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Article

Differences in the Climate-Growth Relationship of Scots Pine: A Case Study from Poland and Hungary

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Abstract: Scots pine is an adaptable and prevalent European tree species that grows naturally throughout Europe and has been planted in a wide range of environments. Previous studies have indicated that climatic variables affect tree-ring growth patterns in this species, but it is also possible that certain aspects of the growth environment moderate this response. In order to understand the potential impact a shifting climate has on this important species, this study compared the growth response of two populations of Scots pine. Trees from similar bioclimatic regions in Hungary and Poland were compared using the hypothesis that differences in the association between climate and growth would be reflected by the degree of tree-ring width variation. We also wanted to know how changing climatic conditions influenced the temporal stability of the climate–growth signal in the most important periods for tree growth. Clear similarities in the effect of temperature and precipitation on tree-ring width variation were found between the two sites, but there were also some interesting differences. In the late winter to early spring period both populations reacted to warming with a decreasing association with temperature. Summer precipitation was shown to be the dominant factor in controlling ring-width. A decreasing trend in summer precipitation values at both Hungarian and Polish sites resulted in a weakening in correspondence for the Hungarian trees, while the Polish trees showed a significant increase in correlation with summer precipitation. The results indicated that changes in climate influenced the studied trees in different ways which has implications for the future balance of Scots pine growth in Europe.

Keywords: dendroclimatology; dendroecology; *Pinus sylvestris* L.; tree rings; climate change

1. Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most dominant tree species in central and eastern Europe, with the widest natural range among all pines. The fact that it can grow well on nutrient poor sites has led to planting outside its natural range while richer forest sites, more suitable for deciduous species, have been adapted for agriculture. As a result of this process, of all the pine species in Hungary, Scots pine represents the largest group, and although it is not native, it plays an essential role in Hungarian forest composition (NFCSO Forestry Directorate Forest Inventory 2010–2014 (<http://portal.nebih.gov.hu/documents/531011/531862/2001101001000.pdf/c9a01ba7->

184a-4a76-9400-59387aee2df8). Nowadays it is difficult to establish a definitive border for the natural range of Scots pine due to over two hundred years of intensive cultivation of this species [1]. For the purpose of our study we used the distribution map from the EUFORGEN project (<http://www.euforgen.org/species/>). Even if both Poland and Hungary are under the influence of the same temperate continental bioclimatological zones [2], the relationship between Scots pine and climate may differ. In Poland the species grows within the natural range while in Hungary it is anthropologically dispersed (Figure 1). However, the future distribution of this species in terms of climate change and the economics of forest management may change significantly. According to models, the species will probably grow further north, and will be replaced by *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. [3]

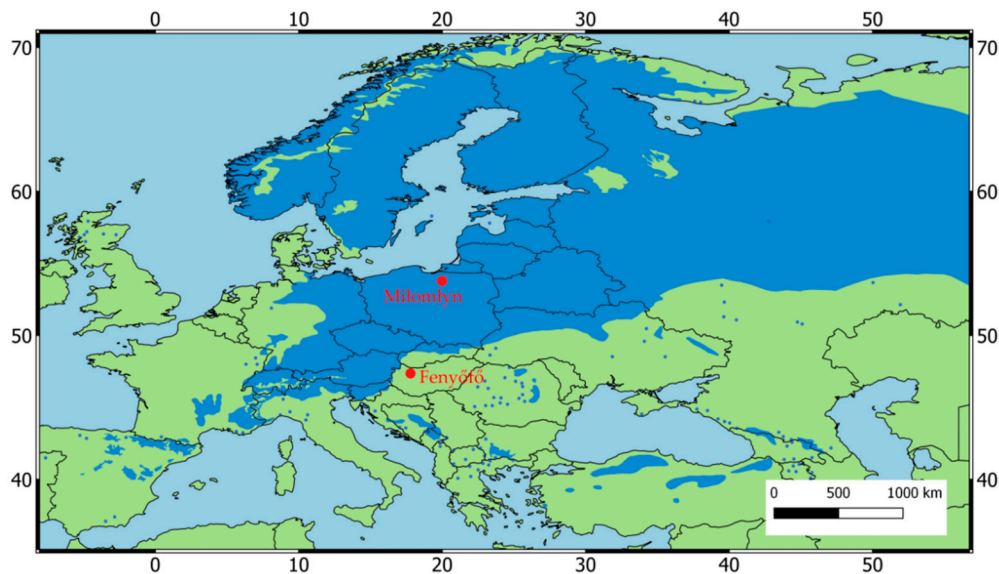


Figure 1. Distribution of *Pinus sylvestris* in Europe (dark blue shading) and locations of study sites (red circles) [Courtesy of EUFORGEN, (<http://www.euforgen.org/species/pinus-sylvestris/>)].

Scots pine is well recognized according to its ability to grow in a wide range of relatively extreme conditions. However, a provenance study has shown that within the distribution of *P. sylvestris*, different subpopulations develop adaptations to specific local climate conditions. For example, frost resistant sub-species from Lapland, could not be replaced with pines from southern Spain. Interestingly, where *P. sylvestris* grows in optimum conditions the trees demonstrate high plasticity and adaptability [1]. From dendroclimatological studies on pine and other species we can observe some common effects of temperature and precipitation on tree-ring growth across different regions. In Poland, wide rings are formed especially when higher temperatures are observed in February and March and when there is precipitation during the vegetation season [4]. In Hungary, precipitation from June to July and temperatures from February to March, are the dominant controls with summer temperatures playing the secondary role [5–8]. In northeastern Germany wide rings are formed during wet/warm February and wet/cool June [9]. In the Czech Republic February and March temperatures and summer precipitation are critical factors favorable to growth [10], and in Lithuania low precipitation in May and June is the key limiting factor [11]. Several of these studies also confirm that such climatic variables are indeed the dominant factors for growth, even where trees are growing on a range of soil types with a number of different nutrient regimes [11,12].

In the current study, we hypothesized that, despite the different distribution history, there would be no difference between the long-term reaction to temperature and precipitation of tree populations for this species because the trees as from the same bioclimatic zone. As the populations in Hungary, growing outside their natural range, have seen years of cultivation and are by now well adapted to local environmental conditions, the growth response to climate will not differ significantly from Scots pine growing in Poland. Interspecific differentiation in adaptation to environmental factors is

an important issue in predicting the response of Scots pine to projected changes in environmental conditions [13].

The main aim of our work was to analyze the two populations and evaluate which climatic parameters most influence tree-ring growth of Scots pine in these distributions. In addition, we investigated the temporal stability of the climate–growth signal and determined its dependence on changes of temperature and precipitation trends to inform future predictions related to climate change.

2. Materials and Methods

2.1. Study Sites and Climate Data

The Hungarian site (Fenyőfő: 47°21' N, 17°45' E) is situated in the northern part of Western Hungary (Figure 1), on the northern slopes of the Bakony Mountains. The site is well-known for the distribution of Scots pine and the forest is the oldest pine stand in Hungary. The substrate is a secondarily evolved dune sand with a weakly humic sandy soil, overlying a calcareous sand bedrock [14]. The forest is mixed with oak (*Quercus cerris* L., *Q. robur*, *Q. petraea*), silver birch (*Betula pendula* Roth) and ash (*Fraxinus ornus* L.), but the canopy is dominated by the pine population, which varies widely in age. Though younger individuals dominate, it is not difficult to find trees more than 120 years in age with trunks over 2.4 m in diameter. The climate of the region is moderately warm (Köppen code: Cfbx), and highly affected by the Bakony Mountains [15]. The long-term annual average temperature (1965–2010) in the area is 10.3 °C and the total long-term average annual precipitation is 650 mm. The warmest month is July (20.6 °C) and the coldest month is January (−1.1 °C). Most of the precipitation falls in the late spring to early summer (May–July) period with a maximum in July (75 mm).

The Polish site is situated in the northeastern part of Poland, in the Mazury Lakeland (53°46' N, 19°58' E). The site is also well-known and the trees have been specially named Taborska pine to distinguish them from other pine trees because of the quality of the timber. In particular their suitability to be used for ships masts led to a well-documented purchase by a Dutch queen in the 16th century AD. The underlying substrate in this region is glacial clay overlain by humic rich soils, providing a rich to moderately rich growth environment for the pines to grow along with *Fagus sylvatica* L. [16]. Climate data for the region shows that the long-term (1951–1965) annual average temperature is 7 °C and the total long-term average annual precipitation is 554 mm. The warmest month is July (22.5 °C), and the coldest is February (−6.9 °C). Most of the precipitation falls in the late spring to early summer (July–August) period with the maximum in July (91 mm) [17]. For both sites we used CRU TS 3.23 0.5° × 0.5° [18] gridded monthly and seasonal temperature and precipitation data which were extracted for the areas encompassed by the coordinates 47°–47.5° N and 17.5°–18° E and 53°–53.5° N and 19.5°–20° E using the KNMI (The Royal Netherlands Meteorological Institute) Climate Explorer (<http://climexp.knmi.nl/>).

To determine the differences between the study site in western Hungary and the native range in northern Poland we used the values of climatic indexes from the BIOCLIM 1.4 dataset (<http://worldclim.org>) [19]. These variables are considered limiting factors of the spatial distribution of Scots pine and they are commonly used for niche modeling or analysis of the tolerance of species for differing climatic conditions (e.g., [20–22]). The climatic variables were extracted from gridded maps of 2.5 arcmin resolution with DIVA-GIS 7.5 software [23,24]. We used 273 localities of *P. sylvestris* ssp. *sylvestris* from the native range taken from Conifers of the World as reference points for climatic conditions, along with the Resources for Conifer Research online database (<http://herbaria.plants.ox.ac.uk/bol/conifers>) [25]. The selected annual and seasonal BIOCLIM indexes were paired and presented on graphs (Figure S1). Moreover, BIOCLIM values of both study sites were compared (Table 1).

Table 1. Selected for comparison of study sites bioclimatic variables [19].

Bioclimatic Variable	Poland	Hungary
Isothermality $(2/7) \times 100$	24	30
Temperature Seasonality (Standard deviation $\times 100$)	816.9	759.6
Max Temperature of Warmest Month	23 °C	25.8 °C
Min Temperature of Coldest Month	−7.6 °C	−4.8 °C
Temperature Annual Range (5–6)	30.6 °C	30.6 °C
Mean Temperature of Wettest Quarter	17.1 °C	19 °C
Mean Temperature of Driest Quarter	1.8 °C	0.9 °C
Mean Temperature of Warmest Quarter	17.1 °C	19 °C
Mean Temperature of Coldest Quarter	−3.9 °C	−0.6 °C
Precipitation of Wettest Month	81 mm	71 mm
Precipitation of Driest Month	31 mm	32 mm
Precipitation Seasonality (Coefficient of Variation)	30	27
Precipitation of Wettest Quarter	235 mm	189 mm
Precipitation of Driest Quarter	103 mm	103 mm
Precipitation of Warmest Quarter	235 mm	189 mm
Precipitation of Coldest Quarter	120 mm	117 mm

2.2. Chronology Building

Old, dominant and healthy trees were selected for sampling and two cores per tree were extracted at breast height using standard techniques. All of the samples were air-dried, then sanded and polished to enhance the tree ring structure. A LINTAB measurement station (<http://www.rinntech.de>) was used for the measurement of ring widths at 0.01 mm precision [26] for the Hungarian samples. The Polish cores were scanned first at the resolution of 1200 dpi using a standard scanner (Epson Perfection V700 Photo) then tree-ring widths were measured to the nearest 0.01 mm using CooRecorder software and the related CDendro program (<http://www.cybis.se>). On-screen cross-dating of individual series was performed using the programs TSAPX and TSAP-Win [26]; series intercorrelation, missing ring identification and detection of possible dating errors were checked using the program COFECHA 6.06P [27].

Non-climatic trends preserved in the tree-ring growth patterns related to the tree's age, size and the effect of stand dynamics, were removed by fitting a cubic smoothing spline with a 50% frequency response at 67% length of the individual series [28]. Autocorrelation was removed from each individual index, then all residual series (obtained from the autoregressive modeling of the detrended measurement series) were averaged for the site chronology using the bi-weight robust mean [29]. For the examination of stability of the climate-related signal preserved in the index series, an Expressed Population Signal (EPS) calculation was applied with a 25-year window lagged by 1 year using 0.85 as a widely accepted threshold [30]. In addition, mean inter-series correlation (R_{bar}) was computed with the same window and lag as EPS values. Standardization and the index calculation procedure were carried out using ARSTAN 4.1b_XP [31].

In order to investigate climate growth relationships, we applied the response function correlation from the R package TREECLIM [32] using a bootstrap procedure as one solution to obtain more robust parameter estimates. The independent variable, sum of precipitation and mean monthly temperature from May of the previous year to September of the current year. The time series for each parameter was used as the dependent variable. To evaluate the connection between the climate data and the tree-ring index, a Pearson correlation was calculated from May of the previous year to September of the current year of tree-ring formation. The temporal stability of the climate–growth relationship was investigated by computing a 30-year moving window correlation of temperature, precipitation and the tree-ring index for selected periods.

3. Results

3.1. Tree-Ring Chronology

Site chronologies were constructed at each location. At the Hungarian site, 96 samples from 48 trees formed a chronology with a time-span of 100 years (1914–2013), and at the Polish site 25 samples from 15 trees covered the period 1855–2015.

Inter-series correlation was low ($r = 0.13$, $p < 0.05$) (Figure 2).

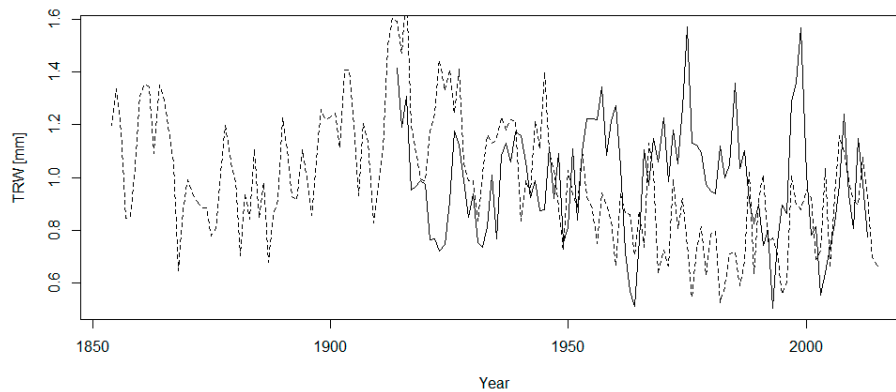


Figure 2. Tree-ring widths (TRW) of Scots pine from Hungary (solid line) and Poland (dashed line).

The biggest difference between the two chronologies was the mean ring width. Tree-rings at the Hungarian site were almost one millimeter wider on average than at the Polish site. The standard deviation was also higher at the Hungarian site but autocorrelation and mean sensitivity were similar (Table 2).

Table 2. Comparison of descriptive statistics for both raw data chronologies. Correlation with Master: the average correlation of each series with a master chronology derived from all other series; Standard deviation: Year-to-year differences in tree-ring measurement; Autocorrelation: a measure of the previous year's influence on current year's growth; Mean sensitivity: the relative change in ring-width from one year to the next; Expressed Population Signal (EPS): climate-related signal preserved in the index series, Rbar: mean inter-series correlation.

	Hungarian Site	Polish Site
Time-span	1914–2013	1855–2015
Correlation with Master	0.651	0.571
Mean ring width	2.36	1.44
Standard deviation	1.168	0.734
Autocorrelation	0.785	0.797
Mean sensitivity	0.234	0.244
Expressed Population Signal (EPS)	0.97	0.87
Rbar	0.44	0.46

3.2. Climate-Growth Relationship

At both sites, the Expressed Population Signal (EPS) was above the widely accepted 0.85 threshold indicating a high degree of common forcing [30]. Similar to EPS, the mean inter-series correlation (Rbar) also demonstrated a strong relationship between the individual series with mean values of 0.46 and 0.44 in Poland and Hungary, respectively. These results demonstrated that both chronologies had a high degree of common forcing and were suitable to represent the whole pine stand.

According to the BIOCLIM variables, the mean annual temperature in Fenyőfő was higher (about 2.2 °C) and the annual precipitation lower (75 mm) than in Tabórz (Table 1). The mean temperature of the wettest quarter at the Hungarian site was higher by 1.7 °C but the precipitation was lower by 43 mm. The mean temperature of the coldest quarter in Fenyőfő was higher by 3.1 °C than in Tabórz

and precipitation was lower by 2 mm than at the Polish site. The temperature in the warmest quarter in Fenyőfő was higher by 1.7 °C and precipitation was lower by 43 mm. In the driest quarter, mean temperature in Fenyőfő was lower by 0.9 °C and precipitation was higher by 1 mm than at the Polish site. The analysis of bioclimatic indices showed that both study sites were included in 95% of the variability for the most limiting variables for the occurrence of Scots pine. In light of the variability of all the distribution ranges, the differences between the two study sites were insignificant. We found higher differences in climatic conditions among sites within the native range than between our study sites which were relatively similar in values of bioclimatic indices (Table 1, Figure S1).

According to the results of the correlation analysis of tree-ring indices and climate, summer precipitation plays a major role in the formation of tree-rings at both sites (Figure 3). At the Hungarian site every summer month was important, especially July, and in addition, May moisture also had a significant effect on radial increment development. For the summer period, precipitation in June and July exceeded the significance level in Poland. A relatively high correspondence between February moisture and tree growth was also observed.

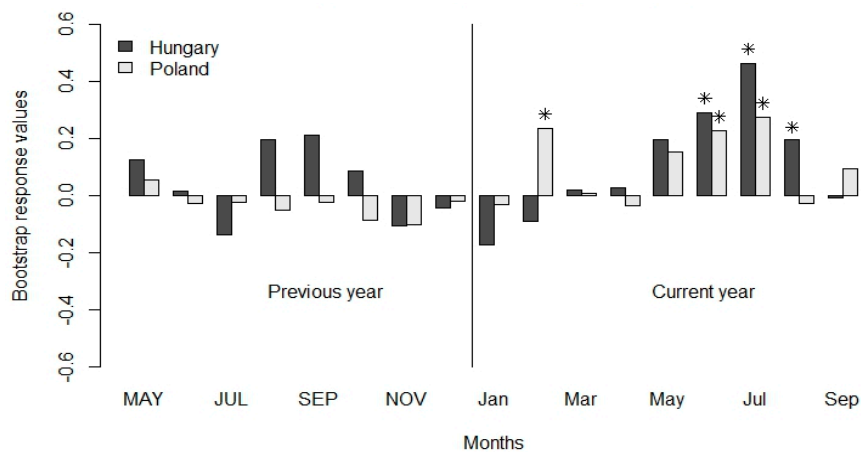


Figure 3. Relationship between tree growth and precipitation in Hungary and Poland. Asterisk (*) indicates significance at $p < 0.05$.

There were bigger differences in the role of temperature on tree-ring formation. Thermal conditions of the late winter-early spring period had a positive effect at both the study sites, but the typical negative effect of summer temperature on ring-widths was only visible at the Hungarian site. Moreover, a significant positive relationship was found between tree-ring growth and temperature in December of the preceding growth year at the Polish site (Figure 4).

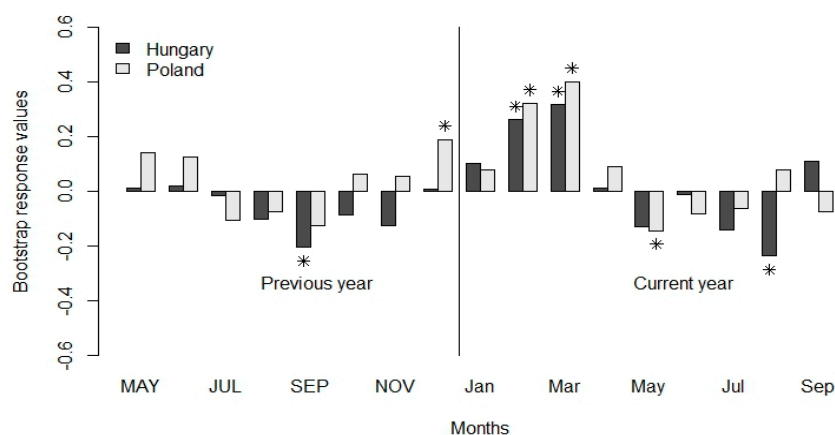


Figure 4. Relationship between growth and temperature in Hungary and Poland. Asterisk (*) indicates significance at $p < 0.05$.

Although there were similarities in the climate–growth connection on longer time-scales between the sites, there were also interesting temporal differences. Precipitation in June and July was important for radial increment development at both sites but its role has changed significantly within the last 100 years. The decreasing trend in the amount of precipitation at both sites (Figure S2), resulted in a weakening in correspondence in Hungary, but a significant increase in correlation in Poland (Figure 5).

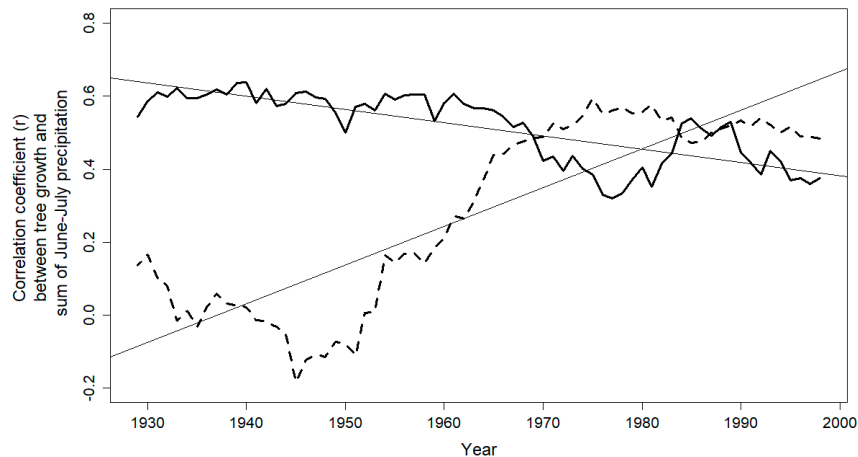


Figure 5. Changes in the influence of the sum of June–July precipitation on tree-ring widths in sequential 30-year time intervals. The solid line represents correlations of tree-ring widths from the Hungarian site with precipitation data. The dashed line shows the correspondence of radial increment development in Polish samples with rainfall.

At the Hungarian site, the significant correlation of June–July precipitation to tree-ring growth ($\sim r = 0.6$, $p < 0.05$) decreased by the end of our study period, however, in Poland, despite the decreasing amount of precipitation, June–July precipitation became almost as important as it was in Hungary in the first part of the last century. Similar to summer precipitation, the role of temperature in the late winter to early spring period has changed remarkably during the last 100 years. In this case, the two populations responded in the same way. The temperature in February and March has been increasing since the middle of the last century (Figure S3). Parallel to the warming trend, the connection between temperature and tree-growth has decreased and become insignificant by the second half of the last 100 years (Figure 6).

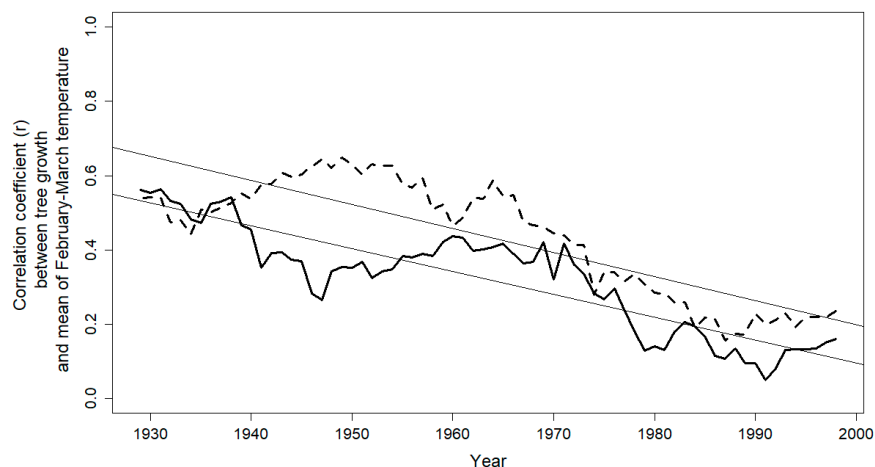


Figure 6. Changes in influence of mean February–March temperature on tree-ring widths in sequential 30-year time intervals. The solid line shows the correlation variability between tree-ring widths and temperature data at the Hungarian site, the dashed line represents the changing correspondence of thermal conditions to tree-ring growth in Polish site.

4. Discussion

Contrary to expectation, our results show that differences in climatic conditions between our study site in Poland and some other sites in the native range are higher than between the Polish and Hungarian site (Figure S1). The climatic conditions in Miłomłyn and Fenyőfő are included in 95% of variability of BIOCLIM indices from the whole native range. This result is consistent with ecological niche models which suggest that the Hungarian site is located within the optimal or close to optimal climatic conditions for *P. sylvestris* [33]. Also, the natural regeneration of Scots pine in Fenyőfő confirms that environmental conditions in this region are particularly favorable for this species. High similarity between BIOCLIM indices for the two study sites (Figure S1, Table 1) suggests that these two populations are controlled by the same climatic factors.

However, some bioclimatic values considered as limiting factors may be less favorable to species establishment and growth in Fenyőfő than in Miłomłyn. The Hungarian site is not only warmer but, its precipitation amount is lower in the warmest period (Table 1, Figure S1). Research conducted on the Iberian Peninsula and in Mongolia show that rainfall deficiency in summer can be the most limiting factor for species establishment in southernmost localities, with low summer rainfall directly leading to high mortality of seedlings [34–36]. Water deficiency in the warmest quarter is also important as a limiting factor for radial growth of this species [7,8,37,38]. This might be the most important reason behind the absence of native populations of Scots pine in Hungary. However, climatic conditions in Fenyőfő are not statistically different from climatic conditions in the full native range (Figure S1), which suggests that the occurrence of Scots pine may be limited by other factors. A smaller realized than potential niche as result of interactions between species is commonly known in ecology [39,40]. In the case of this species for seedling establishment, several biotic factors are important, like suitable microhabitat availability, occurrence of a dense herb layer, shading trees and seed predators [34–36]. It is interesting that we also found differences in the indicators related to winter and early spring temperatures (Table 1). Our findings agree with previous studies [5–7,41] that these factors have the most influence on tree-ring width formation of Scots pine. At the Polish site, the values of minimum temperature in the coldest month and mean temperature in the coldest quarter are lower than in Hungary (Table 1). Hence, winter and early spring meteorological conditions should be more favorable in western Hungary, than in northern Poland. Generally, sites within natural range are more affected by rainfall in the warmest and wettest quarters of the year. Even if the run off correlation is still high, some clear differences are visible. In term of previous findings crucial differences seem to refer to the minimum temperature of the coldest months, mean temperature of the coldest quarter and precipitation of wettest and warmest quarter (Table 1).

According to the results of the climate-growth relationship analysis there are analogous and differing effects in certain months of tree-ring formation. At low elevations and in a moderate climate, a positive dominance of summer precipitation and negative effect of higher summer temperature on tree growth is typical, as previously reported for Scots pine (e.g., [37,38]) and other conifer species [42,43] under similar climatic and environmental conditions to our sites. Our study shows that this pattern is stronger at the Hungarian site. The correlation analysis shows that for trees growing in Hungary, summer precipitation is the most important limiting factor, its amount principally influences incremental growth (Figure 3). A negative effect of temperature prevails in September of the previous year and August of the current year (Figure 4). In contrast, in Poland summer precipitation has a lower impact on tree growth and is confined to June and July, with some residual influence in February (Figure 3). The typical negative pattern of summer temperature is missing in this dataset; the effect of temperature was found to be significantly negative in May only (Figure 4). This difference between tree-ring width variation and temperature may also reflect higher sensitivity to aridity and summer drought at the Hungarian site. February and March temperature is the most important factor in tree-ring formation in Poland, but it has a significant role in Hungary as well (Figure 4). One explanation to this direct relationship may be the increased demand for photosynthesis in the early stage of the vegetation period

because higher winter temperatures reduce the snow cover and promote infiltration of moisture into the soil. This leads to a higher rate of photosynthesis at the beginning of the growing season [44].

Over the course of a climate–growth relationship analysis, the temporal stability of the detected correspondence is always a core issue. In parallel with international trends [45–47], the climate at our study sites has changed significantly over the last 100 years, which has already had significant impact for Scots pine forests in Hungary [8,48,49]. In spite of the increasing temperature in the February–March period, its role on tree-ring growth has been decreasing since the middle of the 20th century. At the same time as the warming, correlations in both months started to decline, and the temperature of February and March has become an insignificant factor in terms of tree-ring width variation. Earlier studies [42,50] on Norway spruce in the lowland area of Poland found the same reaction to warming conditions in the late winter to early spring period. These studies noted that this was likely a result of higher temperatures disturbing the hardening of trees, so that they become more vulnerable to late winter and early spring frosts. A similar process has been observed at high northern latitudes as well [51], where summer temperature is the main limiting factor of tree growth. According to tree-ring widths and density data, the increasing temperature after the 1950s may have caused a weakening response between conifers' tree-ring growth and mean summer temperatures [52–54]. This phenomenon is called the divergence problem. Although there are still questions concerning the exact mechanism of this phenomenon, studies by D'Arrigo et al. [54] have noted that temperature alone is not always sufficient to characterize the trees' thermal environment, since they will also be influenced by other factors such as soil moisture, soil temperature or insolation. While thermal conditions in the late winter to early spring period are substantially similar, precipitation trends in June–July differ considerably, just like the strength of relationship between moisture and tree growth in summer. The recorded reaction of the Hungarian pine stand to precipitation decline is in line with our expectations. Owing to the emphasized role of summer precipitation on tree-ring growth (especially in July), decreasing precipitation reduced its positive effect on radial increment development. June–July precipitation has lower impact on tree-growth at our Polish site on a longer time-scale, but it is interesting that in the last 50 years, regardless of the similar decreasing trend of precipitation, in Poland the pine stand reacted in an entirely different way to these changing environmental conditions. Such alterations in the climate–growth relationship are particularly important because they impact the most important periods of tree-ring growth at both sites and may lead to growth reduction on a longer time-scale. As Bauwe et al. [55] noted, growth trends in Scots pine tree-rings will essentially depend on the growth location in the future. In the northern part of Europe radial increments will most probably increase, but in the central and southern part of the continent, reduced growth is predicted [54]. According to Bauwe et al. [55], growth can remain stable if the positive influence of warming winters can balance the negative effect of summer drought. Based on our results, the positive impact of warming winters was not detected at the Hungarian site, moreover, the role of more frequent arid summers on tree-ring formation is steadily increasing.

5. Conclusions

Our study shows that both bioclimatic predictors and climate–growth relationship produce comparable results in Hungary and Poland. In our case, the growth of Scots pine is controlled by similar climatic conditions and the tree-ring growth response to this is analogous. Late winter and early spring temperature and summer precipitation proved to be the most important factors controlling tree-growth at both sites, but there were temporal differences over the last 100 years. Warming in February and March caused a weakening in the correlation between temperature and tree-ring width variation in Hungary and Poland, but decreasing summer precipitation resulted in a declining growth response at the Hungarian site, but with an increased correlation with summer precipitation at the Polish site. This research supports previous findings that growth response in terms of wood biomass may be differently affected for this important commercial species depending on certain site-specific variables.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/3/243/s1>, Figure S1. Comparative analysis of the most limiting BIOCLIM [19] values: black square–Fenyőfő; black dot–Tabórz; grey dots–273 localities from all distribution range of *P. sylvestris* ssp. *sylvestris* taken from Conifers of the World database [25]. Ellipses indicate 95% variability of climatic values for all sampling sites. Figure S2. Changes in the sum of precipitation from June to July at Fenyőfő (solid line) and the Mazury Lakeland (dashed line). The mean correlation (r) between sites is 0.46 ($p < 0.05$). Figure S3. Changes in the mean of temperature from February to March at Fenyőfő (solid line) and the Mazury Lakeland (dashed line). The mean correlation (r) between sites is 0.93 ($p < 0.05$).

Author Contributions: Conceptualization, D.M., M.K., R.P.; Formal analysis, D.M., R.P. and M.K.; Investigation, D.M., R.P. and M.K.; Methodology, D.M., R.P. and M.K.; Writing—Original draft, D.M., R.P. and M.K.; Writing—Review & editing, C.P. and I.R.

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Conflicts of Interest: The authors declare no conflict of interest.

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