurements; EK performed laboratory analysis; RS1 and GO processed the data and performed the statistical analysis; EK helped to draft the manuscript; MM and RS2 wrote the manuscript. Note: RS1 – Rida Sultanova, RS2 - Regina Sazgutdinova.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ectomycorrhizal Flora Formed by Main Forest Trees in the Irtysh River Region of Central and Northeastern Kazakhstan

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ABSTRACT

In this study, the aim was to determine and identify symbiotically living ectomycorrhizas of the main tree species forming forests in central and northeastern Kazakhstan. Surveys were conducted on the right bank of the Irtysh River in a mixed forest of *Pinus sylvestris*, *Picea obovata* and *Betula pendula* trees. The collection was formed and the primary identification of voucher samples of fruiting bodies of macromycetes collected as ectomycorrhiza forming fungi was completed. In the collection and species identification of fruiting bodies, standard methods were used. A total of 30 ectomycorrhizas belonging to Agaricomycetes were identified. The distribution of 30 species into families is as follows: Suillaceae (8), Russulaceae (7), Cortinariaceae (4), Boletaceae (3), Tricholomataceae (2), Amanitaceae (1), Cantharellaceae (1), Gomphaceae (1), Gomphidiaceae (1), Paxillaceae (1), and Bankeraceae (1). The richest genus on account of the number of species was *Suillus* (8). Concerning the woody host species, 17 mycorrhizas were determined to build symbiosis with *P. sylvestris*, 8 mycorrhizas with *B. pendula*, 6 mycorrhizas with *Populus tremula*, 1 mycorrhiza with *P. obovata*, 1 mycorrhiza with *Quercus robur*, 1 mycorrhiza with *Salix* sp., and 1 mycorrhiza with *Pinus densiflora* Siebold and Zuccarini. Ectomycorrhizas should be used as a major performance-enhancing tool in afforestation and restoration studies in the Irtysh River basin under extreme ecological conditions and under climate change effects.

Keywords: mycorrhiza; forest ecosystem; symbiotic, mycotrophy; continental climate

INTRODUCTION

The degree of mycotrophy of the main forest-forming species on Earth is one of the most important indicators of their growth and development. Over 8000 species of higher plants and 7000-10000 species of mushrooms form ectomycorrhizal relationships (Rossi et al. 2013). The most important symbiotic relationship between plants and fungi is mycorrhiza. Namely, Brundrett (2002) stated that about 80% of higher plants have mycorrhizal formations on the roots. In addition, around 83% of dicotyledons, 79% of monocotyledons and all gymnosperms have mycorrhizal life. Non-mycorrhizal plants appear on very dry or highly salty submerged areas, and habitats where soil fertility is either quite high or too low. However, mycorrhizal life has not been recorded among the members of the

Cruciferae and *Chenopodiaceae* families, even under all environmental conditions (Harley 1975, Brundrett 1991, Marschner 1995). According to recent studies, mycorrhization has a close functional connection with the formation of the structure, diversity, and stability of plant communities (Püschel et al. 2007, Lambers et al. 2008, Veselkin 2012a, 2012b).

This symbiotic partnership allows participating in the circulation of nutrients, optimizing plant metabolism, activating mineral nutrition, and inducing resistance to drought, salinization, heavy metals, and pathogens (Rossi et al. 2013). There is consensus that these plant-fungal associations have profound impacts on nutrient cycling and vegetation dynamics in ecosystems, particularly in temperate forests (Taylor et al. 2016, Bennett et al. 2017, Jo et al. 2018).

The conservation of favorable living conditions in various regions of the world directly depends on the rational and careful use of forest resources. Forest ecosystems of central and northeastern Kazakhstan are one of the most important components of the Earth's biosphere. In forest biogeocenoses, the leading role belongs to ectomycorrhizal relationships (Agerer 2008, Smith and Read 2008).

It is known that for the normal development of any type of tree, specialized strains of macromycetes fungi forming ectomycorrhizal (EcM) and rhizospheric associative microorganisms are necessary (Shubin 1990, Fitter and Garbaye 1994, Brundrett 2002, Shubin 2002, Roman et al. 2005, Cairncy 2005, Wu and Xia 2006, Polenov 2013). In the case of mutualistic relations, EcM mushrooms receive from 10% to 50% of organic carbon from plants and become competitive in the soil, and thus plants have the opportunity to use the underground communication network from the mycelium of EcM mushrooms and root systems of different tree species, along which metabolites, energy sources, cofactors, vitamins, hormones, toxins and possibly genetic information are exchanged. In this case, the integration of populations and even species, communities of plants in a single giant underground communication network of the mycelium of mycorrhizal fungi occurs.

The study of ecology and physiology of EcM has been mainly concentrated in Europe, North America and Australia (Read 1999, Finlay 2005, Smith and Read 2008, Polenov 2013). The studies on the mycobiota and ectomycorrhizal macromycetes within the Republic of Kazakhstan have been carried out by Nam (1998), Abiev et al. (2000), Abiev (2015) and Veselkin et al. (2015). In addition, some pioneer studies regarding the identification and application of mycorrhizal macromycetes, particularly in Zailiysky Alatau region, were conducted by Meshkov et al. (2009a) and Meshkov (2010).

Due to the lack of the data on the mycotrophy, flora of mycorrhiza, and plant-fungi partnership in Kazakhstan forests, the aim of this research was to identify the ectomycorrhizas of the main forest-forming species in the Irtysh River region of central and northeastern Kazakhstan.

MATERIALS AND METHODS

Study Area

This study was carried out on the right bank of the Irtysh River (latitude: N 51˚ 57' 56 92'', and longitude E 78˚ 50' 2 72'') of central and northeastern Kazakhstan in 2018 and 2019. Study sites include mixed forests of Scots pine and birch (Figure 1), with the presence of grassy vegetation in the protected and buffer zones (Anonymous 2009). The Irtysh River is an international river flowing through the territories of China, Kazakhstan, and Russia. Being 4,248 km long, the Irtysh River originates from the Altai Mountains in SinKiang, China, crosses the Chinese border flowing west through Zaysan Lake and northwest across eastern Kazakhstan (Huang et al. 2013). The Kazakhstan Altai covers the eastern part of the Altai range including the right sub-basins of the Irtysh River. This mountainous area is covered by forests consisting of spruce, larch, pine, birch, and aspen,

while *Pinus sibirica* Du Tour occupies the top part of the mountain slopes (Meshkov et. al. 2009b, Sarsekova et al. 2016).

Deciduous trees are located on the edge of the meadow with a predominance of small-leaved *Ulmus* sp. with a shut-in crown forest density of 0.7. The species composition of plants is represented by 38 species of angiosperms and 1 species of gymnosperms belonging to 19 families. The largest number of species belongs to the family *Rosaceae* (4 species) and *Compositae* (9 species). Most of the examined species (21 species) are found in single specimens and 6 species are found very abundantly forming a dense grass cover. Both in open areas and under the canopy of trees, there is a litter layer whose thickness varies from 1-3 mm up to 7 mm under the trees. The moss, lichen and low shrub layer are absent within the study area (Anonymous 2009).

The climate is sharp continental and dry. This is due to the influence of dry and hot winds blowing from the Central Asian deserts in the summer. In winter, the territory of the pine forest is open to cold air flows coming from the North. The climate is characterized by cold and long winters (5.5 months), and short and hot summers with sharp temperature fluctuations in winter and summer (Anonymous 2009). The average annual air temperatures of the coldest month (January) and the warmest month (July) are 2.5°C and 30°C, respectively. The average duration of the warm period is 175 days, while frost-free days' amount to 117 days. The precipitation average is 240–310 mm per year, of which 60–75% of the annual amount falls in May – September. In spring winds from the north and northeast, and in summer, south and southwest winds quickly dry out the soil. Relative humidity in the summer (at 1 p.m.) is about 40%, dropping down to 10% on some days, causing intense transpiration of plants and a large loss of moisture in the soil (Anonymous 2015).

In this region, the pine forest soil is zonal. On low surfaces among tape hogs' meadow deep effervescent chernozem soils are developed, sometimes in a complex with solonets. In general, the soil profile is characterized by a slightly acidic to neutral reaction (pH of aqueous extract 6.5-7.0). Soils are washed from water-soluble salts. The mechanical composition is usually light, although there are layers of heavy and medium loam (Anonymous 2009).

Sampling and Identification of Macromycetes

The materials of macromycetes were collected in the central and northeastern part of Kazakhstan. Two methods of sampling were applied, including route and stationary research method. The routes covered various forest biocenoses — in pure or mixed forests of *P. sylvestris* L. with *B. pendula* Roth. Seven permanent multi-year transects with a total area of 1080 m^2 were marked where the sampling of macromycetes was performed every 10-15 days during the seasons of 2018 and 2019. The collection was formed from the sampled material, and primary identification of voucher samples of fruiting bodies of macromycetes was performed. All the collected macromycetes were sampled as ectomycorrhizal fungi under *P. sylvestris* L., *P. obovata* Ledeb. and *B. pendula* Roth trees.

Figure 1. Pure Scots pine (upper image), and birch and Scots pine mixed forest (lower image) where samples were collected.

During the identification of fruiting bodies, standard methods were applied, using the available identifiers such as the resource www.indexfungorum, as well as the web-sites on "Mushrooms of the Kaluga Region" and "Mushrooms of the Novosibirsk Region". Micromorphological studies of characters were performed under the light microscope (Altami SMO745-T, St. Petersburg, Russia), at 400×–1000× magnification. To identify the natural color of the microstructures, the preparations were viewed in distilled water and in a 3-5% KOH solution. Hyaline structures were stained with a 5% aqueous solution of safranin, while the presence or absence of amyloid and dextrinoid structures was determined using Meltzer's reagent. To determine the type of fungal symbiont in the ectomycorrhiza, the morphotyping method was used according to Agerer (2008). For these purposes, soil blocks (10×10×20 cm in size) were taken within the projection of the crown of mature trees, according to the method of concentric sampling (Smith and Smith 2011). Before sampling, the upper undecomposed layer of the litter was removed. Coniferous seedlings were extracted from the soil with the most intact root system. Moreover, DNA was isolated

from the specimens that could not be identified using the conventional identification tools.

The samples were wrapped in the aluminum foil and stored at a temperature of 4°C. The roots were washed under the running water, cut into 3 to 5 cm segments, and ectomycorrhizal endings were separated under a magnifying glass with tweezers and scissors. Morphotyping of ectomycorrhiza was performed using a stereomicroscope in the binocular and trinocular version (Altami SMO745-T), equipped with an Altami UCMOSO3100KPA video camera. Based on the nature of branching, the color of the ectomycorrhizal ends, features of the mantle surface, the presence or absence of external mycelium and rhizomorph, and morphotyping were performed according to the DEEMY system (Agerer 2008). The data were entered into a specially developed checklist. The selected ectomycorrhizas were photographed and fixed in 70% ethanol for DNA isolation (Gardes and Bruns 1993). In determining the species affiliation of voucher samples of fruiting bodies, consultations were made by personal communication with leading mycologists from Russia, Sweden, and Norway during the XV International Meeting on Macromycetes held in August 2018 in Tomsk.

In Vitro Culture and Cultivation

For the mycelial development, Khudyakov-Voznyakovskaya, Hagem, and Wort-agar media optimized for the C/N ratio and thiamine was used (Kõljalg et al. 2013). Aseptic conditions were maintained during the superficial and deep cultivation of mycelium (Bukhalo 1988). At the same time, optimal cultivation modes have been optimized and used. In total, 25 strains were developed into *in vitro* cultures.

RESULTS

A total of 30 ectomycorrhizas belonging to Agaricomycetes were identified (Table 1). The distribution of 30 species into families was as follows; Suillaceae (8), Russulaceae (7), Cortinariaceae (4), Boletaceae (3), Tricholomataceae (2), Amanitaceae (1), Cantharellaceae (1), Gomphaceae (1), Gomphidiaceae (1), Paxillaceae (1), and Bankeraceae (1). The genus with the highest number of recorded species was *Suillus* (8) (Figure 2). The photos of some fungi identified in the research are shown in Figure 3.

In the study area, three of the sampled tree species were coniferous and four were broadleaved. In total, 63.3% of all recorded mycorrhizae have established a symbiotic life with a coniferous host species (Table 2). Following the sampling and identification, 17 ectomycorrhizas were recorded under *P. sylvestris*, 8 under *B. pendula*, 6 under *P. tremula*, 1 under *P. obovata*, 1 under *Q. robur*, 1 under *Salix* sp., and 1 under *P. densiflora*. It should be noted that some species such as *Paxillus involutus*, *Russula undulata*, *Cortinarius* sp. and *Cantharellus cibarius* formed a symbiotic relationship with both coniferous and broadleaved tree species (Table 2, Figure 4).

Table 1. The list of recorded ectomycorrhizas in the Irtysh River region.

№	Scientific name	Class	Family
1	*Amanita muscaria* (L.) Lam. 1783		Amanitaceae
2	*Boletus satanas* Lenz, 1831		Boletaceae
3	*Cantharellus cibarius* Fr. 1821		Cantharellaceae
4	*Chroogomphus rutilus* (Schaeff.) OK Mill. 1964		Gomphidiaceae
5	*Cortinarius nemorensis* (Fr.) JE Lange 1940		Cortinariaceae
6	*Cortinarius* sp.		Cortinariaceae
7	*Cortinarius* sp. (Bull.) J. Kickxf. 1867		Cortinariaceae
8	*Cortinarius* sp. sensu NCL (1960)		Cortinariaceae
9	*Hydnellum ferrugineum* (Fr.) P. Karst. 1879		Bankeraceae
10	*Lactarius controversus* Pers. 1800		Russulaceae
11	*Lactarius deliciosus* (L.) Grey 1821s		Russulaceae
12	*Lactarius torminosus* (Schaeff.) Grey 1821		Russulaceae
13	*Lactifluus flexuosus* (PERS.) KUNTZE 1891		Russulaceae
14	*Leccinum aurantiacum* (Bull.) Grey 1821		Boletaceae
15	*Leccinum scabrum* (Bull.) Grey 1821		Boletaceae
16	*Paxillus involutus* (Batsch) Fr., 1838	Agaricomycetes	Paxillaceae
17	*Ramaria stricta* (Pers.) Quél. 1888		Gomphaceae
18	*Russula grisea* Fr. 1838		Russulaceae
19	*Russula undulata* Velen. 1920		Russulaceae
20	*Russula vesca* Fr. 1836		Russulaceae
21	*Suillus bovinus* (L.) Roussel 1796		Suillaceae
22	*Suillus granulatus* (L.) Roussel 1796		Suillaceae
23	*Suillus luteus* (L.) Roussel 1796		Suillaceae
24	*Suillus placidus* (Bonord.) Singer 1945		Suillaceae
25	*Suillus salmonicolor* (Frost) Halling 1983		Suillaceae
26	*Suillus sibiricus* (Singer) Singer 1945		Suillaceae
27	*Suillus tridentinus* (Bres.) Singer 1945		Suillaceae
28	*Suillus variegatus* (Sw.) Richon & Roze 1888		Suillacaee
29	*Tricholoma* sp.		Tricholomataceae
30	*Tricholomae questre* (L.) P. Kumm. 1871		Tricholomataceae
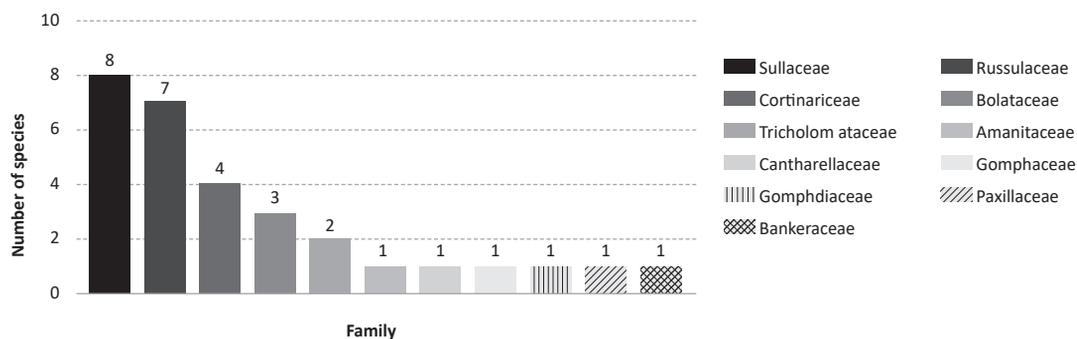
Figure 2. The number of ectomycorrhiza by families.

Figure 3. Representative photos of the macromycetes identified in this study: **(a)** Amanita muscaria; **(b)** Cantharellus cibarius; **(c)** Cortinarius nemorensis; **(d)** C. mucosus; **(e)** Lactarius delicious; **(f)** Leccinium aurantiacum; **(g)** L. scabrum; **(h)** Suillus bovinus; **(i)** S. luteus; **(j)** S. sibiricus; **(k)** S. variegatus; **(l)** Tricholoma equestre.
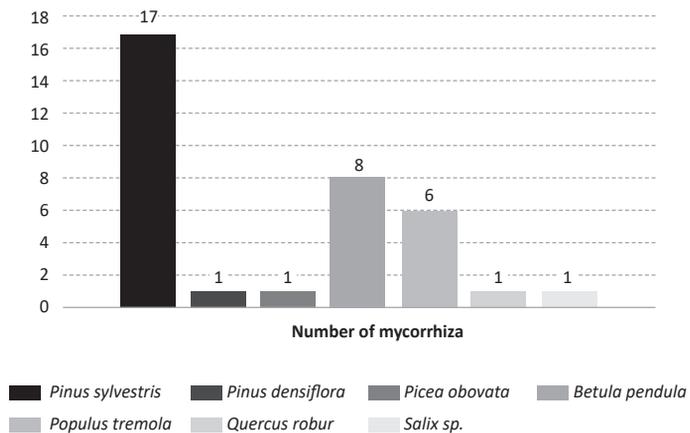
Figure 4. The number of ectomycorrhiza by tree species.

Table 2. Host trees and status of the detected and diagnosed mycorrhizas.

Latin name of the macromycetes	Common name	Mycorrhizal status	Latin name of host tree
Amanita muscaria	Fly agaric (Fly amanita)	Mycorrhiza	*Pinus sylvestris* L.
Boletus satanas	Satan's bolete (Devil's bolete)	Not mycorrhiza	-
Cantharellus cibarius	Chanterelle	Mycorrhiza	*Pinus sylvestris* L., *Betula pendula* Roth., *Picea obovata* Ledeb.
Chroogomphus rutilus	Brown slimecap (Copper spike)	Mycorrhiza	*Pinus sylvestris* L.
Cortinarius nemorensis	Contrary webcap	Mycorrhiza	*Quercus robur* L.
Cortinarius sp.	Orange webcap (Slimy cortinarius)	Mycorrhiza	*Pinus sylvestris* L.
Cortinarius sp. sensu	Cortinar (webcap)	Mycorrhiza	*Pinus sylvestris* L. *Betula pendula* Roth.
Hydnellum ferrugineum	Mealy tooth (Reddish-brown corky spine)	Not mycorrhiza	-
Lactarius controversus	Blushing milk cap	Mycorrhiza	*Salix* L. *Populus tremula* L.
Lactarius deliciosus	Saffron milk cap	Mycorrhiza	*Pinus sylvestris* L.
Lactarius torminosus	Bearded milk cap	Mycorrhiza	*Betula pendula* Roth.
Lactifluus flexuosus	Stumpy milk cap	Mycorrhiza	*Populus tremula* L
Leccinum aurantiacum	Orange oak bolete	Mycorrhiza	*Populus tremula* L.
Leccinum scabrum	Brown birch bolete	Mycorrhiza	*Betula pendula* Roth.
Paxillus involutus	Brown roll-rim (common roll-rim, poisonpax)	Mycorrhiza	*Betula pendula* Roth., *Populus tremula* L*., Pinus sylvestris* L.
Ramaria stricta	Strict-branch coral	Not mycorrhiza	-
Russula grisea	Milk-white brittlegill	Mycorrhiza	-
Russula undulata	Brittlegill	Mycorrhiza	*Betula pendula* Roth., *Pinus sylvestris* L.
Russula vesca	Bare-toothed russula (flirt)	Mycorrhiza	*Betula pendula* Roth.
Suillus bovinus	Bovine bolete	Mycorrhiza	*Pinus sylvestris* L.
Suillus granulatus	Weeping bolete	Mycorrhiza	*Pinus sylvestris* L. *P. densiflora* Siebold et Zucc.
Suillus luteus	Slippery Jack	Mycorrhiza	*Pinus sylvestris* L.
Suillus placidus	Slippery white bolete	Mycorrhiza	*Pinus sylvestris* L.
Suillus salmonicolor	Slippery jill	Mycorrhiza	*Pinus sylvestris* L.
Suillus sibiricus	Siberian slippery jack	Mycorrhiza	*Pinus sylvestris* L.
Suillus tridentinus	Orange larch bolete	Mycorrhiza	*Pinus sylvestris* L.
Suillus variegatus	Velvet bolete	Mycorrhiza	*Pinus sylvestris* L.
Tricholoma equestre	Man on horseback (Yellow knight)	Mycorrhiza	*Populus tremula* L.
Tricholoma sp.	Grey knight (Dirty tricholoma)	Mycorrhiza	*Betula pendula* Roth., *Populus tremula* L.

DISCUSSION

A rich diversity of 30 different mycorrhiza species was recorded in this study. Of these 30 mycorrhizae, 17 were determined under *P. sylvestris*, 8 under *B. pendula*, 6 under *P. tremula*, 1 under *P. obovata*, 1 under *Q. robur*, 1 under *Salix* sp., and 1 under *P. densiflora*. As determined within the scope of research, all *Suillus* species, *Amanita muscaria* and *Chroogomphus rutilus* associated a partnership with conifers, while the species of *Leccinum*, *Tricholoma* and *Lactifluus flexuosus* formed a partnership with hardwoods. In addition, *Cantharellus cibarius*, *Paxillus involutus*, the species of *Cortinarius* and *Lactarius* presented a partnership with both conifers and hardwoods on a broad spectrum.

Some macromycetes are selective in forming a partnership, while others may form a partnership in a very large spectrum. For example, of the genus *Laccaria, Suillus* form ectomycorrhiza on coniferous seedlings (early-stage fungi), fungi from the genera *Russula*, *Boletus* mycorrhizal roots of older conifers (late-stage fungi). For example, *Amanita muscaria* and *Boletus edulis* can form mycorrhiza with trees that are systematically distant from each other, such as conifers and hardwoods. There is a definite connection between the systematic groups of fungi and plants. Thus, fungi of the genus *Gomphidius* form mycorrhiza mainly with coniferous trees, while in *Suillus sibiricus*, mycoses with *Pinus sibirica* are more fixed than with *Pinus sylvestris. Leccinum chromapes* is confined to black birch (*Betula dahurica* Pall.) and does not form mycorrhiza with white birch (*Betula pubescens* Ehrh.) On the contrary, representatives of the genera *Cortinarius*, *Inocybe*, as well as *Laccaria laccata*, *Paxiluus involutus*, *Amanita vaginata*, and *Hebeloma crustuliniforme* possess a wide valence in relation to tree species (Singer 1938).

Valuable coniferous forests of the Irtysh River regions and Kazakhstan Altai have been exhausted by timber cutting and fires. The continuous felling of forests in the river basins of Bukhtarma and Uba in the East Kazakhstan region is responsible for the considerable loss of water in the Irtysh River. Because of the increasing demand for energy firewood, harvesting of these important forests has considerably increased (Meshkov et al. 2009b). Most ectomycorrhizal fungi cannot remain viable for a long time unless they find a host plant and establish mutually beneficial contact (Shemakhanova 1962, Shubin 1990, Timonen and Marschner 2005). The biodiversity of the fungi forming ectomycorrhiza with woody plants decreases rapidly due to clear-cutting operations and fires. The restoration of the natural diversity of this important component of forest biogeocenosis requires a long period of time if human-made and natural disturbances are severe in these forest areas. A number of researchers have shown that direct and indirect anthropogenic effects can very negatively affect the development of ectomycorrhizal mushrooms (Massicotte et al. 1998). The findings of this study are very important in this respect, in the sense of easier restoration of afforested areas.

Meshkov et al. (2009b) have emphasized that the priority should be given to forest rehabilitation on burned areas and lands where the forest was previously removed, including in the ribbon-like relict pine forests of the Irtysh region of the Kazakh Uplands (Akmola and Karaganda Provinces), plain forests of Kostanay Province. In addition, Meshkov et al (2009b) and Sarsekova et al. (2016) recommended that in many parts of Kazakhstan, in the degraded forest areas, mycorrhizas should be used as a major improvement tool.

Applied aspects of the application of mycorrhization were investigated by Meshkov who was the first in Kazakhstan not only to define four species of macromycetes into the culture, but also to develop a technology for their scaling and application in the form of mycorrhized compost for reforestation in Zailiysky Alatau

(Meshkov et al. 2009a, Meshkov 2010). Such practical projects should be increased and implemented as soon as possible. In this study, due to higher atmospheric and soil drought during the autumn-summer period in 2018 and 2019, no mass fruits of fungi were formed at the end of September. Following the rains in late September, the fruit-bearing macromycetes' organs started to appear. Moreover, most of the recorded mycorrhizae (63.3%) were found under the coniferous host species (Table 2). However, this could be associated with the dominance of coniferous tree species in the studied region, and the most sampled hosts in this study belonged to conifers. Vaishlya et al. (2017) emphasized that coniferous trees are able to form symbiotic relationship with 200-300 species of ectomycorrhizal fungi. There are nearly 50 ectomycorrhizal fungi species which are capable of forming ectomycorrhizas with *Pinus sibirica* in Tomsk region of west Siberia.

Species of *Suillus* are found all over the northern hemisphere where members of the *Pinaceae* tree family can be found. Although a few species are distributed in mainly mountainous regions of tropical regions, most are limited to temperate areas (Singer 1986). The *Russulaceae* have a worldwide distribution, but there are differences among the distribution of genera. *Russula* is the most widespread, found in North, Central and South America (Buyck and Ovrebo 2002), Europe, temperate (Gorbunova 2014) and tropical Asia, Africa (Natarajan et al. 2005), and Australasia (McNabb 1973). It is the only *Russulaceae* genus that occurs in the *Nothofagus* zone of temperate South America (Singer 1953). *Lactarius* is mainly known from the north temperate zone, but some species also occur in tropical Asia and Africa. *Lactifluus* has a more tropical distribution than *Lactarius*, with most species known from tropical Africa, Asia, South America, and Australasia, but some also occurring in the north temperate zone (Verbeken and Nuytinck 2013).

In addition, Shi et al. (2016) stated that there are still critical gaps remaining in our understanding of biogeographic patterns of mycorrhizal associations, and our limited knowledge of the anthropogenic factors responsible for shifting plant-mycorrhizal distributions has hindered the efforts to predict the ecosystem feedbacks to climate change.

CONCLUSION

The species of ectomycorrhizae identified in the study and the knowledge about which tree species they form partnership with have great critical importance, especially for the propagation of ectomycorrhizal seedlings to be grown in the central and northeastern Kazakhstan regions where environmental conditions and anthropogenic effects are severe. As a result, ectomycorrhizas must be used as a major performance-enhancing tool in afforestation and restoration studies in the Irtysh River basin, under extreme ecological conditions and climate change effects.

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Conflicts of Interest

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Long-Term Forest Dynamics of Oromediterranean Fir Forests in Greece

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ABSTRACT

The present study illustrates the situation of the genus *Abies* in the Mediterranean and in Greece, focusing in detail on the oromediterranean forest of the southern Peloponnese, at Mt. Taygetos. The existing pattern of forest dynamics (mosaic cycle) and also the timberline dynamics are presented and explained. Since fir forests are highly susceptible to drought-related impacts (fire and fir dieback: insect outbreaks/ forest pathogens), the analysis of the present situation is put into perspective by looking at the role of direct human influence and the climatic fluctuations of the past, taking into consideration dendrochronological findings and archival climate records. In view of climate warming the question is whether in recent decades the fingerprints of climate change can already be observed in the Greek fir forests.
The study concludes that drought periods and climatic extremes have been an essential part of Greek climate for many centuries, causing high natural forest vulnerability. Therefore, fir dieback and fires are not a new phenomenon either – but in recent decades the accumulation of fuel, caused by land abandonment, has increased the danger of large wildfires. Nevertheless, the Greek mountain forests are highly endangered by increases in aridity and/or more frequent climate extremes (heat waves), together with increased risk of wildfires. Recommendations for an active forest management (counteracting expected adverse effects of climate change, by focusing on the establishment of an *Abies cephalonica* Loudon /*Pinus nigra* J. F. Arnold mosaic cycle) and further scientific research are provided.

Keywords: Greece; *Abies* sp.; *Pinus nigra*; timberline; dendrochronology; forest management

INTRODUCTION

The consequences of global warming on forest ecosystems and vegetation dynamics are an important ecological issue (Peters 1990, Lindner et al. 2010) that should be considered under adaptive forest management (Lindner et al. 2014, Yousefpour et al. 2017).

All Mediterranean *Abies* species grow in relatively moist and cool mountain areas, where they withdrew after the last ice age, forced by the increasingly dry and warm Mediterranean climate. The disjunction of their habitats caused the formation of various endemic species. Today, some of them (such as the fir species in northern Africa and *A. pinsapo* Boiss. in southern Spain) have already been reduced to small relict areas, while in the case of *Abies nebrodensis* (Lojac.) Mattei, on Sicily, actually only a few specimens have survived (Mayer 1985, Schütt 1994, Brandes and Ise 2007). Therefore, already 40 years ago, Quézel (1977) viewed the chances of survival for some of the Mediterranean *Abies* species with great skepticism.

Due to geographical isolation and the special climatic conditions, oromediterranean fir forests are restricted to mountain areas. Growing at its ecological boundary makes the genus particularly sensitive in regard to climate-ecological fluctuations or even profound climatic changes. For this reason Brandes and Ise (2007) identified the *Abies* forests as highly suitable study objects, in order to detect fingerprints of climate change (Walther 2001) in the vegetation of the Mediterranean. Global warming is most likely to exacerbate tree stress near the margins of the geographical or altitudinal species ranges, i.e. observations should be concentrated at ecological boundaries of zonal tree species (Delcourt and Delcourt 1992, Paine and Baker 1993).

Fire, as well as insect-outbreaks and pathogens, influence the health, the dynamics and the regeneration of Mediterranean fir forests. Several fir diebacks and wildfires were recorded in the last decades in the fir forests in Greece. But, are these processes already a consequence of a changing climate, or (still) part of natural climatic fluctuations, as they have existed for a

long time? In order to assess this question, a broad scientific approach, considering many factors, is necessary.

The present study deals with the fir and pine/fir forests of the oromediterranean altitudinal zone (ca. 900-2000 m a.s.l.) on the Hellenic peninsula. They are of great ecological and economic importance, representing one of the most important forest types in Greece. Their existence depends on climate-ecological conditions differing from the lowlands by higher precipitation and lower temperatures (Efthimiou et al. 2014). Any climatic impacts changing these favorable hygric conditions would have negative ecological consequences on the mountain forests, especially in combination with land-use changes, leading to the accumulation of fuel for wildfires (Sarris et al. 2014). The aim of the current study is to give a synoptical, wide-angle view on the situation of the genus *Abies* in the Mediterranean and more precisely on the oromediterranean forests of the southern Peloponnese, at Mt. Taygetos, based on the results from earlier studies (Block and Brandes 2001, Brandes 2007, 2008, 2009, Brandes and Ise 2007). In addition, more recent ecological and forestry studies conducted after the 2007 wildfire were taken into account (Arianoutsou et al. 2010, Christopoulou et al. 2013, 2014, 2019, Sarris et al. 2014).

Dominant Tree Species in the Oromediterranean Forests of Greece

In Greece, rather extended fir forests, estimated at almost 200000 ha (Aussenac 2002), still exist in the higher mountain areas, many of them under close-to-natural conditions. Three fir species are present on the Hellenic peninsula, namely *Abies alba* Mill., *Abies borisii-regis* Mattf. and *Abies cephalonica* Loudon, replacing each other from north to south, displaying an increasing drought-tolerance (Horvat et al. 1974, Brandes 2007, Korakis 2015, Mitsopoulos et al. 2015, Papadopoulos 2016). *Abies alba* reaches only the northernmost mountains (Rhodopi) at the Bulgarian border, seldom forming pure stands. The Balkan endemic *Abies borisii-regis* appears first in mixed forests (pines, fir and beeches) of Mt. Olympus and the northern Pindus (Smolikas, Tymphi), between ca. 900-1500 m a.s.l. Further south, this fir species forms huge, pure forests in the central and southern Pindus Mountains of Western Greece (Pertouli area, Tzoumerka, Tymphristos) between ca. 900 m a.s.l. and the treeline at ca. 1800 m altitude.

Greek fir (*Abies cephalonica*) is an endemic Greek species, appearing first in the high mountains of Central Greece (Vardoussia, Giona, Parnassos), often in mixed stands with *A. borisii-regis*. Due to intermediate morphological features, the two species are sometimes difficult to distinguish in this common distribution region (requiring a genetically-based differentiation) (Papadopoulos 2016). Eventually, in Southern Greece (i.e. mountains of the Peloponnese; Mt. Aenos/ island of Cephalonia; Mt. Parnitha in Attica; island of Euboea) *A. cephalonica* is the only existing fir species.

Pure fir forests, pure black pine (*Pinus nigra* J. F. Arnold) forests as well as mixed forests of fir and black pine are characteristic for the oromediterranean altitudinal zone of the Peloponnese (between ca. 900 m and the timberline at ca. 1900-2000 m a.s.l.). On extremely dry and rocky sites also *Juniperus foetidissima* Willd. can appear in the upper margin of the oromediterranean forests, forming the treeline in some areas of the Peloponnese (Erymanthos, Kyllini), in Central Greece (Giona) and also in the Pindus (Tymphi, Tzoumerka) (Brandes

2007, 2009). This reveals that for centuries some juniper-sites have obviously already been too dry for the establishment of *A. cephalonica* or *P. nigra*.

The Oromediterranean Fir Forests Endangered by Climate Change

In recent decades, the oromediterranean forests of Greece have suffered not only from drought-related fir decline, but also from severe wildfires. The catastrophic fire year of 2007, destroying huge forest areas also in the mountains (e.g. at the National Park of Mt. Parnitha, near Athens, or at Mt. Taygetos on the Peloponnese) (Koutsias et al. 2012), caused the well-founded concern that the mountain forests, i.e. ecosystems not adapted to high intensity crown fires, will be highly endangered by any future climate changes, which increases aridity (Arainoutsou et al. 2010, Christopoulou et al. 2013, 2014, 2018, Sarris et al. 2014).

Several climate studies predict for the 21st century a warming trend in the Mediterranean basin, being associated with a decrease in summer precipitation and an increase in summer temperatures, as well as in the frequency of drought events and heat waves (Gao and Giorgi 2008, Giorgi and Lionello 2008, Fischer and Schar 2010, Diffenbaugh and Giorgi 2012, Nastos and Kapsomenakis 2015). This makes the area highly vulnerable to consequences of global change, including an increase of wildfires (Giannakopoulos et al. 2009, Dury et al. 2011, Lelieveld et al. 2012). In this context it must be stressed that the change of certain extreme values could already have an influence on vegetation – long before any climatic change becomes evident in climatic mean values (Walker 1991, Walther 2001).

THE CASE STUDY OF MT TAYGETOS: CLIMATE, TREE SPECIES AND EXISTING FOREST PATTERN IN THE ORO-MEDITERRANEAN ZONE

Mt. Taygetos (at ca. 36°57´N/22°21´E) is the highest (max. 2407 m a.s.l.) and longest mountain range on the Peloponnese and the southernmost on the Greek mainland (for location see Figure 1). Because of the immediate closeness to the Messenian Gulf the roughly north-south stretching mountain range is not the driest of the Peloponnese, though.

Climatologically Mt. Taygetos is an exemplary Mediterranean high mountain area. It underlies the characteristic Mediterranean summer incision in precipitation (causing intense dryness), a high full-year variability of precipitation and strong insolation.

Winters are cold, bringing frost and snow (> ca. 1000 m a.s.l.). Spring and autumn are characterized by higher precipitation and more cloud cover than in the lowlands (e.g. Bolle 2003). Based on data from a meteorological station situated at 1310 m a.s.l. mean annual precipitation is 983.4 mm, but during the driest summer month (July) precipitation can be lower than 3 mm. Only this special Mediterranean mountain climate renders the existence of the oromediterranean coniferous forests possible. Reliable, long-term climatic data from high elevations in Greece are missing, but Figure 2 gives an approximate idea about the oromediterranean climate (Artemisia is a Taygetos village at 750 m altitude, i.e. at the lower margin of the oromediterranean zone).
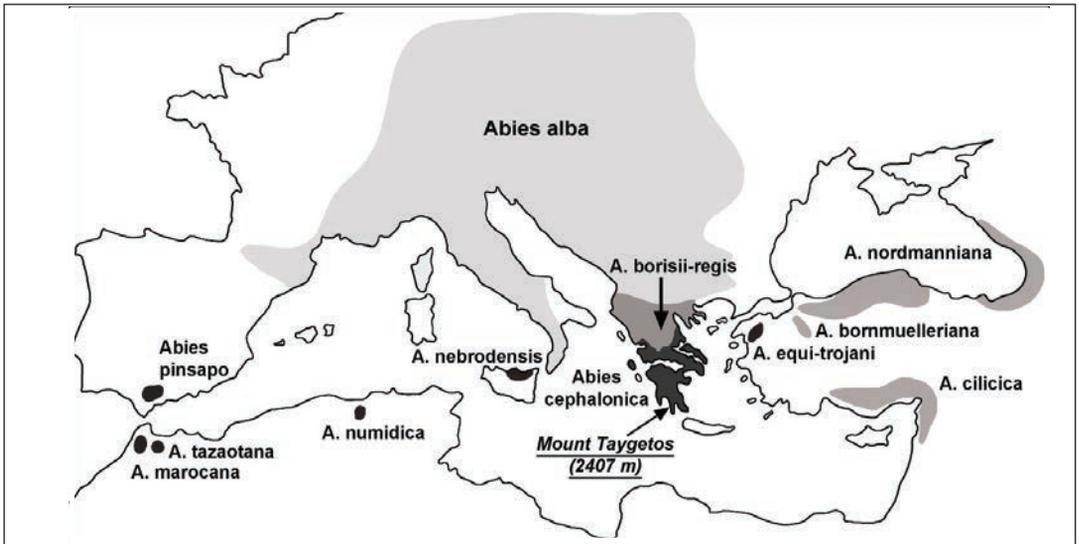
Figure 1. The study area (Mt. Taygetos, Greece) in regard to the circum-Mediterranean distribution of *Abies* species (from Brandes and Ise 2007, based on Schütt 1994).

Geologically, limestones (including dolomite and marble) prevail and often create rocky, dry soil conditions. In steep terrain this can impede the formation of dense forest cover. However, in flat or hollow areas with thick soil cover and in places where water-impermeable schist forms the bedrock, dense forests can grow.

The vegetation of the oromediterranean altitudinal zone is dominated by coniferous forest, which at Mt. Taygetos is formed by *A. cephalonica* and *P. nigra*, reaching from ca. 900 m a.s.l. to the timberline, at ca. 1800-1900(-2000) m a.s.l. (Brandes 2007). The two species differ from each other concerning their adaptation strategies and ecological behavior. Yet, exactly this makes them complementary in forest dynamics.

A. cephalonica is a shade-tolerant species and does not regenerate (well) on open sunny sites and on dry soils. Even though the Greek fir is a quite drought-resistant *Abies* species, it remains relatively demanding in regard to water supply, compared to black pine or juniper. Drought stress also explains why it is quite susceptible to pathogens (fungi, insects, mistletoe). In this regard it is no surprise that healthy *A. cephalonica* specimens of very old age (> 200 years) are hard to find on Mt. Taygetos.

Under fully natural conditions, wildfires are not a frequent event in the oromediterranean forests. If they were, the Greek fir could not be a characteristic tree of the mountain forests since it has no morphological adaptations against fire: the bark is thin, not providing insulation against extreme heat, and the branches reach far down on the stem (no self-pruning), facilitating crown fires. Accordingly, wildfires usually lead to the (almost) total destruction of fir stands. Natural post-fire recovery of fir is difficult, since the tree forms no serotinous cones, which could play a role as a canopy seed bank (Arianoutsou et al. 2010, Mitsopoulos et al. 2015, Christopoulou et al. 2018).

P. nigra, the other important tree of the mountain forest, is a typical pioneer species, invading successfully open, sunny
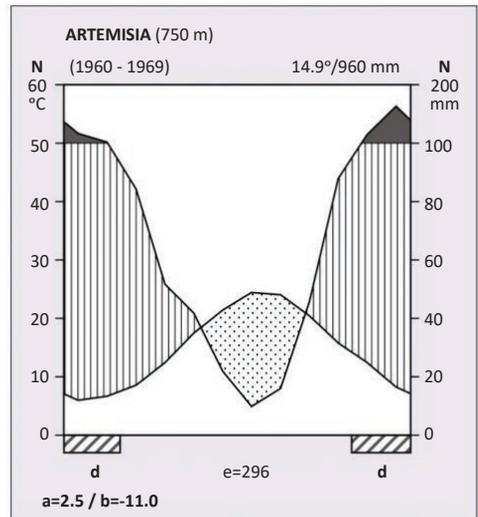
a = mean daily minimum of the coldest month (°C)
b = absolute minimum (°C)
d = months with absolute minimum < 0 °C
e = mean length of frost-free period (days)

Figure 2. Mediterranean mountain climate: diagram from Artemisia, Mt. Taygetos, ca. (750 m a.s.l.) (Brandes 2009).

and dry, even rocky sites – but it is almost unable to regenerate in the shade. Moreover, the heliophilous black pine is quite drought-resistant and shows little damage by pathogens (fungi, insects). Only mass outbreaks of *Thaumetopoea pityocampa* Schiff. (processionary moth), the most important insect pest in Greek pine forests, can lead to intense, but usually non-lethal

defoliation of black pines (Avtzis 1983, Markalas 1987). The effect of processionary moth on black pine tree growth has been documented in several studies in the Mediterranean Basin, with the range of defoliation varying from 10 to 100% (Jacquet 2012). *P. nigra* has a very thick bark (up to 10-15 cm) which protects the cambium from lethal heat damage and also allows older specimens to withstand low intensity surface fires (Tapias et al. 2004, Fulé et al. 2008, Christopoulou et al. 2013). Yet, like fir, black pine does not produce serotinous cones and does not maintain a canopy seed bank, so it cannot really be qualified as a typical pyrophyte (Christopoulou et al. 2013, 2014). Still, the overall excellent adaptation to its environment enables *P. nigra* to reach an age of 400-500 years and even more, making it very suitable for dendrochronological studies (Brandes 2007).

For both species it must be stressed that their seeds ripen in autumn. Thus, if a devastating forest fire strikes in summer (when most wildfires happen in Greece), there are no mature seeds to ensure regeneration. At least in large wildfires, i.e. fires that are larger than 500 ha (San-Miguel-Ayanz et al. 2013), this leads to an increased risk of non-potential re-establishment of the burned forest, due to regeneration failure (Ganatsas et al. 2012, Christopoulou et al. 2019).

The existing forest pattern at Mt. Taygetos constitutes a mosaic of distinct patches of different sizes, formed by pure stands of both species (of various age groups), and of various types of *A. cephalonica/P. nigra* mixed stands with different crown layers.

This type of oromediterranean coniferous forest, as we find it on Mt. Taygetos, exists also in the other mountain areas of the Peloponnese (Horvat et al. 1974, Brandes 2007). Therefore, conclusions from this contribution are not only meaningful for Mt. Taygetos, but for the whole Peloponnese, as well as for fir/black pine forests in central and western Greece in general.

Current Forest Dynamics and Its Drivers: Mosaic Cycle, Fire and Fir Dieback

Various types of forest patches exist in the oromediterranean fir/black pine forest of Mt. Taygetos (Figure 3). Analyzing this pattern, it becomes obvious that the forest regenerates by means of an exemplary mosaic cycle, with fire being its main driving force (Pickett and White 1985, Block and Brandes 2001, Brandes 2007, Brandes and Ise 2007).

Black pine usually starts the forest recovery of open sites. Not only after fire, but also on abandoned field terraces, e.g. stone-built threshing floors (*alonia* in Greek), which are to be found under forest cover on the eastern side of the mountain at ca. 1300 m a.s.l. As the pines grow, forming stands with (semi-) shady conditions on the ground, the heliophilous *P. nigra* cannot regenerate anymore and *A. cephalonica* starts to invade the shady sites. Without disturbance, these patches will develop
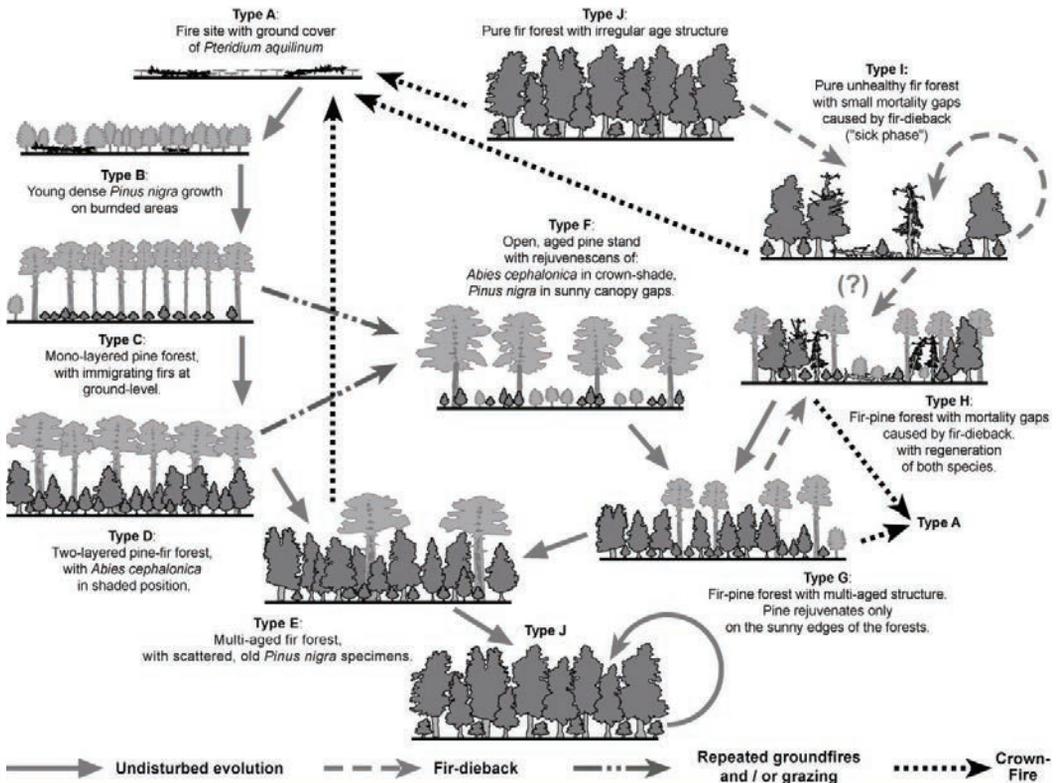

Figure 3. Fir/black pine mosaic cycle in the oromediterranean forest of Mt. Taygetos (from Brandes and Ise 2007, modified).

into a fir forest with a few single old black pine specimens remaining. At the end of an undisturbed succession patches of pure, dense fir stands can be observed. These can fall into a so-called sick phase, caused by fir dieback.

It also becomes obvious that grazing and surface fires explain the shape of the patches, where *P. nigra* is growing to quite fire-resistant, old specimens in open stands (with little regeneration of *Abies* in the crown-shade and of black pine in the sunny spots). Dendrochronological research by Christopoulou et al. (2013) confirmed that low intensity surface fires, with mean fire intervals of ca. 30 years (8-103 years on single tree analysis), have been a common phenomenon at Mt. Taygetos for a long time, even during the 20th century.

Fir dieback, the second driving force of the mosaic cycle, is a slow process caused by various factors (e.g. climate, air pollution, pathogenic fungi), out of which drought plays the key role. Indeed, a close temporal and causal connection between drought years and wide-spread fir dieback has been documented in fir stands of various regions in Greece for several years of the 20th century and also in 2001 and 2002 (Markalas 1992, Brofas and Economidou 1994, Raftoyannis and Spanos 2015). The lack of water causes stress to the trees and thus favors the attack of various biotic factors. Figure 4 shows the complex process framework of the fir dieback (Brandes and Ise 2007).
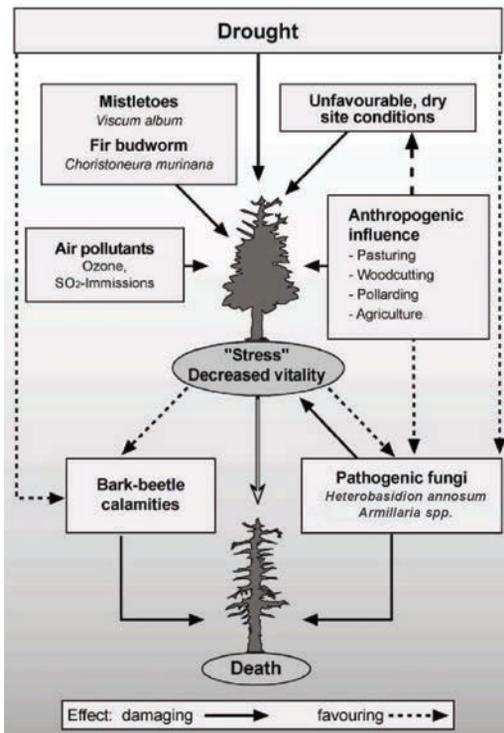
Figure 4. Process framework of fir dieback in Greek *Abies* forests (from Brandes and Ise 2007, modified).

Unfavorable site conditions (dry, rocky soils on limestones) can easily lead to stress, if there is a lack of rainfall. On dry sites plants usually develop higher root-to-shoot ratios in order to secure access to water (Brunner et al. 2015). Nevertheless, in some cases, especially on rocky sites, the root system cannot grow enough anymore in order to sustain the above-ground parts of a tall tree and to supply it with water and nutrients. In this case the tree starts to die from the top, which sometimes leads to so-called spike top growth forms (Fritts 1976, Brandes 2007).

Anthropogenic influences such as air pollution (Larsen et al. 1990, Lelieveld et al. 2002) and grazing have contributed in various ways to the fir forests damage (e.g. mechanical damages to trees, site deterioration, spreading of pathogenic fungi). Fir budworm (*Choristoneura murinana*) and mistletoe (*Viscum album*) are also species that can cause damages to *Abies* in Greece (Sinclair et al. 1987, Barbu 2010, Tsopelas et al. 2004, Ganatsas et al. 2012, Smidla et al. 2019).

The stress caused by the already mentioned factors greatly facilitates a successful attack by pathogenic fungi. Only *Armillaria* species kill single firs rather quickly. The more widely spread fungus in the Greek fir forests is root rot (*Heterobasidion annosum*). By slowly destroying the root system of a tree, it leads to difficulties in water uptake and dieback of the crown. Therefore this fungus strongly contributes to the widespread phenomenon of unhealthy looking firs, being in a sick-phase which can last for many years or even decades (Tsopelas and Korhonen 1996, Tsopelas 1999). Numerous fir individuals within the forest of Taygetos are in a poor state of health. The same can be observed in other mountain forests of the Peloponnese, as well as in Central Greece and the Pindus Mountains (Brandes 2007).

Eventually, the outbreak of bark beetle is also very closely connected with drought years, mainly if the rainfall deficit occurs in spring and early summer. The insects profit from the weakened defense mechanism of the stressed *Abies* trees and also from higher temperatures during periods of drought. Episodic bark beetle calamities, on regional or even nation-wide scale, have been documented by the Greek forests authorities since 1929 (Kailidis and Markalas 1988). The last intense and nation-wide bark beetle outbreak, with losses of up to 40% of the trees in certain regions, happened between 1987 and 1990 and was connected to the drought period of 1986-1990 (Markalas 1992).

Fir dieback leads to the slow decay of single trees, but also to distinct small mortality gaps inside the dense forest (Brandes 2007, Brandes and Ise 2007). Especially the selective dying of firs in mixed stands with black pine clearly points out that pathogenic fungi (which only rarely do any damage to *P. nigra*) are an important factor in the process framework of fir dieback. Since in southern Greece the mortality gaps are rather small (max. diameter ca. 100 m), they get closed again by the establishment of black pines or even firs.

Timberline Dynamics

A study of 11 high mountain areas of Greece revealed that the ecology of timberlines in Greece is not only influenced by the climatic conditions of winter (snow, frost), but also by the complex dryness, which corresponds to rocky, dry sites; high insolation, dry summers and drought events. The

complex dryness plays a limiting role, influencing the health of established trees and of seedling establishment / tree regeneration (Brandes 2007).

The dieback of firs is the most important dynamic process at the timberline. In some areas, among them Mt. Taygetos, the decay of fir stands has already led to a local treeline decline of 50-200 m of altitude. At the upper margin of the timberline ecotone, where rocky, dry sites prevail, the slow dying of older firs is accompanied by a general insufficiency or even total lack of regeneration. Only at the lower part of the timberline ecotone, in flat or hollow landforms, with deeper soils and snow/moisture accumulation, the rejuvenation of firs and black pines, caused by the reduction of grazing, can be vigorous. Often former, sometimes even terraced pasture grounds and fields are reconquered by young dense tree stands. Figure 5 and 6 illustrate the situation at Mt Taygetos.

The important role of the ecological complex dryness at the timberlines in southern and central Greece is confirmed by contrasting treeline observations in Albania, Montenegro, Spain and Italy. There, a high altitude tree densification or even a locally starting treeline advance has been documented (Vitali et al. 2019). This recent process, synchronic at all sites (last 30 years), is mainly a consequence of reduced grazing, with still unclear role of the climate. Even though the sites examined by Vitali et al. (2019) belong culturally (transhumant land use) and geographically to the Mediterranean world, heat and summer drought are not major limiting ecological factors in them. This becomes clear by looking at the ecological profiles and the geographical distribution of the tree species forming the timberlines: *P. heldreichii*, *P. peuce*, *P. sylvestris* and *P. uncinata*. The last one does not exist in southeastern Europe at all, so it cannot be compared. But *P. peuce* and *P. sylvestris* are only to be found in the very north of Greece (Rhodopi mountains), i.e. in areas without intense summer drought. *P. heldreichii* reaches its southern geographical limit at ca. 40° latitude, on Monte Pollino in Italy and in Greece on Mt. Olympus and in the northern Pindus (north face of Tymphi, Smolikas), also forming the treeline. Further south, the climate-ecological situation, even at high altitudes, becomes too dry for Bosnian pine (Brandes and Ise 2007).

The regressive timberline dynamics, caused by fir dieback and lack of fir regeneration, make it obvious that in recent decades the climatic situation for the survival and the rejuvenation of fir at the timberline ecotone has been unfavorable. Human impact (mainly by grazing of sheep and goats) cannot explain these dynamics, because it has been strongly reduced since the second half of the 20th century. Today, fir stands are disintegrating and slowly disappearing, which had grown in times of higher population in the mountain villages – and therefore in times of much stronger grazing impact (ca. 1750-1950 AD), but probably of more favourable climatic conditions for rejuvenation. Since *A. cephalonica* (similar to *P. nigra*) depends very much on precipitation in late spring and summer (Papadopoulos 2016, Fyllas et al. 2017), it may be concluded that single severe drought years and longer periods of sub-normal rainfall conditions, as revealed by tree-ring chronologies from *Pinus nigra* at Mt. Taygetos (Brandes 2007), also determine the conditions for survival and regeneration of *Abies*, especially at the timberline ecotone. At this ecological boundary the winter climate conditions are an additional aspect, reducing the number of climatically favourable years even more. All together it is hard to assess, which factors

exactly are decisive in impeding regeneration. For sure it can only be said that climatic influences, i.e. a series of favourable years (in winter and summer climate), obviously play a decisive role in successful tree seedling establishment in the ecotone. It is remarkable that the treeline in Greece does not advance in altitude, which is caused by reduced grazing and/or warmer temperatures. It seems that in recent decades the ecological complex of dryness stands against a treeline advance, as it is documented in climatically wetter mountains on the Balkans and as it is generally expected for many treelines in other parts of the world (Holtmeier 2003, Vitali et al. 2019).

Climatic Fluctuations and Their Effects on Forest Dynamics in the Past

Since drought years play a central role in fir dieback and wildfires, it is of special interest to ask if these events are a new phenomenon of the 20th and 21st century, related to global warming – or if they occurred in the past as well?

The limited information capacity of meteorological data (records in Greece start only since the end of the 19th century) makes it necessary to switch to methods of dendrochronology, combined with information from historical sources (Sarris et al. 2014). Both yield proxy data of climate from the past (Glaser et al. 2000).

Climatic Information from Dendrochronology

Dendrochronological research carried out on *P. nigra* in Mt. Taygetos (sampled on 1400-1700 m a.s.l.) provided the data for tree-ring width chronologies of this area (Brandes 2007, 2009). Figure 7 shows a 472 years chronology going back to 1538 AD, together with a 10-year low-pass filter. The figure was made by R using the dplr package (Bunn et al. 2019). Statistically revealed climate-growth relations indicated that the precipitation during the growing season (first of all in May/June) has the strongest influence on the tree-ring width of black pines of Taygetos (Brandes 2007, 2009). Similarly, Fyllas et al. (2017) found that the growth of both *A. cephalonica* and *P. nigra* at Mt. Taygetos was positively correlated with May precipitation.

The periods of suppressed growth in the low-pass filtered chronology may be interpreted, under simplifying assumptions, as relatively dry phases, mainly concerning spring and early summer precipitation (and vice versa).

As a whole the dendrochronological results from Mt. Taygetos prove the occurrence of marked long-term cyclic fluctuations in precipitation, superimposed by high interannual rainfall variability, a characteristic feature of the Mediterranean climate. Hence the drought years at the end of the 20th century are to be seen as part of an intense and long-lasting climatically unfavourable dry period. Nevertheless, set into a view of the total this period is not at all to be classified as exceptional.

These dendroclimatological results from Mt. Taygetos fit into the general picture drawn by other tree-ring analyses from the eastern Mediterranean (Waldner and Schweingruber 1996, Hughes et al. 2001, Papadopoulos 2016, Klippel et al. 2018).

Climatic Information from Historical Archives

Historical sources are well suited for the verification of dendroclimatological findings (as demonstrated, e.g. Richter and Eckstein 1990) and yield valuable information on their own. By evaluating Venetian archives, Grove and Conterio (1995) made it evident for Crete (ca. 200 km south of Mt. Taygetos) that in some years of the 16th and 17th centuries severe winters
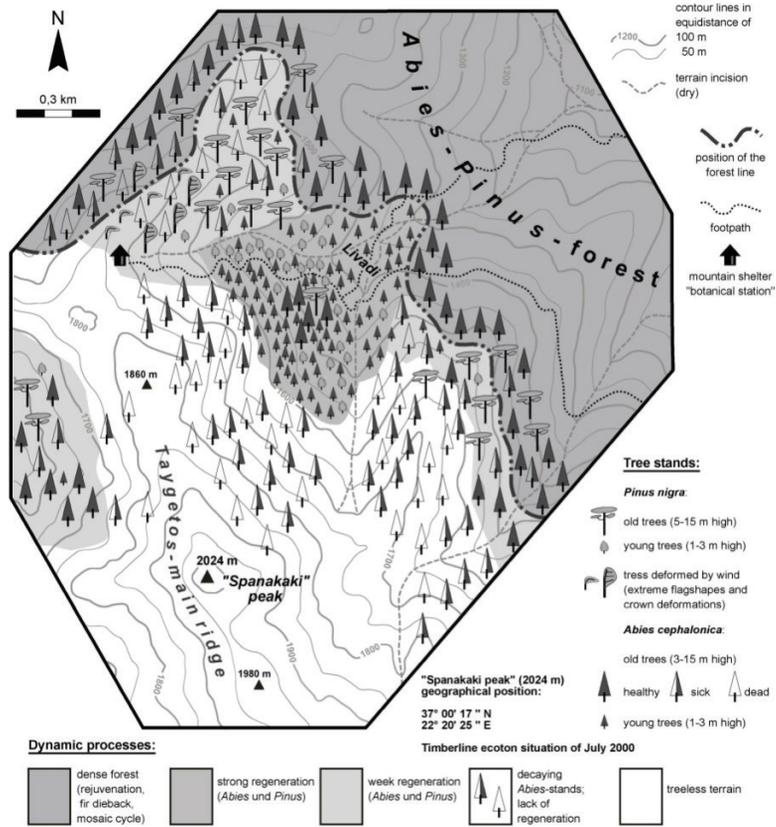
Figure 5. Exemplary situation of dynamics at the timberline ecotone, near Spanakaki peak (2024 m a.s.l.) on the eastern side of Mt. Taygetos, situation of July 2000. Based on Brandes (2007), modified.
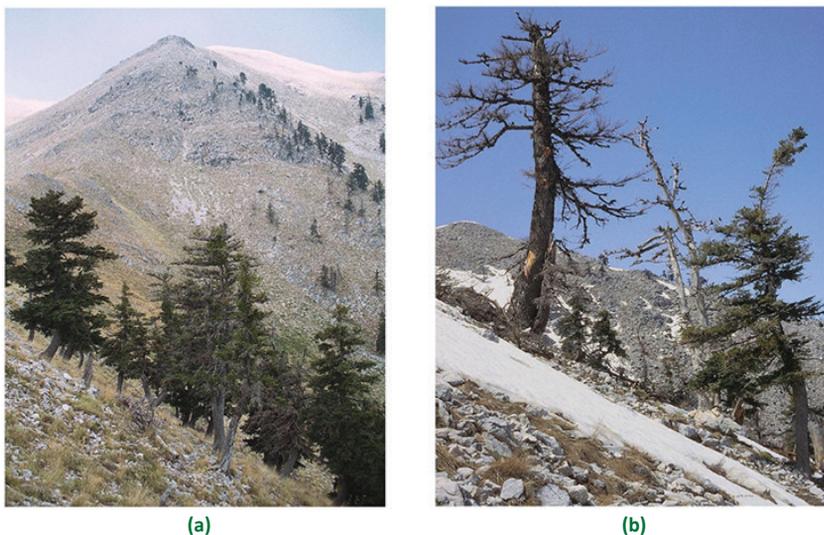

Figure 6. Lack of regeneration and fir dieback, NE of Spanakaki peak, Mt. Taygetos, ca. 1700-1800 m a.s.l., situation of July 2000 **(a)** and April 2000 **(b)**.
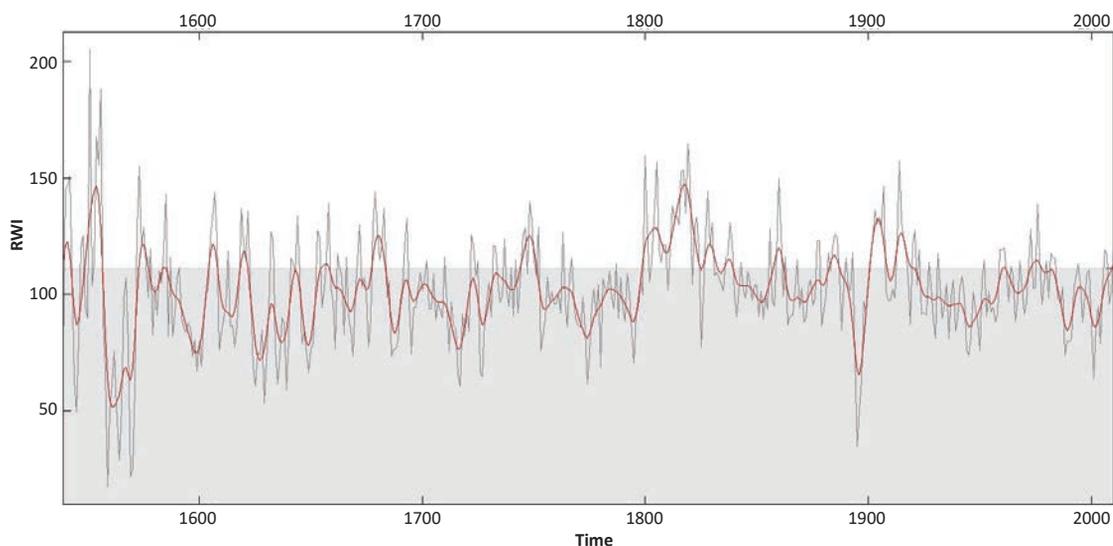
Figure 7. Year ring chronology from *Pinus nigra*, Mt. Taygetos, 1538-2010 AD, smoothed by a 10-year low-pass filter. Small sample replication (8 trees) contributes to strong oscillation amplitudes before the year 1640.

and heavy summer rains, unusually intense compared to 20th century standards, occurred there. Concerning the periods of drought, the authors even state that none of the 20th century drought years have experienced such long rainless periods as those in the period 1547-1645 AD, nor such a complete lack of winter rain.

Another example is 1715 AD, according to Xoplaki et al. (2001) a year of great famine in central and southern Greece, following dryness all over the country from November 1712 to summer 1714. In 1873/74 thousands of people and animals died of starvation in Turkey. According to Kuniholm (1990), tree-ring chronologies from all over the Aegean show subnormal growth for 1873 and 1874, following four other years of significantly subnormal growth. The Taygetos chronologies too identify 1861-1874 as a period of unfavourable rainfall conditions. These examples, and also other well-known drought events in the Aegean region, are mirrored in the tree-ring chronologies from Taygetos, showing years/periods of weak tree growth (Kuniholm 1990, Grove and Grove 1992, Brandes 2007).

In general, information from dendrochronology, historical archives and archaeology clearly affirms that for millennia drought years, as well as decennial and centennial precipitation fluctuations, were essential parts in the spectrum of natural climatic variability in the Aegean. Obviously no lasting climatic changes have happened in this region since the last sub-pluvial (2000-3000 BC). This relative constancy of climate also manifests itself in the good agreement between ancient descriptions and today's plant cover and wind trajectories (Lamb 1977, Thirgood 1981, Hempel 1990, 2000, Kuniholm 1990).

Based on dendrochronological and historical information, it may be concluded that for hundreds of years drought events and drought periods have been an integral part of Greek climate. Therefore, it must be assumed that seasonal water deficits would always have had negative influences on the physical condition of *Abies* on the Hellenic peninsula. And indeed, indications of this

have been found even in statements from antiquity. Theophrast (370-285 BC) and Plinius (first century AD) say that the best timber used in Greece came from the geographical region of Macedonia, in northern Greece (Rhodopi mountains, Chalkidiki) and the Turkish black-sea coast, the worst from Parnassos, Euboea and Arcadia. There is a good reason to assume that they were referring to *Abies* wood, since in antiquity firs were the most important timber for the making of ships, as well as for the construction of buildings (cf. Meiggs 1982). It would fit perfectly into the ecological picture that *A. alba* from the Rhodopi mountains or *A. bornmuelleriana* and *A. nordmanniana* from the wet slopes of the Pontic mountains had delivered superior quality wood, compared to *A. cephalonica*-trunks (from Parnassos, Euboea, Arcadia). In Central and Southern Greece, where summer dryness and episodic drought events are typical characteristics of climate, bark beetle epidemics and pathogenic fungi, easily spread by human activities in forests well above their natural rate, probably have always played a major role in making the wood of relatively tall old firs technically quite useless (rot inside the trunks caused by fungi), exactly like today.

This consideration is confirmed by the example of Parnes (today Parnitha National Park), a mountain of 1413 m altitude, only 20 km north of the Greek capital. During the 5th century BC, when Athens already depended on timber imports from northern Greece in order to realize huge building programs, the timber resources of mount Parnes were not drawn upon. The import by sea was probably preferred not only because of transport difficulties by land (which would have required huge wagon-building programs first), but also because of the firs' poor timber quality (Meiggs 1982).

In the further course of antiquity not even charcoal works seemed to have caused a total destruction of the forest on mount Parnes. This can be concluded from the fact that the area was used for hunting bears and wild boar during the times of Pausanias (late 2nd century AD; Meiggs 1982). Reports from the

early 19th century even spoke of inexhaustible supply of timber there (McNeill 1992). Extended fir forests covered the mountain even during the 20th century. Nevertheless, almost 50% of the fir forest was burned during the 2007 wildfire and even 10 years after the fire *A. cephalonica* recruitment remains rather poor (Christopoulou et al. 2018).

Assessing the Role of Direct and Indirect Human Influences on Forest Dynamics

Based on the above-mentioned, periods of drought, fir dieback or bark beetle epidemics in the forests of Greece should not be readily interpreted as a per se new (i.e. climate change related) phenomenon. Moreover, the direct and indirect human influences, affecting the health of fir stands in Greece in a negative way, should also be taken into account. First of all it must be highly considered that the period of ca. 1750-1950 AD was a time of relative overpopulation in the mountain villages of Greece, which means it was the absolute historical peak period of direct human impact on the mountain forests (McNeill 1992, Brandes 2007). Since the middle of the 20th century sometimes total depopulation of the mountain villages took place, together with land abandonment. Therefore, all recent processes in the Greek oromediterranean forests must always be viewed against the background of former land use.

Direct human impact on the oromediterranean forest consisted mainly in grazing (forest pasture of sheep and goats), which did restrain regeneration, leading sometimes to an over-ageing of stands in the forest and especially at the timberline. Systematic timber-cutting (lumbering for timber or fire-wood, charcoal working and even opening small fields for grain and corn farming up to 1300 m a.s.l.) remained restricted to those parts of the terrain which also allowed a profitable removal of the wood. In the pathless mountain regions this meant that many fir forests were spared the axe because of their remote location and their inaccessibility for wheels. On the Hellenic peninsula most forest roads and even many road connections between mountain villages had not been built before the second half of the 20th century, often only after ca. 1970. Anthropogenous fires contributed to an impairment of the natural forests as well; at least they lead to a considerable increase in the frequency of (low intensity) ground fires, keeping available fuel low.

In some places these direct anthropogenic influences resulted in erosion and site-degradation. But even where this was not the case and the *Abies* forest has fully recuperated (partly involving a temporary species change with *P. nigra* as pioneer on open sites), former timber cutting as well as grazing had a long-term effect which is not very widely known: the spread of pathogenic fungi, above all *Heterobasidion annosum* and *Armillaria* spp.

Since these pathogens damage the root system, they increase the susceptibility of the trees to climatically caused hygric stress. Once airborne basidiospores of the fungi have infected stumps or wounds at the base of the stem, the infections colonize the root system and spread also to healthy trees via root contacts. The significance of pathogenic fungi for today's poor physical condition of Mediterranean fir forests must not be underestimated because they form a permanent disease which is very hard to eliminate from once infested stands (Harrington 1993, Tsopelas and Korhonen 1996, Woodward et al. 1998, Tsopelas 1999).

Since the 20th century has been an additional non-climatic factor related to human activities: air pollution. Even though Greece is not heavily industrialized, nevertheless air pollution is an important factor there because of emissions from Western and Eastern Europe drifting into the Eastern Mediterranean. Thus even in Crete ozone-values can reach the amount which is 10-15 % above the usual levels for Central Europe (Lelieveld et al. 2002). Detailed research for the assessment of the O3-impact on Greek forests has yet to come, though. Experiments by Larsen et al. (1990) on *Abies alba* give an indication of the adverse effects on firs. They yielded the result that ozone fumigation causes a reduction in photosynthesis and the rate of transpiration, but a strong increase in the rate of respiration. Moreover, frost hardiness is reduced, shoot is retarded and the increment in height and diameter is reduced.

In combination with very dry weather conditions (drying out normally rather moist dead wood) even in the mountain forests devastating fires can occur (Mitsopoulos et al. 2015). However, the factor of fire is also closely connected to human action. The high mountains of Greece are embedded into a cultural landscape, which has been populated for thousands of years – and the reduction of human influence (grazing, wood-cutting) since ca. 1950, as well as many years of fire exclusion, have led to an accumulation of fuel in the mountain forests in the last decades (dead wood remaining in the forests; denser tree stands by natural rejuvenation). In the northwestern part of Mt. Taygetos, for example, the longest fire-free period lasted 34 years, from 1973 until high severity crown fire in 2007 (Christopoulou et al. 2013). This development has also lead to more pure stands of Greek fir, dominating many forests today, and increasing the likelihood of large fires (Sarris et al. 2014, Raftoyannis and Spanos 2015).

Most fires occurring in Greece are caused by humans, often by arson. For the period 1983-2008 only about 4.1% of all fires in Greece originated from lightning (Tsagari et al. 2011).

Therefore, there is no doubt that heat waves and general lack of rain heavily increase the risk of wildfires in the oromediterranean forests – but without the spark being caused by humans, it remains doubtful how often wildfires would originate if they were restricted to purely natural ignition (lightning). Against the proven historical background of dry weather periods being a fully normal feature of the Greek climate, we must be careful to interpret extreme fire-years, like the last one in 2007, mainly as a consequence of climate warming. Interestingly, in the last 12 years no major fires have occurred in the mountain forests of Greece.

The Importance of the Mosaic Cycle under Climate Change Conditions

The unusually severe and devastating wildfires of the year 2007 destroyed huge forest areas of the oromediterranean altitudinal zone, formed by *A. cephalonica* and/or *P. nigra*. Especially at the National Park of Mt. Parnitha and in the northwestern part of Mt. Taygetos a few thousand hectares of fir and black pine forest burned down (Arianoutsou et al. 2010). At Taygetos, some of these areas had already been affected by fire in 1998 (Christopoulou et al. 2013). Several studies have monitored the process of natural regeneration and of active reforestation in these two prominent areas (Ganatsas et al. 2012, Christopoulou et al. 2014, 2018, 2019, Efthimiou et al. 2014, Siorokou et al. 2015).

The probably most important finding of these studies is that natural regeneration of both fir and black pine depends first of all on the distance to unburned forest patches, corresponding to the anemochorous seed dispersal of the two species (Ganatsas et al. 2012, Christopoulou et al. 2014, 2018). Therefore, the survival of mature and tall trees plays a paramount role in supplying seeds and shade for the establishment of seedlings.

It also became clear that forest patches, which had been burned twice within a few years at Mt. Taygetos, showed extremely low regeneration (Christopoulou et al. 2014). Of course, a number of biotic and abiotic factors (vegetation cover, soil conditions, competition, seed predation by animals, insolation, climatic conditions in the first years following a fire) also mediate the recruitment of young trees. In microhabitats formed by coarse woody debris (trunks, dead branches etc. covering the soil), where seedlings and saplings are better protected from high temperatures and excessive water loss, regeneration density proved to be higher (Christopoulou et al. 2014, 2018), a fact that does not support the widespread praxis of Greek forestry to remove dead trees after a fire.

Even 10 years after the fires, natural regeneration in the former *A. cephalonica* forests of Mt. Parnitha and Mt. Taygetos was still quite poor. The low levels of regeneration can be mainly attributed to the ecological characteristics of the tree species in combination with the unfavorable, i.e. dry, site conditions in the post-fire environment. The poor recruitment density, especially on sites of formerly pure *Abies* stands, makes it questionable if natural processes alone can ensure the full recovery of the forest. At least on dry sites this seems rather unlikely. Only on sites with favorable conditions, i.e. high rainfall and deep soils (as in Central Greece), a natural regeneration of fir forests is expected to happen within ca. 50 years after a fire (Raftoyannis and Spanos 2015).

If regeneration fails, there is a high risk that former forest sites might turn into open mesoxerophytic grassland, phrygana dominated by *Genista acanthoclada*, *Sarcopoterium spinosum* and *Thymbra capitata* or into areas densely covered by *Pteridium aquilinum* (Christopoulou et al. 2019). This has led to efforts of active reforestation. Planting seedlings is very difficult and costly. It requires careful planning, but often yields very poor results. Moreover, seedling establishment and survival of young Greek firs in time is better accomplished in the protective shade offered by elder plants (Politi et al. 2009). Therefore, at the southern exposures and drier sites of Parnitha, *Pinus nigra* (promising to be more drought- and heat-tolerant than fir) was planted with the aim of *Abies* to return later, under the canopy of black pines. However, the survival rate of the pine seedlings hardly reached 50%, not fulfilling the expectations (Siorokou et al. 2015).

The above considerations make it clear that devastating wildfires are the most serious threat to the oromediterranean forests. This is based on fuel accumulation by land abandonment, combined with drought intensification projected for the Mediterranean region under global warming (Sarris et al. 2014).

Fire suppression and reforestation are difficult steps with highly uncertain results. Therefore, the focus of forest protection must be directed towards fire prevention and active management of the forest structures wherever the terrain is accessible.

A survey by Greek forest experts revealed restrictions in climate change forest management, caused by budget and staff constraints, as well as by the need for the establishment of a new firefighting organization and for the better coordination of all involved parties during fire suppression (Mitsopoulos et al. 2015). Against the background of the discussion about CO_2 emissions and CO_2 sinks, it should be a priority task of national and European authorities to allocate sufficient financial resources and manpower to the Greek foresters. According to the same study, Greek forest experts say that fire prevention in fir forests under climate change should be focused on public awareness and fuel management.

The expected rapidity of climate change makes unmanaged natural ecosystems probably more endangered than managed ecosystems (Gates 1993). Therefore, the results of the present study suggest that forestry measures should not only aim at an improved fuel management, but also at the active establishment of an *A. cephalonica*/*P. nigra* mosaic cycle, as presented in Figure 3. Under optimal conditions, the size of the patches should be aligned with the structures of topography. In relatively flat terrain, fire aisles should form the borders of the patches. Since the risk of fires in the mountain forests will increase with climate change, it is most important to avoid that the fires reach catastrophic size and lead to a total forest destruction on a large scale, making forest regeneration difficult or even impossible.

Yet, on a small scale and of low intensity, fires can even help to establish the mosaic cycle more easily than it is achieved only by mechanical stand thinning. The legislation in Greece does not allow the use of prescribed burning in forest management, although it could possibly provide an important tool to forest managers. Where pure fir stands form huge forests, *P. nigra* islands could be planted to create a start for the establishment of a future mosaic cycle.

Where mixed fir/black pine or pure pine forests already exist, the creation of stand types corresponding to type F in Figure3 should have the highest priority. The study by Christopoulou et al. (2013) confirmed that stands with large *P. nigra* trees having a very thick bark are most likely to survive low intensity surface fires, and therefore they are decisive for natural postfire regeneration of the forest (Christopoulou et al. 2014). Thus, the authors saw it as an important forest management issue to maintain fire resistant stands through appropriate understory fuel treatment (by stand thinning, selective understory removal, moderate grazing and even prescribed burning). In the event of a wildfire this will lead only to low intensity or patchy burning.

At Mt. Taygetos an exemplary mosaic cycle of relative small patches exists on the eastern flank of the mountain. In contrast, in the northwestern part of Taygetos, where topography is much less structured, fires destroyed huge, relatively homogenous forest areas which will need a very long time to recover, if they will recover at all. From this situation the following conclusions can be drawn:

The mosaic cycle of *P. nigra* and *A. cephalonica* makes the ecosystem of the oromediterranean forest at Mt. Taygetos relatively resistant to the impacts of fire and fir dieback. The disturbances in the various single patches give a greater stability to the forest as a whole, compared to pure *Abies* forests (Block and Brandes 2001, Brandes 2007, Brandes and Ise 2007).

As long as forest fires do not surpass a certain fire interval and as long as the burned surface is not too big, the disturbance fire creates patches of different, asynchronous stages of forest succession in which a change of the key species, *A. cephalonica* and *P. nigra,* takes place. This patchwork makes the ecosystem as a whole very stable. As it is typical for many mosaic cycles, at Taygetos it can also be seen that the forest ecosystem exists and

evolves under the headline stability by disturbance (Pickett and White 1985, Remmert 1991, Block and Brandes 2001). For many centuries the forest has adapted to frequent, but low-intensity surface fires (mainly caused by humans). Thus, neither wildfires per se nor the fir dieback will put the existence of the fir/pine forest at risk – as long as these disturbances do not surpass a certain scale or frequency. Since climate change will lead to devastating and more frequent fires, active forest management is necessary in order to counteract this risk.

CONCLUSIONS

The oromediterranean forests of Greece, particularly where formed purely by *Abies*, are not (well) adapted to fires, especially in cases of severe or high-intensity fires. The wildfires of the year 2007, which were connected to exceptional drought and heatwaves, have revealed this clearly. Moreover, the drought-related process framework of fir dieback presents a threat to the existence of fir forests in Greece. Eventually, the regressive timberline dynamics are an indication that in recent decades drought has caused the decay of older firs and also prevented regeneration on dry, rocky sites. All this fits to the expectation that climatically induced effects on vegetation will become visible first at the ecological boundary of a certain species (i.e. at the margin of geographical distribution or at ecotones).

Yet, we must be careful to assign these observations readily as the fingerprints of climate change. Information from dendrochronology and historical archives leave no doubt that severe drought events and dry periods have been characteristic of the climate in Greece for many centuries. This makes it highly likely that fir dieback (in relation with bark beetle outbreaks) is a completely natural phenomenon in the mountain forests of the Hellenic peninsula, not just occurring since the 20th century.

Also, devastating wildfires are to be evaluated in a careful way. There is no doubt that heat waves and lack of rain play a major role in the occurrence of forest fires, but it is wrong to hold climate (change) alone responsible. Two other factors, the accumulation of fuel (caused by land abandonment) and the source of ignition (mainly caused by human impact, often by arson), have to be highly considered. Since both can be controlled by forest management these two factors should be in the focus of actions aiming at the protection of mountain forests.

Nevertheless, there is no doubt that climatic changes, by causing a generally more arid climate in Greece or only by increasing the frequency of extreme heat events, would exacerbate the occurrence of bark beetle outbreaks and fir dieback as well as the risk for devastating wildfires. Since several climate studies expect such a scenario, it will be the task for forest managers to take preventive steps in order to protect the oromediterranean fir/black pine forests, which are highly vulnerable to negative climate change impacts.

Since fire suppression and reforestation have proven to be difficult and expensive tasks (often with very disappointing results), more promising steps should be of first order, such as: education of the population, fuel management, blocking the access to forests during heat waves, and the establishment of a small-scale mosaic cycle by active forest management. Scientific studies on patch dynamics and regeneration mechanisms in the Greek mountain forests should accompany these efforts to enlarge the scientific basis. Moreover, fixed plots for long-term monitoring should be installed to gain more knowledge about the vegetation dynamics inside the forests and on fire sites.

Conflicts of Interest

The authors declare no conflict of interest.

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A Non-Destructive Prediction Method for Wood Density Variations of Silver Birch Trees Growing in the Middle Volga Region, Russia

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ABSTRACT

The density of wood inside the stick varies greatly according to the diameter and height of the tree. The development of a non-destructive method of predicting an average density of wood in all specific breed stem-base density in parts of the tree is of great practical importance. For this purpose, the research was conducted to identify the patterns of wood density dynamics against the height and the diameter of birch trees growing in the conditions of the Middle Volga Region in Russia. Fourteen model birch trees (*Betula pendula*) were selected as research material on two plots in the plantations of the Scientific-Experimental Forest District of the Volga State University of Technology (VSUT) in the Republic of Mari El of Russia. From each felled model tree 50.0 mm cross-section discs were carved at a distance of 1.3 m from the root collar and at relative heights of 0.25H, 0.5H, and 0.75H. Prototypes in the form of 20x20x30 mm rectangular prisms (long side along the wood grain) were made from their north and south sides at a distance of 0.25R, 0.5R and 0.75R from the stem core along the NS diagonal. Density studies were conducted by measuring the density of the wood sample immersed in liquid against the buoyant force. It has been revealed that there are both differences and patterns of change in density in the longitudinal and cross-section directions of the trunk of a birch tree growing in the conditions of the Middle Volga Region in Russia. Based on the results obtained, a mathematical model of these laws has been formulated. Close relationship between the average density of the stick and its diameter at breast height (DBH) cross-section average density, i.e. ρ_{tree}=178.52+0.641ρ_{dbh} (r=0.922) has been revealed. By applying a well-known method of determining the density of cross-section radial core extracted from the wood by an age borer at this height, wood density of the birch trees growing in the Middle Volga Region can be calculated according to this equation.

Keywords: birch wood; tree slenderness coefficient method; height density of a tree; stick diameter density; non-destructive diagnosis of wood medium density.

INTRODUCTION

Scientists and experts from many countries across the world are engaged in the research of wood density of different species of growing trees and timber (Heräjärvi 2004, Repola 2006, Missanjo and Matsumura 2016, Beets et al. 2018, Lachowicz et al. 2019, Farias et al. 2020). Recently, this indicator has particularly gained fundamental importance in tackling global environmental problems, namely, in biomass inventory and carbon balance monitoring in various terrestrial ecosystems (Beets et al. 2018, Farias et al. 2020).

Up to the 1970s in Russia, no particular importance has been given to the research of the technical properties of wood, when wood raw material was in excess, and its cost was negligible. Industry needs regarding high-quality

raw material were met mainly at the expense of pioneer development of new plantations. In subsequent years, the situation changed dramatically, as an acute shortage of high-quality wood raw material was felt in the European part of the country. This initiated the execution of research work (Poluboyarinov 1976, Denisov 1999, Golyakov 2003, Kozlov et al. 2009, Danilov 2016, Platonov et al. 2018).

One of the priority studies of wood density variability inside the trunk of a tree was the fundamental work of Poluboyarinov (1976). Although the results were obtained in the plantations of the North-Western part of Russia, they have been of great scientific and practical interest for other regions.

In the forests of Russia birch is mostly widespread among the hardwood species. Two species, silver birch (*Betula pendula* Roth) and white birch (*Betula pubescens* Ehrh), predominate in growth area size and stock of standing wood among 70 species growing in these forests. Birch wood, especially of these species, has good physical and mechanical properties and is of great industrial importance. It is widely used for the production of sliced veneer and plywood, parquet, chipboard and fiberboard, pellets, as well as for manufacturing rifle stocks, skis, and construction elements. Birch is a valuable raw material for wood chemical and papermaking industries in obtaining charcoal, methyl alcohol, acetone, furfural, cellulose and many other products.

In this regard, wood density is extremely important for its industrial use. This is related to its impact on both the technology of wood raw material processing and the quality of the final product. For example, in terms of the problems of wood cutting, density significantly affects lumber manufacturing technology, as well as wear and tear of saws and other cutting tools (Pesotsky 1970, Chernov et al. 2019).

Nowadays, laminated structures made of wood are widespread, where the density also largely determines their characteristics. For example, a 60% increase in strength and a 45% increase in stiffness of the finished laminated product can be achieved only by the rational arrangement of bonded parts in beams taking into account wood density (Poluboyarinov 1976).

Many physical and mechanical operational characteristics of wood correlate with density: compressive and flexural strength, hardness, rigidity, durability, ability to hold metal fasteners, etc. (Volynsky 1991). In Russian (Interstate) standards the density of birch wood is normalized for lumber and aircraft work pieces according to GOST 968-68 (1968) and GOST 2996-79 (1979), as well as for blanks for sporting and hunting shooting gun stocks according to GOST 16424-83 (1983). In the pulp-and-paper and wood chemical industries the density is the main indicator of the quality of wood raw material, since it determines the relative yield per the unit of its volume.

The maximum density of raw material is of great practical importance for the production of all of the above-mentioned and many other products. It is the density that determines many physical and mechanical properties and specific yield of products from a unit of its volume. One promising way to achieve this goal is the implementation of a non-destructive diagnosis method of standing wood quality and the selection of the most promising trees with maximum density for further cultivation.

The situation is aggravated by the fact that even within the same species of wood density inside the stick along its height and diameter varies depending on the direct or indirect effect of the complex of factors - genetics, soil and climatic conditions of the habitat, forest indices of the stands themselves and, most importantly, geographic region of growth of the specific stands (Poluboyarinov 1976, Lachowicz et al. 2019).

Despite the significant prevalence of birch stands in the forests of Middle Volga Region (occupying about 32% of the forested area), the studies of wood density have been rare hitherto (Denisov 1999) and there are no works directed at identifying the patterns of its variability (densitograms) inside the growing trees. Meanwhile, in recent years there has been an intensive use of birch stands as a source of raw material for wood processing enterprises in this region of Russia, denser wood with high technical characteristics being in special demand.

The purpose of the present work was to develop a non-destructive method for predicting standing wood density for rational use of available inventory of birch stands and, most importantly, their cultivation with specific intended purpose in the conditions of the Middle Volga Region in Russia.

MATERIALS AND METHODS

Birch stands of natural (seed) origin served as the object of the research on the territory of the Scientific-Experimental Forest District of the Volga State University of Technology (VSUT). Experimental plots are presented by birch and lime stands of 11 capacity classes characteristic for the Middle Volga Region. The type of site conditions is C_2; the soil is sod-cryptopodzolic, loamy, and fresh. Silver birch (*Betula pendula* Roth) dominates in the stand composition, while European aspen (*Populus tremula* Link) and Norway spruce (*Picea excels* Link) are sporadically found as well. The average age of birch trees is 70 years; their diameter at breast height (DBH) is approximately 30 cm and the height is 28.5 m.

Two test areas were established in these stands according to GOST 16483.0-80. Seven sample trees based on the 'average model' were selected on each plot for characterizing the plot trees according to the main valuation factors, such as average diameter, height, crown shape, etc.

Before felling each model tree DBH was measured in two mutually perpendicular directions within the accuracy of 0.5 cm, and blazing was made on the north side. The total length, the distance from the butt end to the first open dead branch to the live crown, as well as the stick diameter at relative heights of 0.25H, 0.5H and 0.75H were measured for each felled model tree. At each of these heights and at a height of 1.3 m a disc of about 50 mm was cut out from the stick (Figure 1).

The relative height sampling method has several advantages over the method of fixed height sampling typically used in taxational studies when wood samples are taken in 1, 2 or 3 m. This former method simplifies the data processing on a computer and, more importantly, it allows to directly compare the density of different trees irrespective of their actual height by applying appropriate indicators on the graphs in the form of a densitorgam (Poluboyarinov 1976).

The discs were divided into halves as 'north' and 'south' parts. From each of them test samples were made in the form of rectangular prisms 20×20×30 mm at a distance of 0.25R, 0.5R and 0.75R from the pith (Figure 2).
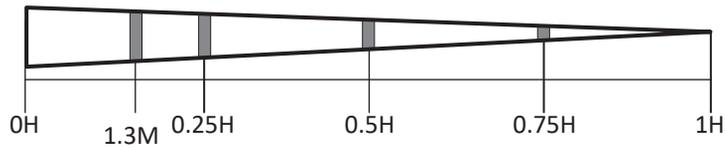
Figure 1. Cross-section severing of a model for taking test discs scheme.

In the study of physical properties of birch wood its density was determined by its basic density (ρ_{bas}, kg·m^{-3}) which is expressed by the ratio of the weight of an oven-dry sample (m_0) to its maximum volume (V_{max}) at humidity equal to or above the saturation limit of the cell walls:

$$P_{bas} = m_0 / V_{max} \tag{1}$$

The physical meaning of the basic density of wood is in the fact that it shows the amount of absolutely dry wood substance contained in a unit of its wet volume; this rate is independent of the humidity of the sample, which simplifies the process of laboratory determination compared with the conventional density ρ_w.

There are several ways of determining basic density, but the method in which the volume is calculated by measuring the buoyancy force of the samples immersed in liquid is the most convenient (a sample can have different geometrical shape) (Poluboyarinov 1976). A detailed description of this method is presented in our previously published work (Fedyukov et al. 2019).

The average density of the cross-section of a stick (ρ_{cs}) was determined by the method of Poluboyarinov (1976) as a weighted average of densities $\rho_{0.25R}$, $\rho_{0.5R}$, $\rho_{0.75R}$. If we take the cross-sectional area of the stick as 100%, the average density of the cross-section will equal to:

$$P_{cs} = \frac{\rho_{0.25R} * 7 + \rho_{0.5R} * 29 + \rho_{0.75R} * 64}{100} \tag{2}$$

where $\rho_{1.3m}$, $\rho_{0.25R}$, $\rho_{0.5R}$, $\rho_{0.75R}$ are wood densities at distances of 0.25R, 0.5R and 0.75R from the pith; $V_{1.3 m}$, $V_{0.25H}$, $V_{0.5H}$, $V_{0.75H}$ are the areas (in per cent) occupied by wood ring area.

The average basal stick density was determined as the weighted average of the densities of the individual parts (discs) along its height:

$$P_{tree} = \frac{\rho_{1.3m} * V_{1.3m} + \rho_{0.25H} * V_{0.25H} + \rho_{0.5H} * V_{0.5H} + \rho_{0.75H} * V_{0.75H}}{V_{1.3m} + V_{0.25H} + V_{0.5H} + V_{0.75H}} \tag{3}$$

where $\rho_{1.3m}$, $\rho_{0.25H}$, $\rho_{0.5H}$, $\rho_{0.75H}$ are stick cross-section (disc) density at 1.3 m, 0.25H, 0.5H and 0.75H height; $V_{1.3 m}$, $V_{0.25H}$, $V_{0.5H}$, $V_{0.75H}$ are the volumes of separate sections (discs) along the stick height.

To determine the basic density by Equation 1 the method based on measuring the buoyancy force of the samples immersed in liquid was used. A detailed description of this method is presented below (Figure 3).
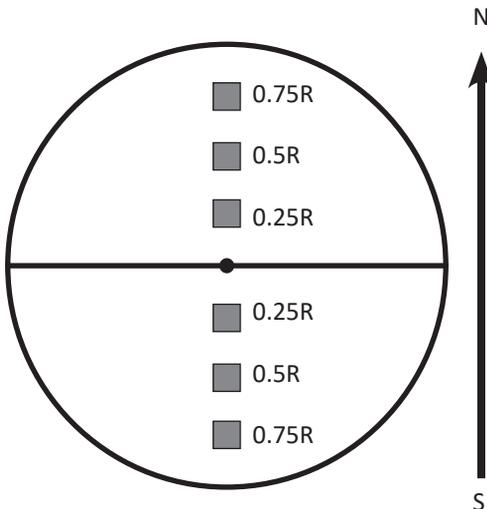

**Figure 2.** Making of test samples from the discs.
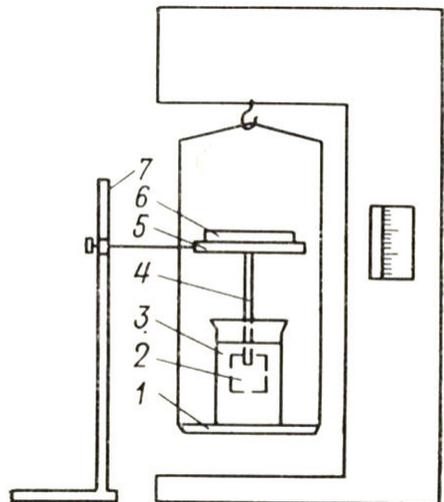
Figure 3. Diagram of the device for determining wood density by measuring the buoyancy: 1 - weighing pan; 2 - sample; 3 - a vessel with water; 4 - needle; 5 - ring; 6 - holder; 7 - tripod.

A vessel with water (3) is placed on the weighing pan (1). The ring (5) fixed to the tripod (7) is placed above the vessel. The ring serves as a support for the holder (6) which has a needle (4) with its end doused into water. In this position, the vessel with water is weighed. After that, the holder is removed and the sample (2), previously held in water, is placed onto the needle to determine its volume. After immersing the sample, a second report is taken. An additional load on the weighing pan is created by overcoming the buoyant force acting on the sample (Archimedes' principle) and numerically equal (if we take the density of water equal to 1) to the sample volume. Consequently, the difference in readings on the scale gives the desired volume in cubic centimeters.

The sample mass in dry conditions will be determined by weighing on an electronic scale after drying at 103° C for about 10 hours.

RESULTS AND DISCUSSION

Changing of Wood Density along the Trunk Radius

According to Table 1, the general character of wood density distribution along the trunk diameter coincides approximately with the results of modern research in other countries (Repola 2006, Missanjo and Matsumura 2016). However, the densitogram received by Poluboyarinov (1976) for this species in the conditions of the North-West region of Russia (Leningrad and Novgorod regions) (Poluboyarinov 1976) is the closest to it.

Meanwhile, there are some differences in wood density variation in the transverse direction, which is observed in the apical part where density gradually increases from the center towards the periphery, while for the birch in the above-mentioned region this occurs only up to 0.4 part of the radius, and there is a further slight decline.

Changing of Wood Density along the Trunk Height

In absolute terms the average wood DBH is close to the results previously obtained in the region of research (Denisov 1999), i.e. about 500.0 kg·m^{-3} for silver birch, which is higher than that of white birch (476.0 kg·m^{-3}). Table 2 shows that birch wood density decreases in a smooth manner along the trunk height.

This densitogram is consistent with the results obtained by other researchers in the past (Ugolev 2001, Danilov 2016) and nowadays (Heräjärvi 2004, Repola 2006, Kozlov et al. 2009, Platonov et al. 2018). For example, in Repola (2006) data are given on lower vertical dependence of birch density compared with spruce and pine. This is explained by the crown structure's specific nature and the way of natural pruning of the stick from the knots. The birch as a light-demanding species undergoes a relatively fast natural pruning of the stick from the knots, and, consequently, the stick with a high raised crown is formed. The annual layers formed at a considerable distance from the live crown have a high percentage of late wood and, accordingly, high density.

As for the wood decrease in density along the birch stick, it appears due to the increase of the vessel area and percentage of parenchyma when approaching the top of a tree (Platonov et al. 2018).

A more detailed analysis presented in Table 1 and Table 2 shows that the reduction in density from the peripheral zone to the core wood is approximately 9.5%, and from the butt end to the top of the tree it is 5.9%. From a technical point of view, e.g. when severing entire tree length into timber assortments, wood density changes along the tree height within 6% are not significant. Density dynamics in horizontal planes of a stick cross-section are of greater practical importance, because they determine the significant difference of specific yield of high-level assortments, as well as the dry matter of the core and peripheral areas of the stick.

Table 1. Changing of the birch wood basic density along the trunk radius.

Cross-section height of stick height fractions	Basic density of stick cross-section fractions (kg·m^{-3})		
	0.25R	0.5R	0.75R
1.30 m	483	509	543
0.25H	479	502	521
0.50H	474	508	517
0.75H	461	484	507

Note: the average density of the stick section is obtained as the arithmetic mean of the zone sections.

Table 2. Changing of the birch wood basic density along the trunk height.

Cross-section height of stick height fractions	Statistical indicator values				
	Arithmetical average (kg·m^{-3})	Standard deviation, $\sigma \pm$ (kg·m^{-3})	Coefficient of variation, V (%)	Error of mean, $m \pm$ (kg·m^{-3})	Accuracy factor, P (%)
1.30 m	512.0	39.74	7.76	8.67	1.69
0.25H	501.0	32.23	6.44	7.03	1.4
0.50H	495.0	28.15	5.69	6.63	1.34
0.75H	482.0	28.11	5.83	6.45	1.34

Consequently, there are less sharp dynamics of decline in density along the height of the tree than the increase in density from the center to the peripheral area in the horizontal plane in all sections of the tree trunk.

The results obtained confirm that the general nature of the change (decrease) in wood density along the trunk height is also consistent with the densitogram of the birch growing in the North-Eastern part of Russia (Poluboyarinov 1976), as well as elsewhere in the world (Repola 2006, Lachowicz et al. 2019).

Mathematical Models for Predicting Wood Density Inside the Tree Trunk

Previous studies also found out that there is a close correlation between the average density of the whole stick and individual density of its levels. Thus, for ripe even-aged stand of wood sorrel spruce forest in Leningrad region the dependence is obtained (Poluboyarinov 1976):

$$y = 34.09 + 0.911 \cdot x \qquad (4)$$

where y is stick average density in $kg \cdot m^{-3}$; x is density at breast height in $kg \cdot m^{-3}$.

In birch and lime forest we also established a close relationship between wood density (ρ_{tree}) and average density of a cross-section at a height of 1.3 m ($\rho_{1.3}$):

$$\rho_{tree} = 178.52 + 0.641 \cdot \rho_{1.3} \qquad r = 0.922 \qquad (5)$$

The average density of the stick was calculated by the above Equation (4); the average cross-sectional density was determined according to Equation (5). Differences in the cross-sectional density according to zones and the areas of these zones were taken into account.

For birch the relationship was established between the average density of the stick cross-section (ρ_{cs}) and wood density in the peripheral zone ($\rho_{0.75R}$) of this cross-section:

$$\rho_{cs} = 67.3 + 0.853 \rho_{0.75R} \qquad r = 0.982 \qquad (6)$$

Based on the findings of the existence of a specific pattern of wood density change according to the zones, we obtained the relationship between tree density and wood density of the peripheral zone at a height of 1.3 meters:

$$\rho_{tree} = 223.3 + 0.542 \, \rho_{\substack{1.3 \\ 0.75R}} \qquad r = 0.839 \qquad (7)$$

It is important to note that in this case it is not required to take cores from the whole radius of a stick, which greatly simplifies the process and, more importantly, is less traumatic for the tree.

To study the pattern of wood density changing inside the stick let us consider basic density of the peripheral cross-section of the stick (0.75R) at the height of 1.3 m to be 100% ($\rho_{\substack{bas\ 1.3 \\ 0.75R}}$). Let us determine the basic density for each stick zone under study (ρ_{bas_i}) with coordinates ($\frac{h}{H}$; $\frac{r}{R}$) as a percentage with respect to $\rho_{\substack{bas\ 1.3 \\ 0.75R}}$. $\frac{h}{H}$ showed the following

values: 0.06 (1.3m); 0.25; 0.5 and 0.75; $\frac{r}{R}$ ratio showed 0.25; 0.5 and 0.75, respectively.

Then the definiendum of the experiment, i.e. the relative basic density of ith zone of the stick, y, is

$$y = \frac{\rho_{bas_i}}{\rho_{\substack{bas\ 1.3 \\ 0.75R}}} * 100 \qquad (8)$$

The following regression equation is obtained with the aid of a computer:

$$y = 85.55 - 7.33 \, \frac{h}{H} + 16.99 \, \frac{r}{R} \qquad (9)$$

After verifying the equation ($F_{calc} = 1.6 < F_{tab} = 2.1$ for q =0.05) and substituting y by the value in Equation (8) we obtain:

$$\rho_{bas_i} = \rho_{\substack{bas\ 1.3 \\ 0.75R}} \cdot 10^{-2} \cdot \left(85.55 - 7.33 \, \frac{h}{H} + 16.99 \, \frac{r}{R} \right) \qquad (10)$$

CONCLUSIONS

The average density of the stick wood can be determined non-destructively by taking a radial cross-section core with the diameter of about 4.0 mm from a growing tree using Bressler's increment borer. The procedure can be limited by the depth of drilling no more than 0.25 DBH. Once the basic density of the sample has been determined by buoyancy force method, the average density of the whole stick can be predicted on the basis of the laws governing the distribution of wood density within a particular wood species tree in respective stands.

This opens the practical possibility to predict wood quality of growing trees, which is the basis of cultivating the target forest with specified technical properties (density) of raw material for strictly special purposes.

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First Record of *Cacopsylla pulchella* (Hemiptera, Psyllidae) in Croatia

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ABSTRACT

The aim of this study is to identify plant lice *Cacopsylla pulchella* a new alien insect in the fauna of Croatia. We propose a new method of infestation level assessment in order to assess the potential of this insect as a pest. In May 2020 samples were collected from eleven locations in Croatia, where *Cercis siliquastrum* is grown in parks. Adults and nymphs of *C. pulchella* were collected together with a plant material and brought to entomological laboratory for further analysis. Field research was performed in order to assess the distribution and infestation intensity across various localities in Croatia. The intensity of infestation was assessed heuristically by visual examination and was categorized in four categories: 0) no infestation, 1) low, 2) moderate and 3) high infestation. *C. pulchella* was confirmed on *C. siliquastrum* in eleven localities in Croatia. Most of the infested trees had a low or moderate intensity of infestation. Damages caused by this insect are due to the emission of honey dew, a small spherical drop covered with waxy secretion causing necrotic areas which could lead to the premature fall of the leaves. It is not known what kind of progress in infestation intensity could be expected so infested trees found in this study should be checked closely in the coming years. It is strongly suggested to continue the monitoring of this alien insect on C. *siliquastrum* in Croatia and to estimate its potential of become an invasive pest which could endanger ornamental and other values of its hosts.

Keywords: alien pest; *Cercis siliquastrum*; Judas tree; damage, infestation level

INTRODUCTION

Insects have been documented as a successful invaders and one of the most important alien invasive species group, in term of numbers as well as in impact (Brockerhoff and Liebhold 2017). In Europe more than 400 alien invasive insect species feeding on woody plants have been identified (Roques et al. 2016), with new species constantly introduced with no signs of saturation (Seebens et al. 2017). In Croatia 101 species of insect and mite alien species on woody plants have been recorded until 2013 (Matošević and Pajač 2013) with constant influx of new species in the following years (Matošević unpublished data). Members of all insect orders have been introduced and established in Europe, mainly from Asia and Northern America but also Mediterranean species have been spreading northwards and invading new habitats and making damages (Matošević and Pajač 2013, Roques et al. 2015). Until now only one alien species from the family of Psyllidae (Hemiptera) has been recorded in Croatia (*Acizzia jamatonica* Kuwayama 1908) (Seljak et al. 2004, Seljak 2006).

Cacopsylla pulchella Low (Hemiptera, Psyllidae) is a jumping plant–lice that is considered as an alien species in Europe (Mifsud et al. 2010). It is probably native in Eastern Mediterranean basin and Asia Minor (Mifsud et al. 2010). The first confirmed record of *C. pulchella* in Europe dates to 1964 in France (Hodkinson and White 1979) and afterwards in many European countries, such as: Austria, France, Great Britain, Greece, Swizerland (Zeidan-Gèze and Burckhardt 1998), Germany, Italy, Ukraine (Burckhardt 2010), Spain, Portugal (Sánchez 2011), Slovenia (Seljak 2006), Hungary (Ripka 2008), Serbia (Jerinić-Prodanović 2011) and Russia (Balakhnina et al. 2015). It was found also in Israel (Spodek et al. 2017) and Lebanon (Zeidan-Gèze and Burckhardt 1998). As a monophagous species it is feeding on the Judas tree *Cercis siliquastrum* L. (Burckhardt 1999), but has been also found on *C. canadensis* L. (Balakhnina et al. 2015). Judas tree is valued ornamental plant in the Mediterranean regions of Croatia. Attacks of *C. pulchella* reduce the ornamental value of the trees causing chlorosis and wilting of leaves (Rapisarda and Belcari 1997). The nymphs produce sugary honeydew which contaminates everything under the tree and is a nuisance to inhabitants.

The aim of this research is first record of the psyllid *C. pulchella* as a new addition to the alien insect fauna of Croatia and assessment of its potential as a pest causing damages on trees.

MATERIALS AND METHODS

In May 2020 samples were collected from eleven locations in Croatia, mostly in the Mediterranean part, where *C. siliquastrum* is grown in parks. In the continental part trees were checked in Zagreb. Adults and nymphs of *C. pulchella* were collected together with a plant material and brought to the entomological laboratory in Croatian Forest Research Institute for further analysis. They were identified according to the keys by Hodkinson and White (1979), Loginova (1964) and Burckhardt (1999). Images of infested leaves and branches were taken with Olympus E-30 camera and detailed images of adults and nymphs were taken under dissecting microscope (Olympus, model SZX7) with an Olympus XC30 camera. All samples are kept in Croatian Forest Research Institute.

Additional field research was performed in order to assess the distribution and infestation intensity across various localities in Croatia (Table 1). The intensity of infestation was assessed heuristically by visual examination and was categorized in four categories: 0) no symptoms visible from distance or when observing single leaves, 1) low - symptoms are not visible when looking at a whole tree, only few specimens can be detected sporadically on single leaves, 2) moderate – symptoms are barely visible when looking at a whole tree, more than 5 specimens can be counted on 50% of observed leaves, 3) high – symptoms are visible when looking at a whole tree from distance, more than 10 specimens can be counted on at least 50% of observed leaves.

RESULTS AND DISCUSSION

This is the first record *of C. pulchella* in Croatia. Its presence was confirmed in all inspected localities (Table 1). Most of the trees (80%) had a low or moderate intensity of infestation (Table 1). However, the highest infestation level was recorded in Novi Vinodolski and Pula. Introduction pathway of *C. pulchella* to Croatia is unknown and these first records (in Mediterranean and continental Croatia) may be independent from each other. It is possible that *C. pulchella* has been present in Croatia for several years or longer but has been overlooked due to different sampling efforts than in neighbouring countries and interests of the collectors. Wide distribution and intensity of infestation along the Croatian coast (Table 1) indicates this overlooked presence in Croatia.

Damages caused by this insect are due to the emission of honey dew, a small spherical drops covered with waxy secretion (Rapisarda and Belcari 1997). These drops cause a necrotic areas as a consequence that lead sometimes to the premature fall of the leaves. It is not known what kind of progress in infestation intensity could be expected so infested trees found in this study should be checked closely in coming years.

Adults of *C. pulchella* are olive-drab or brownish green with orange stripes on the thorax. The abdomen is dark brown and the intersegmental membranes are orange-red. On the forewings black-brown spots could be found. The genital plates of males are almost straight black colored, while females lower genital plates are yellow, long as the previous three segments and sharpened at the end, the upper genital plate is brown or dark brown, not smaller as the lower (Figure 1a and 1b). Nymphs have a five stages of development, the first are yellow with red eyes which are very active, while the following instars are green, settle more or less without much moving on leaves and suck the sup (Figure 1c-1d).

As *C. siliquastrum* is often planted as ornamental solitary tree or in alleys in Mediterranean parts of Croatia. *C. pulchella* could pose a threat to ornamental values of these trees as well as a nuisance to inhabitants due to high amount of honey dew it secrets. It has been documented that species from the genus
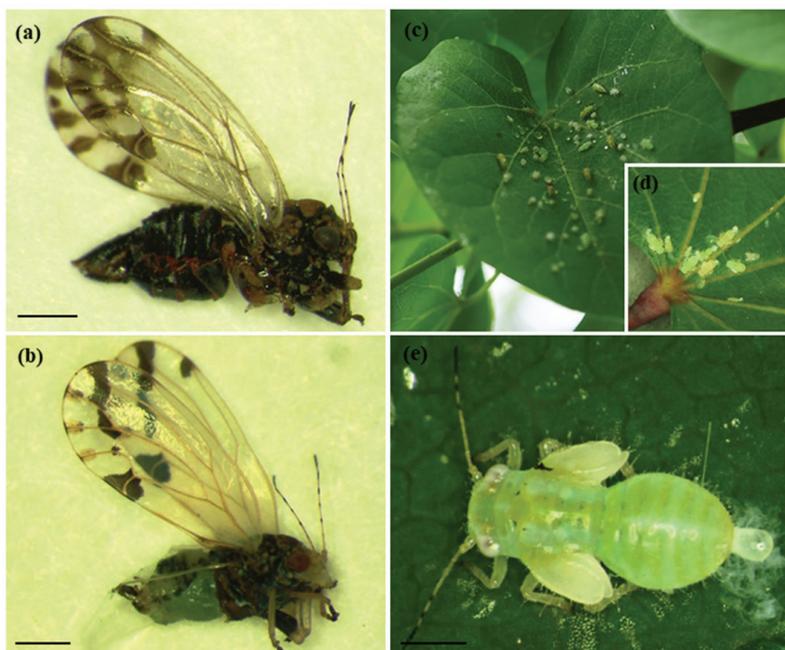
Figure 1. *Cacopsylla pulchella*: **(a)** female; **(b)** male; **(c)** developing stages on the topside of Judas tree leaf; **(d)** nymphs on the underside of Judas tree leaf; **(e)** nymph. Scale bars for (a), (b), (e) = 0.5 mm).

Table 1. Locality, address, geographic coordinates, host, number of checked trees and intensity of infestation.

Locality	Address	Coordinates (φ, λ)	Number of checked trees	Intensity of infestation in percentage	
NOVI VINODOLSKI	Ogulinska street	45.122664; 14.802134	49	1-	18%
				2-	72%
				3-	10%
	Parking school	45.127012; 14.791188	15	1-	7%
				2-	13%
				3-	80%
	Parking bus station	45.125713; 14.790959	4	1-	0%
				2-	25%
				3-	75%
CRIKVENICA	Hrusta	45.169266; 14.705309	5	1-	20%
				2-	80%
				3-	0%
KOSTRENA	Sports hall	45.306444; 14.494731	6	1-	17%
				2-	67%
				3-	17%
	"Nursery" by the graveyard St. Lucija	45.3081855; 14.4996189	18	1-	6%
				2-	83%
				3-	11%
	Graveyard Sv. Barbara	45.2909732; 14.5368178	2	1-	100%
				2-	0%
				3-	0%
OMIŠALJ	Kindergarden	45.211085; 14.559474	1	0-	100%
	Park	45.211217; 14.557224	3	1-	67%
				2-	33%
				3-	0%
NJIVICE	Creska street	45.157090; 14.791188	1	1-	0%
				2-	100%
				3-	0%
VIŠKOVO	Private garden center	45.3846141; 14.3856575	1	0-	100%
	Childrens playground	45.3794035; 14.3849551	11	1-	91%
				2-	9%
				3-	0%
OPATIJA	Park Angelina	45.336511; 14.308946	1	1-	100%
				2-	0%
				3-	0%
PULA	Flanatička street	44.8673611; 13.8498780	10	1-	0%
				2-	20%
				3-	80%
TRIBALJ	Treealley	45.222870; 14.677139	21	1-	90%
				2-	10%
				3-	0%
BRIBIR	Park Hrvatskih branitelja – Vrba	45.161911; 14.760971	1	1-	100%
				2-	0%
				3-	0%
ZAGREB	Botanical garden	45.80463 15.96992	6	1-	83%
				2-	17%
				3-	0%
	Bundek	45.78720 15.98421	3	1-	100%
				2-	0%
				3-	0%

Cacopshylla are carriers of phytoplasma disease (Carraro et al. 1998) but the spread of the diseases transmitted by *C. pulchella* as a vector is still to be studied (Laviña et al. 2004). Phytoplasmas are important insect-transmitted pathogens that cause more than 700 plant diseases, and some of them are lethal (Weintraub and Beanland 2006). It is not known whether or to which extent *C. pulchella* functions as a vector of diseases. Introduction pathway of *C. pulchella* to Croatia is unknown and these first records (in Mediterranean and continental Croatia) may be independent from each other. It is strongly suggested to continue the monitoring of this alien insect on Judas trees in Croatia and to estimate its potential of becoming an invasive pest (Lockwood et al. 2007) that could endanger ornamental and other values of its hosts.

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IN MEMORIAM

Prof. Dr. Nenad Keča
1975 - 2019

Prof. Dr. Nenad Keča (https://www.ponteproject.eu/people/nenad-keca) was born on October 25, 1975, in Senta, Serbia. He completed elementary school in Kanjiža, and high school in Senta. Prof. Dr. Keča received a BSc degree from the University of Belgrade, Faculty of Forestry, Department of Forestry, in 1999 with a grade point average of 9.72. Thereafter, he enrolled in postgraduate studies of "Protection of Forests and Ornamental Plants" in 1999/2000 and defended his master's thesis entitled "The study of the most important fungal diseases of Populus x euramericana Dode (Guinier) and the possibilities of suppression" in 2001. He received his PhD degree in 2005 by defending the doctoral dissertation entitled "Biodiversity of Armillaria species and their role in the decay of trees in conifer and deciduous forests of Serbia and Montenegro" at the University of Belgrade, Faculty of Forestry.

After graduation, Prof. Dr. Keča worked as a forestry engineer in the Public Company "Srbijašume" for six months. Then, he joined the University of Belgrade, Faculty of Forestry as a teaching assistant. In 2016, he was elected full professor of forest and ornamental plant protection. At the age of 41, he became the youngest full professor in the history of the Faculty of Forestry. Prof. Dr. Keča was also involved in teaching Pathology of Forest Trees at the Faculty of Agriculture in East Sarajevo (forestry study program), in the period of 2014–2015. Teaching activities of Prof. Dr. Keča also included mentoring of students at all study levels.

During the period of 2003–2004, Prof. Dr. Keča visited the University of Aberdeen as a researcher in the laboratory of Prof. Dr. Steve Woodward, and was trained in forest pathology. Later, from May to September 2005, he stayed as a Norwegian Government Fellow at the Institute of Forestry (today NIBIO), Department of Forest Protection, with Prof. Dr. Halvor Solheim. During this period, he specialized in forest pathology. During 2018, he stayed for three months at the Institute of Forestry in Poland, specializing in root and tree sprout rot etiology.

Prof. Dr. Nenad Keča participated in the implementation of 20 projects (4 inter-national and 16 national), and 5 COST Actions (FP0801, FP1002, FP1103, FP1203, FP1406), resulting in 104 papers. He made the greatest contribution in studying poplar pathogenic fungi, Armillaria spp., Heterobasidion spp., Phytophthora spp. and Chalara fraxinea. He detected some of those species for the first time in Serbia. He published more than 150 papers in international and national journals, and participated in numerous scientific meetings. Prof. Dr. Keča coauthored two university textbooks (Forest Phytopharmacy in 2010, and Forest Mycology in 2016). Prof. Dr. Keča was also a member of the Editorial Board of the following journals: Annals of Forest Research (SCI, Impact Factor 0.418), SEEFOR (South-East European Forestry) Regional Journal, Acta Scientiarum Polonorum, and Silvarum Colendarum Ratio et Industria Lignaria. He was an editor-in-chief of the Faculty of Forestry's Newsletter from 2009 to 2013, as well as an ad hoc reviewer in the following journals: Forest Pathology, European Journal of Plant Pathology, New Zealand Journal of Forest Science, Baltic Forestry, Annals of Forest Research, SEEFOR, Journal of the Faculty of Forestry and Papers of the Faculty of Forestry in Sarajevo.

The professional career of Prof. Dr. Keča is full of exceptional achievements thanks to his tremendous efforts, endless enthusiasm and devotion to his duties. He was a man of enormous energy, persistence and patience. Furthermore, he maintained high professional and personal standards serving as an example for his students and colleagues. By teaching and sharing his knowledge and expertise, he built an academic foundation out of 20 generations of students that will last for a long time. On August 21, 2019, his family lost a son, a husband and a father, and the society lost a respected and distinguished scientist and professor. Rest in peace, dear friend and colleague.

On behalf of the POnTE University of Belgrade team,
Prof. Dr. Aleksa Obradović
University of Belgrade
Serbia

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