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VARIABILITY OF NEEDLE DENSITY OF DWARF MOUNTAIN PINE (*Pinus mugo*) IN THE EASTERN CARPATHIANS

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Abstract: *The variability of needle density of dwarf mountain pine (Pinus mugo Turra) in relation with shoot type and twig age was analysed using three populations growing on different substrata (volcanic, limestone, and crystalline schists, respectively) in the Eastern Carpathians (Romania). The needle density was evaluated using the total number of needles and the dry mass relative to shoot length and surface area. The number of needles per shoot length unit was significantly lower on volcanic bedrock compared with limestone and crystalline schists, respectively. The mass of needles per shoot length was not significantly influenced by the type of substratum. The dry mass of needles relative to shoot surface area varied significantly between substrata. The pine population on limestone bedrock showed significant differences in needle density between the main and secondary shoots. The needle density relative to shoot surface area decreased significantly with shoot age. The needle mass relative to shoot length did not vary significantly with shoot age.*

Key words: *dwarf mountain pine, needles age, shoot type, needle biomass.*

1. Introduction

Carbon assimilation through photosynthesis depends on foliage density and biomass, which have a direct influence on the capacity of solar energy use [16]. The shape of the crown and the distribution of the needles have a direct effect on the ability to capture light in the case of

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coniferous species. The inclination and length of the shoots influence the needle density, and consequently the photosynthetic capacity [19]. Information on foliage density is required for modelling the photosynthesis and growth processes, and is an indicator of environmental condition [27].

The needle density and biomass of evergreen conifers vary according to species, altitude, and shoot age. Needle age and fascicle density increase with altitude and latitude or in wind exposed sites. These are characteristics of adaptive plasticity to compensate the reduction of shoot length growth due to limitative growing conditions induced by stress factors (e.g., wind, temperature) [11], [15], [18], [22].

Dwarf mountain pine (*Pinus mugo* Turra) is a shrub species adapted to mountainous habitats with poor soils and surface rocks. It has a high resistance to low temperatures and grows mainly in alpine areas, at the upper limit of the treeline [1]. *P. mugo* covers mostly the subalpine belt in the mountains of Central and Eastern Europe (the Alps and the Carpathians), with scattered populations in the Jura, Vosges, Mediterranean, and Balkan Mountains [4]. In the Carpathians, *P. mugo* is an important woody species forming large communities in the alpine area of Eastern and Southern Carpathians [28].

The dwarf mountain pine has major ecological importance in the alpine and sub-alpine ecosystems. The root system consisting of extended roots and the branches stretched to the ground ensure soil stability, prevention of torrents occurrence, avalanches and erosion [4]. Also, *P. mugo* plays an important role in soil formation on steep slopes and areas with rocks on the surface [3].

Most of the studies on dwarf mountain pine morphology variability focus on needles anatomical variation [2], [7-10], [26] or growth dynamics [20, 21], [29, 30]. Little information is available concerning the density and biomass of needles in relation to shoot age or growing bedrock [2].

The dwarf mountain pine represents both a carbon storage system and a valuable ecosystem service provider in alpine areas with high sensibility to climate warming, and therefore should be included in vegetation simulation scenarios. Realistic parameterization of simulation models requires specific regional information on needle density, biomass and morphological traits, not only for the main forest tree species, but also for shrubs and woody species of low economic importance.

The aim of this paper is to compare the needle density (needle number and biomass) on shoots of *P. mugo* growing on different substrata (volcanic, limestone, and crystalline schists) relative to the type and age of the twigs.

2. Material and Methods

The study area is located in the northern part of the Eastern Carpathians (Romania) in three mountain ranges with different bedrock: Călimani, Giumalău, and Rarău (Table 1).

Table 1

Location of Pinus mugo populations

Mountain range	Altitude (m)	Latitude	Longitude	Number of individuals	Type of substrata
Călimani	1,800	47°6´31˝ N	25°13´38˝ E	19	Volcanic
Giumalău	1,750	47°26´5˝ N	25°29´2˝ E	19	Crystalline schists
Rarău	1,550	47°26´51˝ N	25°33´31˝ E	20	Limestone

The climate of the study area is typical for alpine regions with a mean annual temperature between 2-3°C, with a maximum temperature in July and a minimum temperature in January. The average annual precipitation varies between 900 and 1,200 mm with a maximum in June [24]. The snow cover lasts 180-200 days beginning from late October.

A total of 20 individuals of dwarf mountain pine without visible damages and with normal growth were selected in each study site, with a minimum distance between the sampled individuals of 15 m. During the sample preparation, one specimen from Călimani and Giumalău, respectively, was excluded from the analysis due to missing parts of needles. A representative shoot from each specimen was chosen for sampling, which was further sectioned using the following scheme: the last three years growth segments from the long shoot (principal shoot), a secondary twig formed in the current year, a secondary two-year old short shoot (including the current growth and the previous year growth), and a secondary three-year old short twig (including segments with an age of 1, 2, and 3 years) (Figure 1).

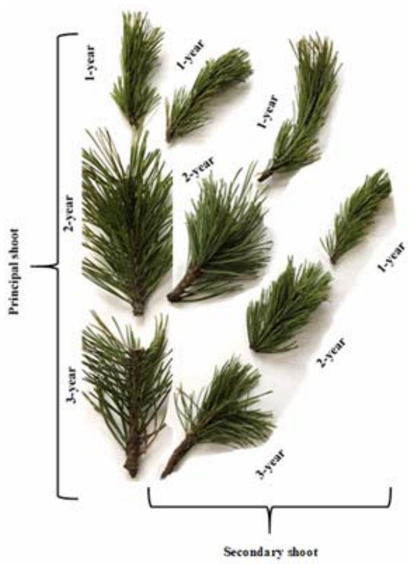

Fig. 1. *Sampling strategy for needle density analysis of dwarf mountain pine*

Having this structure of sampling for data management, a specific coding was used including the mountain range, the number of specimens, the type of shoot (principal and secondary), and the age of the shoot growth segment (1, 2, and 3 years). The samples were collected in late autumn (October – November) 2017 after the end of growing season.

To analyse the density of the foliar system, all needles were separated from the twigs and counted distinctly for each shoot segment. To determine the dry mass of needles, they were dried in a hot air oven at 80°C for 24 hours and weighed using an electronic balance with a precision of 0.01 mg. The total length (mm) and the diameter at the base and top of the shoot segment (mm) were measured for each shoot segment using an electronic precise calliper. The total lateral surface of the shoot segment (mm^2) was derived by assimilating the twig segment with a geometric shape of cone trunk.

The needle density was quantified using four parameters: total number of needles relative to shoot length (NL – no. $needles{\cdot}mm^{-1}$), number of needles relative to shoot segment surface (NS – no. $needles{\cdot}mm^{-2}$), needles mass relative to shoot length (ML - $mg{\cdot}mm^{-1}$), needles mass relative to shoot segment surface (MS - $mg{\cdot}mm^{-2}$).

Exploratory data analysis using statistical parameters (arithmetic mean, standard deviation – SD, and coefficient of variation - CV) and boxplot graphs were applied to identify the outliers due to measurement errors or data recording resulting in a valid database.

Differences between substrata, type of shoots, and twig age were tested using the analysis of variance and specific post-hoc test for mean comparison. To verify the assumptions required to apply the ANOVA, the Shapiro test was used to test the normality, and the Levene test for homogeneity of variance [14]. Due to violation of the normality assumption required by ANOVA, in most of the cases the nonparametric Kruskal-Wallis test was applied, followed by the post-hoc non-parametric Wilcoxon test using a significance level of $p<0.05$ [14].

Statistical analysis of needle data was performed using the R language and specific packages (car and rstatix) in R Studio [23].

3. Results and Discussion

Needle density is defined as the number of fascicles or needles per shoot length or the dry biomass of needles per shoot length. These parameters are a proxy of resource allocation for new needles mass and for shoot length growth [25]. An alternative descriptor of foliar system density in the case of coniferous species is the use of shoot lateral surface as a relative for number or mass of needles.

Needle density varies in relation to the type of shoot, the age of twigs, and the bedrock (mountain range). Exploratory data analysis shows a decrease in the number of needles relative to the length of the shoot with branch age (Figure 2). A possible cause could be needles falling due to time passing and the influence of various abiotic factors. A similar trend is noticed by reporting the number of needles to the surface unit of twigs or if the dry mass of needles is considered an indicator of needle density.

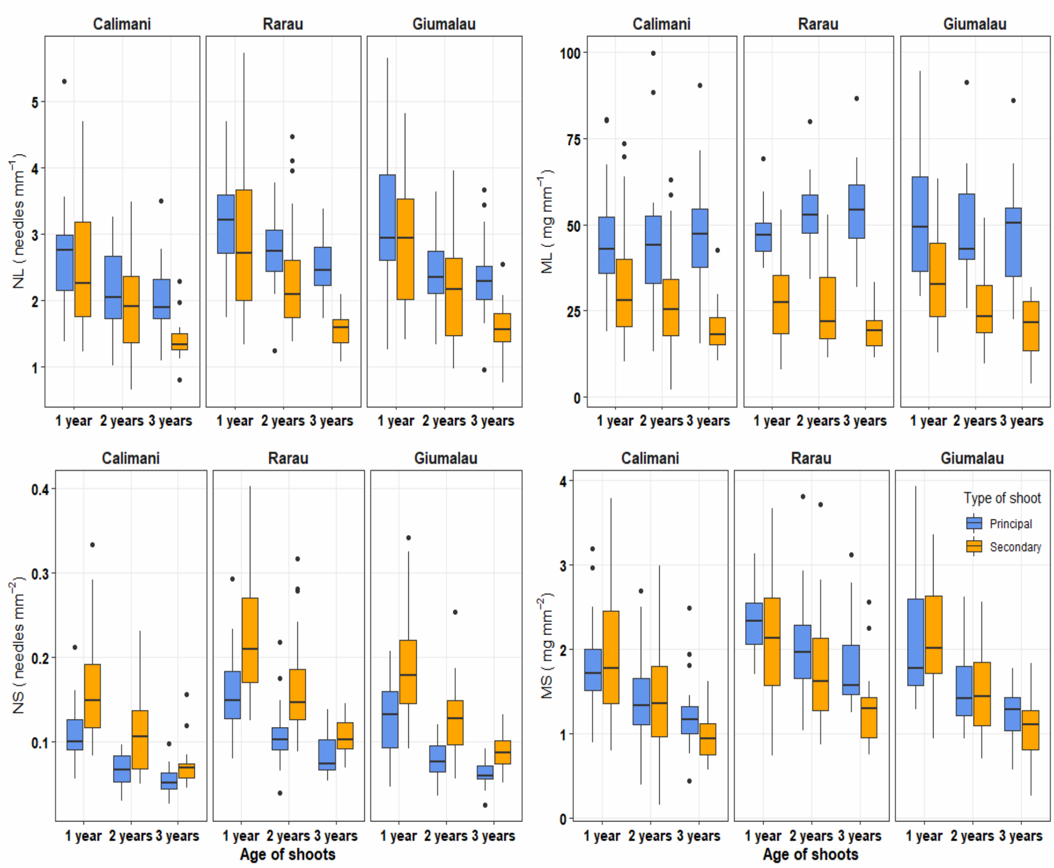

Fig. 2. *Variability of needle density parameters of the dwarf mountain pine in relation to the type and age of the shoot*

The amplitude of variation of needle density parameters was higher in the case of secondary shoots and decreased from the current year short shoots to the third-year shoots. The ML does not show a clear trend depending on the shoot age, but there were differences between the types of twigs.

Normally, the dwarf mountain pine has the needles clustered in fascicles two by two [28]. In our study, shoots with fascicles containing 3 needles were identified. In Călimani, the frequency of abnormal needle shoots is higher (21 out of the 177 shoots analysed have at least a three-needle fascicle) compared with Rarău (11 out of 180) and Giumalău (9 out of 170). The occurrence of more than two needles per fascicle in dwarf mountain pine is considered a remnant of its evolution. The higher frequency of these exceptions is recorded in Carpathian populations (24%), mostly in the locations close to the upper altitudinal limit of the species distribution range [5, 6]. The occurrence of abnormal needle fascicles is related to the higher temperature in winter and lower temperature during shoot growth [12]. The occurrence of shoots containing fascicles with more than 2 needles has also been reported in other species of the genus *Pinus* such as: *P. uncinata*, *P. sylvestris* or *P. pinaster*, species that normally have two needles in fascicles [6].

3.1. Influence of the Bedrock on Needle Density

The shape of the distribution of needle density parameters (NL, NS, ML, MS) showed a left asymmetry in all the considered sites. Statistically it was confirmed that the values of the analysed indicators were not normally distributed (Shapiro test; $p<0.05$). The assumption of the homogeneity of variance was respected with the exception of the NS parameter (Levene test; $p<0.05$). The ratio between the largest variance (Rarău) and the smallest one (Călimani) for NS was not higher than 4, so it was considered that the condition of homoscedasticity in this case is accepted. The analysis of variance revealed a significant difference between bedrock for NL, NS, and MS (Kruskal-Willis test, $p<0.05$) and no difference for ML (Kruskal-Willis test, $p = 0.505$).

NL in the Călimani population (volcanic substrata) had the lowest average value (2.18±0.83 needles·mm^{-1}) with a coefficient of variation of 38% and differed significantly from the other two populations (Wilcoxon test; Rarău $p<0.001$, Giumalău $p<0.01$). There are no statistically significant differences between Giumalău (crystalline schists – 2.49±0.91 needles·mm^{-1}) and Rarău (limestone –2.57±0.87 needles·mm^{-1}). The Wilcoxon post-hoc test showed a significant difference between NS for all three studied substrata ($p<0.05$). The highest mean value of NS was on limestone (0.16±0.07 needles·mm^{-2}) with a CV of 46%, and the lowest on volcanic bedrock (0.11±0.05 needles·mm^{-2}), the crystalline schists having an intermediate mean value (0.12±0.06 needles·mm^{-2}).

The mean needle mass relative to shoot surface (MS) of dwarf mountain pine from limestone (1.93±0.34 mg·mm^{-2}) differed significantly from those from crystalline schists (1.70±0.68 mg·mm^{-2}) as well as from -volcanic bedrock (1.58±0.70 mg·mm^{-2}). The average values of the ML parameter did not differ significantly between substrata, being 34.28±16.28 mg·mm^{-1} on limestone, 35.80±17.23 mg·mm^{-1} on crystalline schists, and 33.99±17.38 mg·mm^{-1} on volcanic substrata. The CV of ML is 48% in the population on limestone and crystalline schists, and 51% for volcanic bedrock.

3.2. Influence of the Type of Shoot on Needle Density

The distribution of needle density parameters considering two-way factors (population and type of shoots) violates the normality assumption required by ANOVA. Moreover, the normality condition of parameter distribution by twig type is not respected even if the analysis was done within each population (Shapiro test; $p<0.05$), with two exceptions: MS and NL for principal shoots in Rarău. The condition of homoscedasticity was accepted because the ratio between the largest and the smallest variance was lower than 2, even if in some cases, the Levene test failed ($p<0.05$).

The nonparametric analysis of variance followed by the post-hoc test showed a nonsignificant difference between twig type (principal and secondary shoots) for NL and MS in Călimani and Giumalău (Kruskal-Willis test and Wilcoxon test, $p>0.05$). In the case of all the others (needle density parameters and population), the differences between shoot types are significant ($p<0.05$). Generally, the principal shoots showed a higher number of needles and needle mass per length unit compared to the secondary shoots. By contrast, the NS is higher in the case of the secondary twigs (Figure 3).

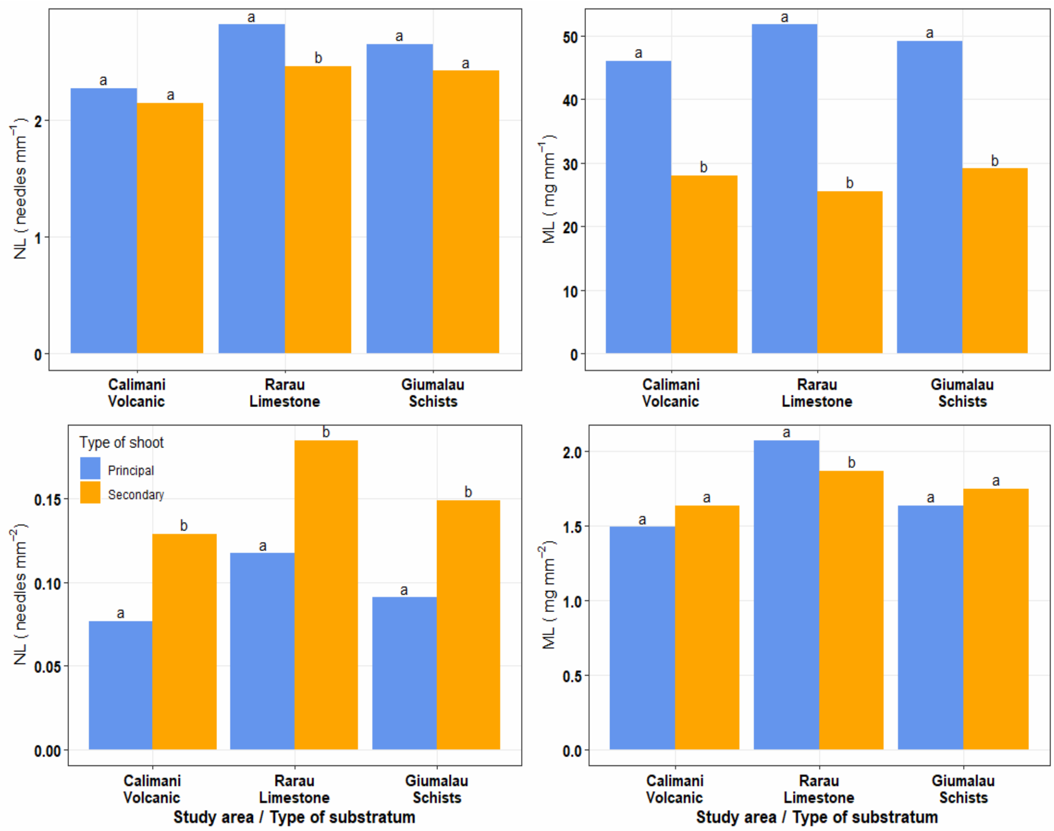

Fig. 3. *Variability of needle density parameters with shoot type (letters indicate significant differences according to the Wilcoxon test)*

The MS ratio between twig types varies between populations. The largest differences between the type of shoot were observed in the case of ML (mean ratio principal versus secondary shoots - 1.78) and for NS (0.61).

The type of shoot is a factor that can induce a fairly high variability on the needle density of dwarf mountain pine. The needle density relative to shoot length is dependent on the growing rate in length which is higher in the case of principal shoots. A strong correlation between the density of the needles and twig length was observed in the case of other coniferous species [13], [15].

3.3. Influence of Shoot Age on Needle Density

The distribution of needle density parameters by twig age within each population follows the normal distribution only in 11 out of 36 cases (Shapiro test, *p>0.05*), and a left asymmetry has been observed in most of the situations. Assumption of homoscedasticity was accepted due to the low ratio between the largest and the smallest variance, even if, in some instances, the Levene test failed (*p<0.05*). Nonparametric analysis of variance indicates significant differences relative to shoot age for all needle density parameters (Kruskal-Willis test, *p<0.05*), apart from ML.

NL of one-year old twigs differs significantly from older shoots in all studied populations (Wilcoxon test, *p<0.05*). There were no significant differences (*p>0.05*) between two- and three-year shoots in the dwarf mountain pine populations from Călimani and Giumalău NL (Figure 4).

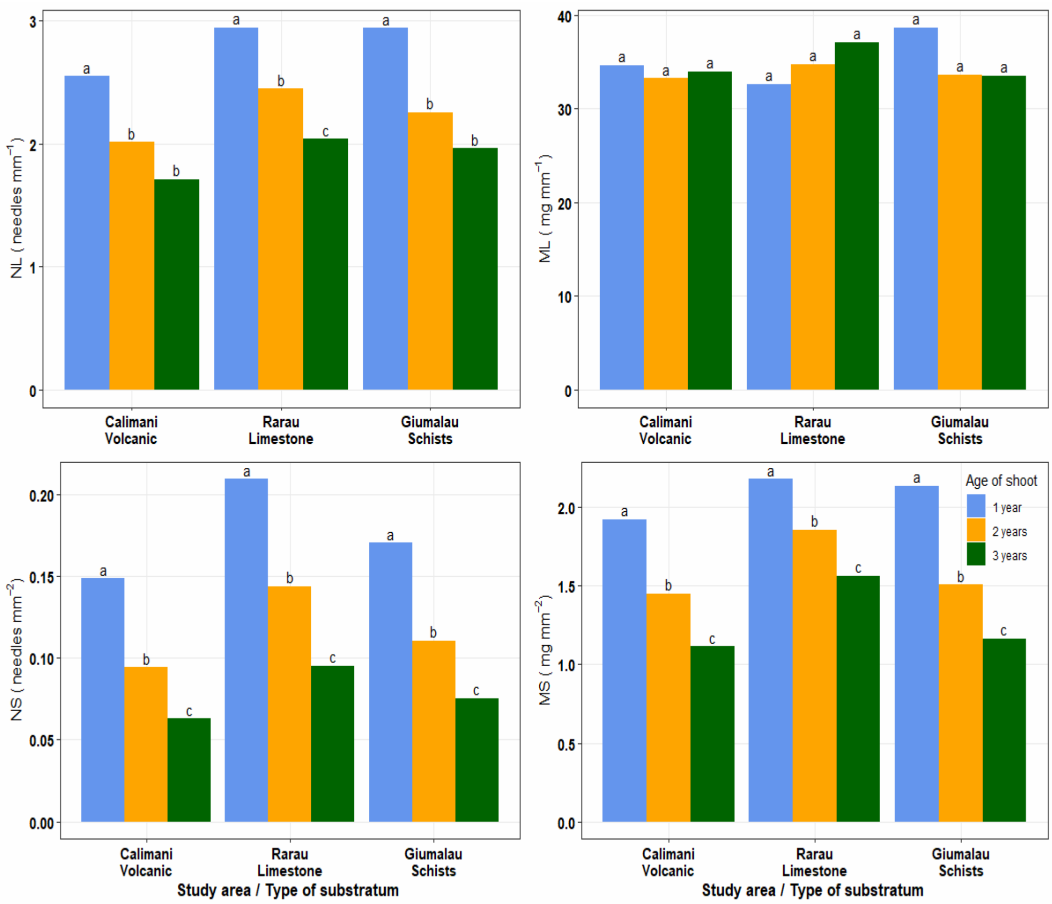

Fig. 4. *Variability of needle density parameters with shoot age (letters indicate significant differences according to the Wilcoxon test)*

NS and MS needle density parameters decreased with shoot age and the differences between them were significant in all populations. ML showed similar values irrespective of twig age. The largest difference was observed between the current year shoots and the older ones, the mean ratio varying from 1.36 for NL, to 1.91 for NS, and 1.47 in the case of MS.

Our data suggest a higher rate of needles loss after the first year of shoot growth. The variability of needle density indicators was higher when using shoot surface as reference comparing with shoot length. One explanation for the increasing differences may be the fact that, unlike length, the shoot surface changes by increasing diameter over the years. Thus, the same number of needles or even a smaller number is distributed over a larger area. The mean CV varies from 32.03% for NL to 35.84% for NS, and 34.63 % for MS.

The needles longevity of *Pinus sp.* varies with species and altitude, and is often between 2 and 15 years, with some exceptions in the case of *P. longaeva* (up to 45 years), with impact on needle biomass allocations and gas exchange rate [17]. For dwarf mountain pine they remain attached to the shoots up to 6 years [4]. The age of the needles depends on genetic and environmental factors. Both for *P. mugo* and *P. cembra*, the age of needles increases with altitude [11], [17].

4. Conclusions

Carbon storage, contribution to soil formation, and natural hazard prevention are among the key ecosystem services provided by dwarf mountain pine in alpine areas. Having more information on the needle density variability between different populations and relative to shoot type and age is required.

Needle number per shoot length units of dwarf mountain pine is significantly lower on volcanic bedrock compared with limestone and crystalline schists. The mass of needles per shoot length was not influenced significantly by the type of substratum. By contrast, the biomass of needles relative to shoot surface varied significantly between substrata.

Significant differences induced by the type of shoots (principal and secondary) were found for each of the four needle density parameters in the Rarău population and only for the needle mass per shoot length and needles number per shoot surface in the Călimani and Giumalău populations. Needle density relative to shoot length was higher on the principal shoot compared to the secondary ones, and the ratio was reversed considering the shoot surface as reference.

The needle density generally decreased significantly with shoot age. Needle mass relative to twig length did not vary with shoot age. A greater difference was found between the current year shoot and older twigs.

Further research in other dwarf mountain pine populations will allow a generalization of the hypotheses revealed in this study.

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