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Adaptive diversity of beech seedlings under climate change scenarios

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Abstract

The ability of beech (Fagus sylvatica L.) populations to adapt to the ongoing climate change is crucial for the maintenance of economic and social benefits and for the conservation of biodiversity in Europe and especially in the southeastern part of the continent, where environmental change is expected to be more intense. Beech populations in the region cover multiple ecological conditions at a small geographical range and have a complex biogeographical background involving several postglacial lineages originating from distant or local refugia. In this study, we tested the existing adaptive potential of eight beech populations from two provenances in N.E. Greece (Evros and Drama), under simulated controlled climate change conditions in a growth chamber and in the field. In the growth chamber, simulated conditions of temperature and precipitation for the year 2050 were applied for three years, under two different irrigation schemes, a non-frequent (A1) and a frequent one (A2). Seedling survival, growth and leaf phenological traits were used as adaptive traits. The results showed that beech seedlings were generally able to survive under climate change conditions and showed adaptive differences among provenances and populations. Furthermore, beech genotypes demonstrated an impressive phenotypic plasticity by changing the duration of their growing season allowing them to avoid environmental stress and high selection pressure. Different populations and provenances were connected with different adaptation strategies, that relate mainly to the temporal distribution patterns of precipitation and temperature, rather than the average annual or monthly values of these measures. Additionally, different adaptive strategies appeared among beech seedlings when the same amount of water was distributed differently within each month. This indicates that the physiological response mechanisms of beech individuals are very complex and depend on several interacting parameters. For this reason, the choice of beech provenances for translocation and use in afforestation or reforestation projects should consider the small scale ecotypic diversity of the species and view multiple environmental and climatic parameters in connection to each other.

Keywords: *Fagus sp.*, phenology, survival, height, adaptation, common garden experiment

1 Introduction

The European beech (*Fagus sylvatica* L.) is widely considered as one of the most important tree species in the continent, both economically and ecologically. Its geographical range extends from Scandinavia to the Mediterranean covering various habitats (Bolte et al., 2007; Willner et al., 2017). It is generally considered as an oceanic species that can grow in areas with mild winters and moist summers, sensitive to intense drought periods in the growing season (Fotelli et al., 2001, Leuschner et al., 2001, Granier et al., 2007, Bolte et al., 2007, Pšidová et al., 2015). Several studies report that beech populations in Southern Europe have faced strong selective pressures during the last decades (Jump et al., 2006, Piovesan et al., 2008), which are expected to become more intense because of future changes in rainfall patterns and temperatures under the forthcoming climate change (Charru et al., 2010, Rita et al., 2014), arising concerns about the survival dynamics of the European populations (Bréda et al., 2006, Geßler et al., 2007). Fotelli et al. (2009) report that the intense drought in 2003 largely affected beech populations across Europe but had a rather insignificant effect on Greek beech populations, leading to the expectation that southern populations may harbor genetic adaptations that allow survival under conditions of increased water stress. Dounavi et al., (2016) report that Greek beech provenances are

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best adapted to prolonged drought periods and emphasize their potential as a source of reproductive material for Europe in the face of climate change.

The adaptive potential of tree populations can be described through various parameters such as growth, survival and shifts in phenology. Seedling growth can be severely affected by abiotic stressors such as temperature and water deficiency. As climate becomes warmer and summer precipitation is expected to decline, beech populations may face intense drought periods. Under water stress, plants usually decrease growth in terms of both height and biomass accumulation because of minimization in carbon fixation through photosynthesis. In addition, low soil water potential affects hydraulic traits (e.g. conductivity) and can create xylem cavities leading to plant mortality (Bolte et al., 2016). Survival under environmental pressure is of primary importance as it directly reflects populations' regeneration dynamics which depend both on genetic and environmental factors (Ngulube, 1989, Sexton et al., 2002, Matías and Jump, 2014).

Although climate is expected to become warmer on average, frost events are not expected to diminish during the 21st century. These events are especially crucial when they take place too early in fall or too late in spring. Under natural conditions, plants are preparing their cryoprotection by incorporating soluble carbohydrates, hydrophilic polypeptides, antioxidants and chaperones (Thomashow 1999) into their cellular membranes to ensure their survival during winter (Hofmann et al., 2015). Species growing in warmer environments are expected to invest less in cryoprotection than those from colder environments. As supported by Kreyling et al., (2012), beech forests in Central Europe are sensitive to late frost events and climate warming may lead to early leaf flushing and more frequent frost damage in the future. For this reason, it is important to investigate marginal beech populations that are expected to be adapted to cold events and extended periods of drought in the growing season at the same time (St Clair and Howe 2007; Eilmann et al., 2014; Thiel et al., 2014).

Leaf phenology is a key adaptive trait that determines carbon balance (production and accumulation) and the overall growth of a plant species, while also affecting ecosystem productivity (Kramer et al., 2000; Larcher, 2003). Bud burst, and leaf senescence are the most important leaf phenological traits used in studies, since they mark the onset, duration and ending of a species growth period. Bud burst reflects the transition phase from the winter dormancy to the onset of next year's growth period and requires a preceding chilling period (Heide 1993; Kramer et al., 2017). Late bud burst can protect from late frosts but can also reduce the growth period (Lechowicz, 1984; Višnjić and Dohrenbusch, 2004). Bud burst is referred to be under genetic-provenance control (Robson et al., 2011), while it is also directly affected by environmental factors such as temperature and photoperiod (e.g. Heide, 1993; Yan and Wallace 1996; Basler and Körner 2014; Schüler and Liesebach, 2015; Kramer et al., 2017). Bud burst includes various metabolic activities, through which carbohydrate reserves, that were invested in buds after their formation during the previous growth period, are getting mobilized. It is known that water deficiency leads to a reduction in carbon fixation and subsequently in diminished growth. However, a limited number of studies exist on the possible effect of irrigation on bud burst timing of temperate forest trees (e.g. Morin et al., 2010; Kuster et al., 2014).

The time of leaf senescence determines the end of the growing period and the onset of winter dormancy and strongly depends on the environmental factors during the current year. For example, premature leaf senescence can be observed under low summer and autumn precipitation to mobilize leaf nutrients (Sedigheh et al., 2011; Chen et al., 2015; Tombesi et al., 2015; Gill et al., 2015), while leaf senescence can be delayed by higher autumnal temperatures (Fu et al., 2017), as well as by an increased photoperiod (Way and Montgomery 2014; Gill et al., 2015). Furthermore, it is also influenced by spring leaf phenology (Panchen et al., 2015; Fu et al., 2014; Keenan and Richardson 2015) and at the same time it can affect leaf flushing in the next year (Heide et al., 2003). Besides genetic adaptations, plants may demonstrate plasticity in their leaf phenology as a response to changes in the environmental conditions (Estiarte and Penuelas, 2014; Mijnsbrugge et al., 2016).

Apart from the existence of specific adaptive variants that fit to the environmental conditions of the locations of populations, a large genetic diversity within populations is expected to play a significant role in plant adaptation under future conditions (adaptability). High genetic diversity in a population guarantees the existence of many alleles at adaptive genes, that may become advantageous when facing environmental stressors in the future (Nicotra et al., 2010). To describe the patterns of diversity in quantitative adaptive traits among populations, plants belonging to different provenances need to be compared in specific common garden tests. When more tests are established on different sites under varying conditions, they can reveal provenance-environment interactions (Scheepens et al., 2010, Malyshev et al., 2014, de Villemereuil et al., 2016), contributing to the selection of reproductive material sources for future restoration and management activities (Carón et al., 2015, Bezděčková and Matějka 2015). Several provenance tests exist in Europe for beech, under field or glasshouse conditions (e.g. von Wühlisch et al., 1995; Nielsen and Jørgensen 2003; Czajkowski and Bolte, 2006; Gömöry and Paule 2011; Liesebach 2012; Schüler et al., 2012; Kreyling et al., 2012, Thiel et al., 2014; Harter et al., 2015; Dounavi et al., 2016; Petkova et al., 2017), providing important information for forest management and conservation of beech populations.

Beech populations in Greece have been found to demonstrate high levels of genetic diversity in non-adaptive gene markers (Papageorgiou et al., 2008; Hatziskakis et al., 2009, Dounavi et al., 2016). One of the reasons for this genetic richness may reside in the existence of several large and small glacial refugia for beech in the country and the complex migration pattern of beech refugial populations after the end of the last glaciation period (Hatziskakis et al., 2009; Papageorgiou et al., 2014). This biogeographical complexity of beech in northern Greece is further enhanced considering a possible influence from the eastern beech (*F. orientalis*) that is reported to form a hybrid zone with European beech (*F. sylvatica*) on the eastern side of the Balkan peninsula (e.g. Gömöry et al., 1999; Hatziskakis et al., 2009; Houston Durrant et al., 2016).

To overcome the negative consequences of the expected increase in drought on the future performance of beech forests in afforestation programs in Europe, several authors suggest testing and using beech ecotypes that are adapted to a less oceanic climate (Nielsen and Jørgensen, 2003; Schraml and Rennenberg, 2002; Meier and Leuschner, 2008; Rose et al., 2009), such as the refugial beech populations from Southern Europe (Rennenberg et al., 2004; Geßler et al., 2007). The aim of our study is to describe the existing adaptive potential of beech in the southeastern part of Europe (N.E. Greece), through the comparison of adaptive traits at seedlings belonging to different provenances with complex biogeographical background representing different postglacial lineages, under simulated controlled climate change conditions in a growth chamber and in the field.

2 Materials and Methods

2.1 Area of the study and selection of populations

Northeastern Greece is a mountainous region, with a topographically diverse landscape covering various altitudes. Mt. Rodopi, a long massif shared between Greece and Bulgaria, dominates this region and extends from the east to the west. In the lowlands, the climate is subhumid and submediterranean, with harsh winters and dry summers, while it becomes more humid and temperate with harsh winters and no summer drought in higher altitudes (Mavromatis, 1980). Beech forests are present all over this region, covering habitats with a large variety of environmental conditions (Bergmeier and Dimopoulos

2001, Tsiripidis et al., 2007). According to genetic and ecological studies, beech populations in N.E. Greece have a complex biogeographic background, since they represent multiple postglacial lineages, originating from different glacial refugia (Hatziskakis et al., 2009; Papageorgiou et al., 2014). At the same time, this region is suggested to be part of a possible introgression zone between two beech species *F. sylvatica* and *F. orientalis* (Gömöry et al., 1999; Hatziskakis et al., 2009; Kandemir and Kaya, 2009; Hatziskakis et al., 2011; Papageorgiou et al., 2014; Houston Durrant et al., 2016), with the former species being present in the western part of the region (west Mt. Rodopi, Mt. Falakro, Mt. Menikio) and the latter characterizing the eastern part of the Mt. Rodopi (Chistensen 1997, Tiripidis and Athanasiadis 2003, Papageorgiou et al., 2008).

Two beech provenances were chosen in N.E. Greece, "Evros", on the eastern side and "Drama" on the western side of the study area (Figure 1 and Supplement 1). Four populations were selected in each provenance (E1 - E4 and D1 - D4 respectively), representing different postglacial lineages, based on genetic studies (Hatziskakis et al., 2009; Papageorgiou et al., 2014; Manolis et al., 2017). Due to the absence of long term meteorological data from the area of the selected populations, we used current climatic data available from worldclim.org in a 30-acr seconds resolution (version 1.4) (Hijmans et al., 2005; Souto et al., 2009). Four basic climatic and 19 bioclimatic variables were extracted for the coordinates of each sampled seed parent and the average values were used to describe each population (Supplements 3, 4 and Figure 2). Provenance Evros represents a climatic environment with moist and cold winters, warm and dry summers with an intense (but not long) drought period. The climate in Drama provenance appears to be more continental with relatively moist and more severe winters as well as warm summers with a less intense dry period (Gouvas and Sakellariou, 2011).

2.2 Seed collection and germination

Seed collection occurred in autumn 2012, a non-mast production year for beech in the study area, since less than 50% of the trees produced nuts. In each population, 60-80 seeds per seed tree were collected from 30 trees, totaling 240 families. We consider seeds and later seedlings originating from the same seed tree as a half-sib family. After their transfer to the laboratory, seeds were air-blown to remove the empty ones. The remaining seeds were immersed for 2 minutes in 35% H_2O_2 (Anand and Chanway, 2013) for disinfection and thoroughly rinsed with tap water for several minutes. After the cleaning procedure, seeds were subjected to cold stratification for 90 days (Baskin and Baskin 2001) at 0°C in petri dishes filled with sterilized sand. Each dish contained 10 randomly selected seeds per family, totaling 300 seeds per population and 1200 seeds per provenance. Germination was completed during the stratification stage. Seeds that germinated were transferred to plastic pots filled with turf, sand and perlite in a ratio of 4:2:1, respectively. The emerging seedlings were evaluated as normal or abnormal according to ISTA (1999) specifications. Only normal seedlings were included in the following experiments.

2.3 Growth chamber tests

In March 2013, the surviving normal seedlings of both provenances were put in a growth chamber under simulated temperature and precipitation levels estimated from the CSIRO MK3 CGM model, according to the expected conditions in the year 2050 (downloaded from Climong.org) (Kriticos et al., 2012) (Supplement 1). The specific model was selected for its relevance with the summer drought periods in the Mediterranean region (Marcos and Tsimplis, 2008; Syktus et al., 2011; Ziv et al., 2013; Pulvento et al., 2015). The reference area for the climate simulation in the growth chamber was the location "Agios Georgios" (Drama, Greece) that corresponds to population D4 in this study. Climate change scenario A1 and storyline A1B were selected, with the assumption that the industrial development in the areas of the populations of this study will remain minimal and that there will be a balanced use of all energy sources until 2050 (IPCC 2007). This model incorporates the indirect effects of greenhouse gases in the change of the estimated future bioclimatic parameters (Kriticos et al., 2012).

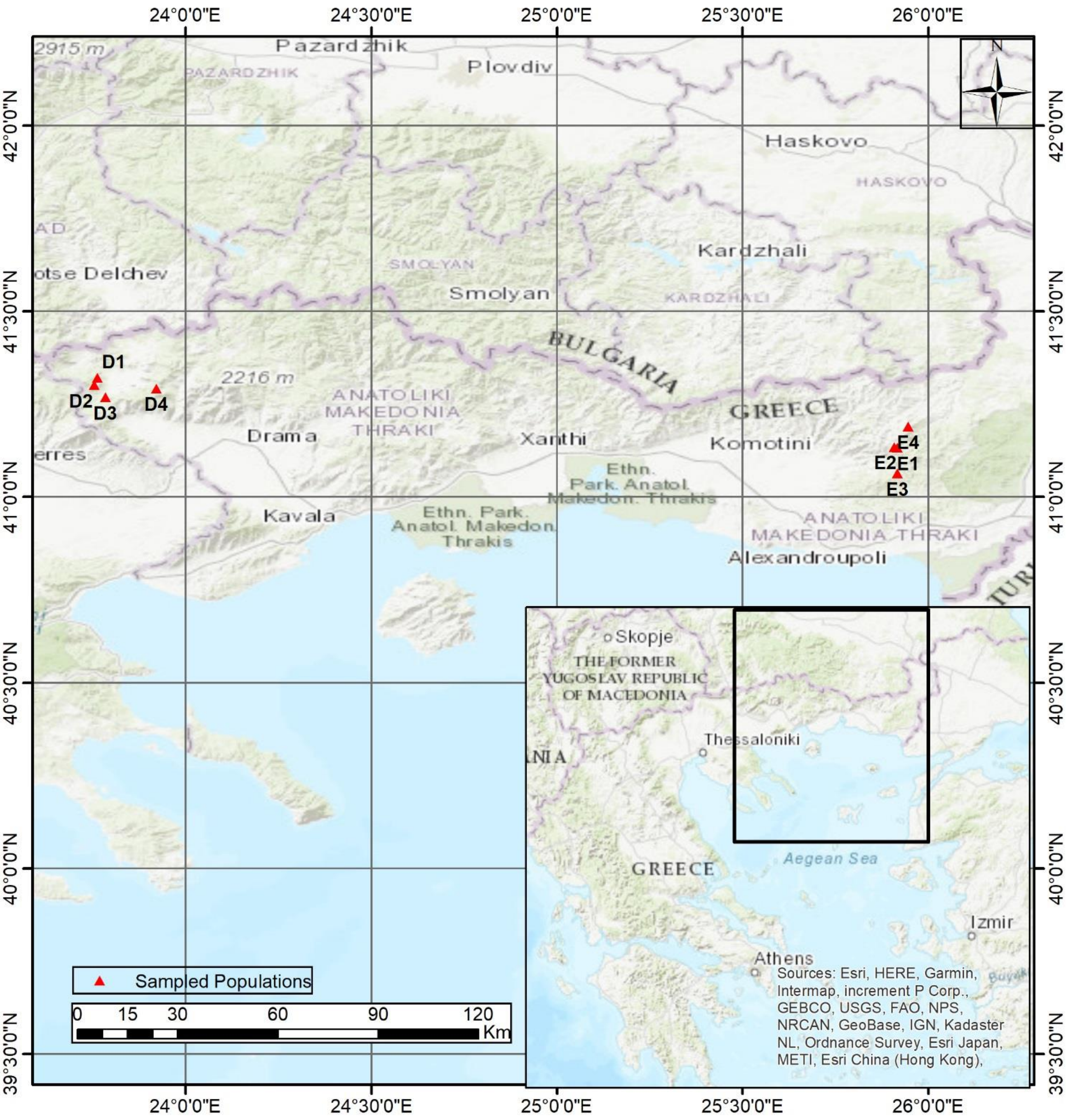

Figure 1. Map of the areas of study.

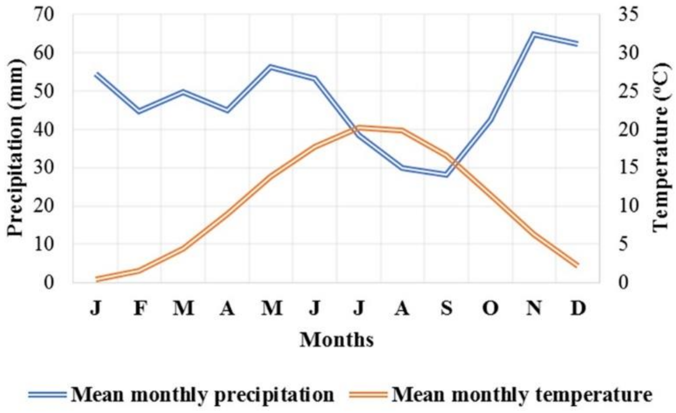

Figure 2-A. Climatic diagram for Drama provenance.

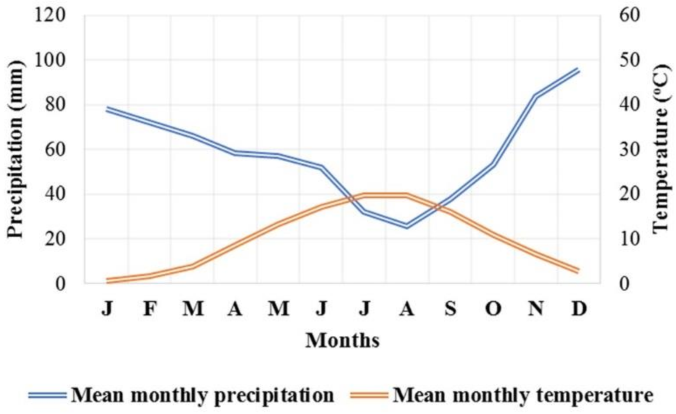

Figure 2-B. Climatic diagram for Evros provenance.

For each month, the estimated maximum, minimum and mean temperatures, as well as the precipitation data were extracted from the above dataset using the DIVA-GIS software (www.diva-gis.org) (Supplement 2). A simulated temperature scheme, with three-hour intervals during a period of 24 hours, was applied in the growth chamber for each month of the experiment, in correspondence with the extracted temperature variables. Illumination intensity during the day inside the chamber was set according to *in situ* observations that took place in the reference location under clear sky. The lengths of day and night for each month were adjusted to those under natural conditions in the reference area.

The simulated monthly precipitation height (mm) was converted into water volume (ml) to regulate seedling irrigation, following Brouwer et al., (1985). Besides precipitation height, we tested the effect of precipitation distribution within a month on seedlings, especially during the summer period, since climate change is expected to destabilize the current precipitation frequency and intensity and cause longer drought periods, as well as climate extremes in the Eastern Mediterranean region (Alpert et al., 2002; Gao et al., 2006; Lelieveld et al., 2012). For this reason, precipitation was distributed within each month according to two different irrigation schemes:

a. Irrigation scheme A1 (non-frequent): irrigation of seedlings every 20 days with the relevant amount of water of the corresponding month (50% of monthly precipitation height when irrigation occurred twice a month, or 100% of monthly precipitation height when irrigation occurred once a month).
b. Irrigation scheme A2 (frequent): irrigation of seedlings every seven days with 25% of the simulated monthly precipitation height.

One seedling per family and per irrigation scheme was included in the experiment. Seedlings representing population D1 were abnormal in a high ratio (66%), producing finally a limited number of viable normal seedlings that were not enough to represent D1 in both irrigation schemes. For this reason, D1 was excluded from the experiment in the growing chamber and the viable normal seedlings from this population were used only in the common garden test under field conditions.

The growth chamber experiment lasted for 3 years (2013-2016). Measurements included seedling height, survival and leaf phenological traits (Madsen 1994; Minnota and Pinzauti 1996; Hiura 1998; Whiteley et al., 2003; Kanaga et al., 2008). Seedling survival was monitored every week and the non-surviving ones were removed. Seedling height was measured at the end of each growing season from October 2014 until October 2016. Phenology measurements included bud burst (leaf emergence), leaf senescence and duration of the growing period. Both parameters were expressed in number of days. The beginning of bud burst was considered to occur when the bud scales opened in a way that the newly emerged leaves were visible. Individual plants were considered entering the senescence stage when at least 50% of their leaves were discolored (Gömöry et al., 2015). Measurements of phenological traits were conducted in 3 day - intervals. Plasticity between the experimental years was also calculated for all the measured traits by subtracting the interim values.

2.4 Common garden experiment under field conditions

A common garden experiment under field conditions was set up in Agios Georgios (Drama, Greece), the same location that was used as a reference for the climate simulation in the growth chamber. An area of 100m^2 was fenced and a total number of 480 seedlings (2 seedlings/family/population) were planted in a natural beech stand with dense crown closure (70-80%). Survival measurements were taken two times per year during the months of October and April for 3 years. The survival percentage at the end of the three-year experiment was used in this study.

2.5 Basic statistical analysis

A comparison of means between populations and provenances and between irrigation schemes was performed for all variables. Differences in seedling survival were described through the chi-square test, since survival was scored as a binary variable. Means in seedling height were compared with the analysis of variance (ANOVA) and the LSD criterion, since normal distribution and homoscedasticity of data were shown. Differences in the number of days for bud burst or leaf senescence were calculated using the non-parametric Mann-Whitney U test, since normal distribution assumptions were not met. For all comparisons, the software STATISTICA v.10 (STATSOFT inc) was used.

2.6 Hierarchical linear multilevel models (HLM)

To assess the effect of provenance, population and irrigation scheme on the dependent variables (traits), we utilized a three-level hierarchical linear modelling approach (HLM; Raudenbush and Bryk, 2002), that considers the nested structure of the data in this study. A major advantage in this type of model over the standard regression models, is the within group and between groups comparison and the improved accuracy of point estimates in model parameters (see, e.g., Katahira, 2016). The framework of the model considers n_{ijk} responses on the dependent variables, nested within the populations ($i = 1, 2, \ldots, 8$) which are again nested within the provenances of Evros and Drama ($j = 1,2$). The last level of this nested modelling structure is completed with the inclusion of the two different irrigation schemes ($k = 1,2$). The first level of the model is described as:

$$\boldsymbol{y_{ijk} = \beta_{0ijk} + \beta_{1ijk} \times X_{ijk} + e_{ijk}} \qquad (1),$$

where $\boldsymbol{y_{ijk}}$ is the trait as a continuous response variable, $\boldsymbol{X_{ijk}}$ denotes the level-1 predictor variable of populations nested within each provenance and $\boldsymbol{e_{ijk} \sim N(0, \sigma_e^2)}$ is the observation-level deviation which is normally distributed. The β- coefficient of the slope in equation (1) is subsequently used as a response variable (second level):

$$\boldsymbol{\beta_{1ijk} = b_{0jk} + b_{1jk} \times X_{jk} + r_{jk}} \qquad (2),$$

where $\boldsymbol{X_{jk}}$ is the level-2 predictor factor (provenance) and $\boldsymbol{r_{jk} \sim N(0, \sigma_r^2)}$ expresses the normally-distributed deviations at the provenance level. Finally:

$$\boldsymbol{b_{1jk} = \gamma_{0k} + \gamma_{1k} \times X_k + u_k} \qquad (3),$$

with $\boldsymbol{X_k}$ denotes the irrigation scheme factor and $\boldsymbol{u_k \sim N(0, \sigma_u^2)}$.

Variables "population", "provenance" and "irrigation scheme" were the fixed effects in our models. For the provenance factors, "Evros" was used as a reference category, while the first population within each provenance was used as a reference category for the population factors. Finally, we have used the irrigation scheme A1 as a reference category of the irrigation scheme.

Model fit was assessed by initially fitting the null model (Model 0), which includes only the grand mean as predictor. One new predictor variable is added for each subsequent model. While Model 0 includes only the intercept, Model 1 introduces the factor of the provenances and Model 2 adds the component of populations. Finally, Model 3 additionally includes the factor of irrigation scheme. Every next model is compared with the previous one for the assessment of the significance of the predictors on the dependent variables. The likelihood ratio test (LRT) is used for the comparison of models, which is calculated through the following likelihood ratio statistic:

$$\boldsymbol{D = 2 \times (\ln(likelihood_{Mi}) - \ln(likelihood_{Mi-1}))} \qquad (4),$$

where Mi-$_1$ denotes the reduced model and Mi the model with the additional parameter. The lme4 library (Bates et al., 2012) of the R software was used to fit the HLM models.

3 Results

3.1 Seedling survival

Survival under field conditions was generally low (average 33.74%) and all populations had survival rates below 60% (Figure 3). Populations E1 and E2 showed the lowest survival in the field (12% and 6%, respectively), while population D3 and the local D4 the highest (57% and 48%, respectively). In the growth chambers under controlled conditions, average survival was generally higher for irrigation scheme A2 than A1 (87.68% and 71.78%, respectively). Besides E1 that demonstrated equal survival for both irrigation schemes, all populations showed lower survival percentages under A1. However, this difference was statistically significant only in populations E4, D2 and D4 (Figure 4).

Table 1. Differences among populations in total seedling survival under irrigation schemes in the end of the experiment[¥].

Population	**Irrigation scheme A1**						
	E1	E2	E3	E4	D2	D3	D4
E1	-----	ns	*	*	***	***	***
E2	ns	-----	ns	ns	*	*	*
E3	*	ns	-----	ns	*	ns	*
E4	*	ns	ns	-----	ns	ns	ns
D2	***	*	*	ns	-----	ns	ns
D3	***	*	ns	ns	ns	-----	ns
D4	***	*	*	ns	ns	ns	-----
	Irrigation scheme A2						
Population	E1	E2	E3	E4	D2	D3	D4
E1	-----	ns	ns	ns	ns	ns	ns
E2	ns	-----	ns	ns	ns	ns	ns
E3	ns	ns	-----	ns	ns	ns	ns
E4	ns	ns	ns	-----	ns	ns	ns
D2	ns	ns	ns	ns	-----	ns	ns
D3	ns	ns	ns	ns	ns	-----	ns
D4	ns	ns	ns	ns	ns	ns	-----

[¥] *Significant at *p<0.05, **p<0.01, ***p<0.001*

In the growth chamber, seedling survival was generally higher in provenance Evros and this difference was more profound under the non-frequent irrigation scheme A1 (Figure 4 and Table 1). Almost all seedlings of E1 survived under both irrigation schemes. Under scheme A1, all populations belonging to Drama showed a significantly lower survival rate than the Evros populations, except for E4 that showed no significant difference in survival with any population besides E1. No significant differences in seedling survival were found between populations under the frequent irrigation scheme A2 (Table 1).

The comparison of the HLM models showed that only the irrigation scheme had a significant effect on seedling survival (Table 2). When the growing environment was considered separately, provenance demonstrated a significant effect on survival under irrigation scheme A1 in the growth chamber, while no effect was recorded under A2. In the field experiment, population was the only significant factor affecting seedling survival. Compared to scheme A1, scheme A2 showed significantly higher survival in the growth chamber and significantly lower survival for beech seedlings in the field experiment (Table 3). Under irrigation scheme A1, provenance Evros demonstrated significantly higher survival than Drama. In the field experiment, populations E3 and E4 had a significantly higher seedling survival than E1 and E2, while seedlings of population D3 and D4 survived better than D1 and D2.

The partition of variance for the overall survival was explained by the different growing environments (irrigation schemes in the growth chamber and field trial) to a great extend (73%), while provenance and population had no effect at all (Figure 5). Variance partitioning was explained more by the fixed effects (population and provenance) in the field trial (36.5%) than in the growth chamber under irrigation scheme A2 (21.1%). A relatively larger proportion of variance was explained by population than by provenance under field conditions and under the irrigation scheme A1 in the growth chamber, while under A2 both provenance and population accounted for a small fraction of the overall variance (10.8% and 10.3%, respectively).

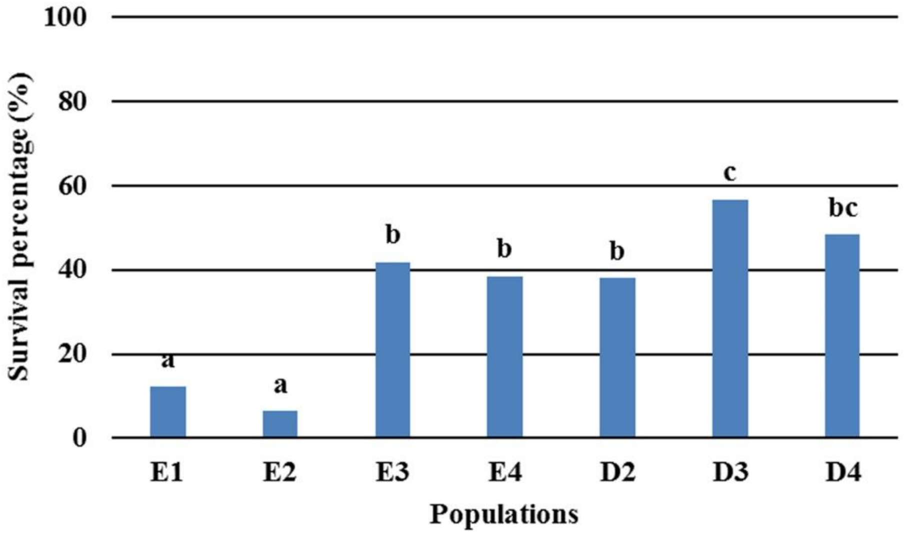

Figure 3. Survival percentages under common field experiment*.

**Values followed by the same letter do not differ among populations at 0.05 level of significance*

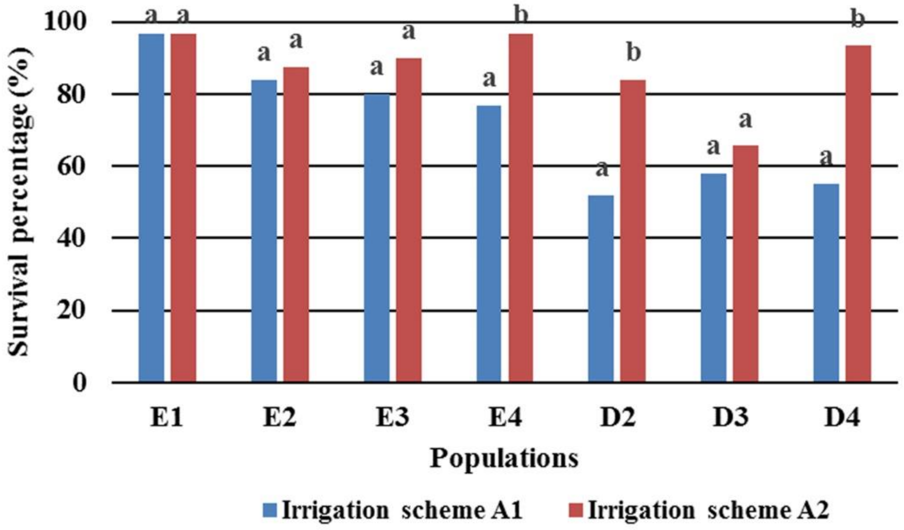

Figure 4. Survival percentages under common chamber experiment*

**Values followed by the same letter do not differ between irrigation schemes for each population separately*

Table 2. Model comparisons of the HLM models for the survival data via LRT (D: likelihood ratio statistic; P: p-value of the statistical significance of LRT test).

Dependent variables	**Model 1 (Null Model)**	**Model 2 (Population effects)**		**Model 3 (Model 2 + Provenance effects)**		**Model 4 (Model 3 + Irrigation scheme effects)**	
	D	***D***	***P***	***D***	***P***	***D***	***P***
Survival (complete)	492.86	487.04	n.s.	485.17	n.s.	469.67	**
Survival under A1 scheme	182.39	178.73	n.s.	167.20	**		
Survival under A2 scheme	69.77	61.23	n.s.	59.41	n.s.		
Survival in field conditions	183.72	165.37	**	162.13	n.s.		

¥Significant at **p<0.01

Table 3. Parameter estimates for the best selected models (5% level of significance) for survival data.

	Dependent variables			
Independent variables	**Survival (complete)**	**Survival under A1 scheme**	**Survival under A2 scheme**	**Survival in field conditions**
Intercept (β_{0jk})	0.76	0.96	0.88	0.23
Plot in Evros (Ref: E1)				
E2	n.s.	n.s.		n.s.
E3	n.s.	n.s.		0.15
E4	n.s.	n.s.		0.16
Plot in Drama (Ref: D2)				**Plot in Drama** (Ref: D1)
D2	---	---		n.s.
D3	n.s.	n.s.		0.32
D4	n.s.	n.s.		0.26
Provenance (Ref: Drama)				
Evros	n.s.	0.41		
Irrigation (ref: Irrigation scheme A1)				
Irrigation scheme A2	0.14			
Field conditions	-0.38			

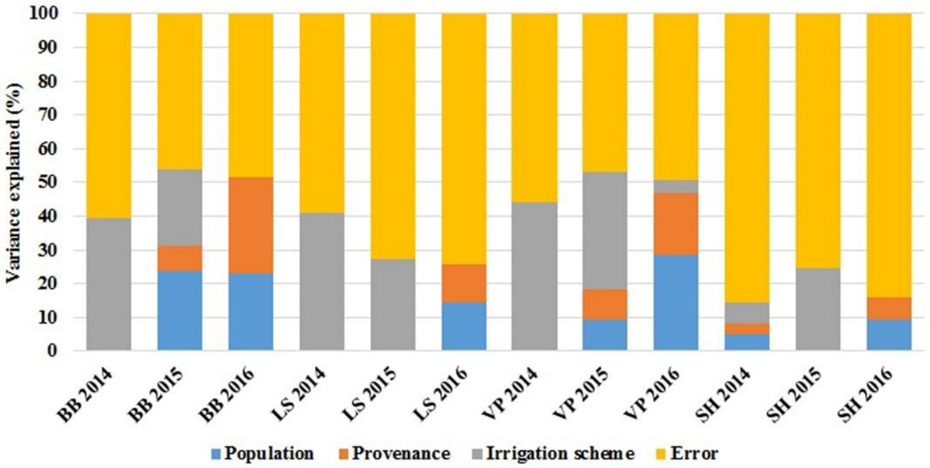

Figure 5-A. Overal partitioning of variance across years of study*.

**Abbreviations: BB (Bud burst), LS (Leaf senescence), VP (Growing period), SH (Seedling height)*

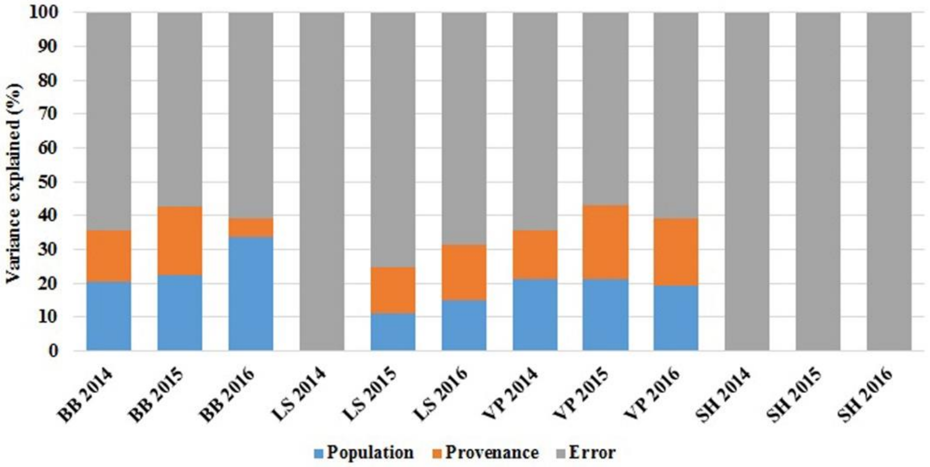

Figure 5-B. Partitioning of variance across years of study under irrigation scheme A1.

**Abbreviations: BB (Bud burst), LS (Leaf senescence), VP (Growing period), SH (Seedling height)*

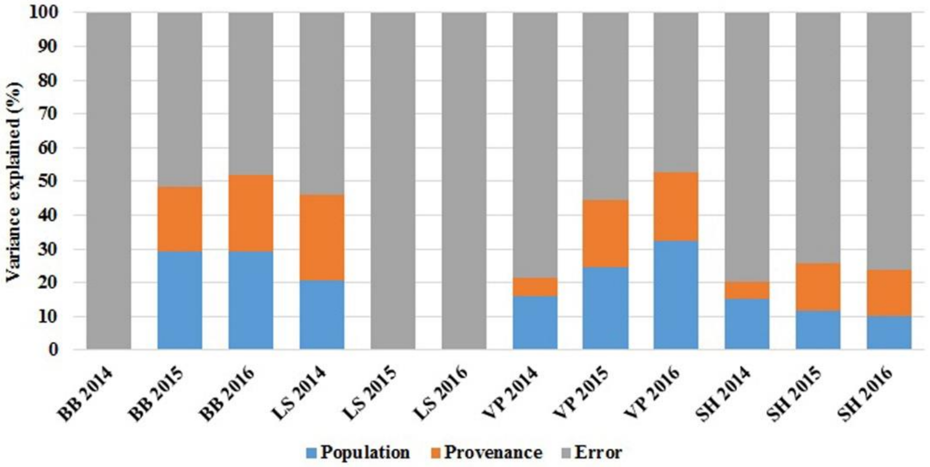

Figure 5-C. Partitioning of variance across years of study under irrigation scheme A2.

**Abbreviations: BB (Bud burst), LS (Leaf senescence), VP (Growing period), SH (Seedling height)*

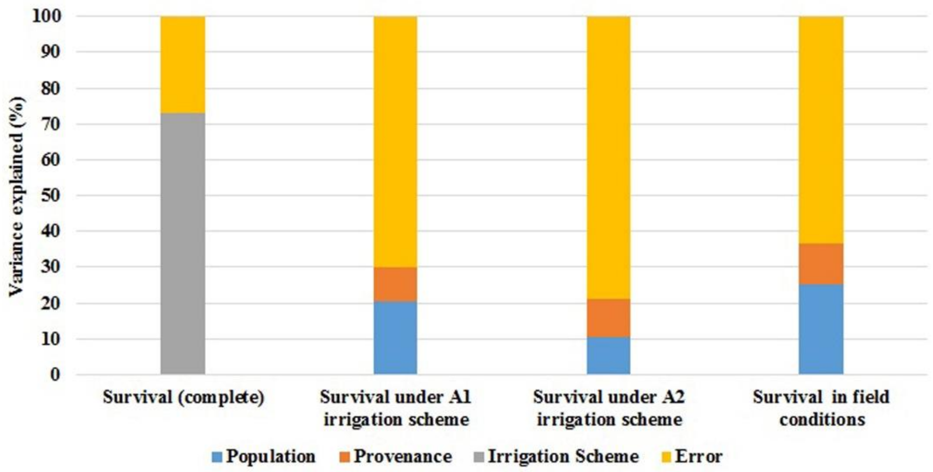

Figure 5-D. Partitioning of variance across years of study for seedling survival.

**Abbreviations: BB (Bud burst), LS (Leaf senescence), VP (Growing period), SH (Seedling height)*

3.2 Seedling height and growth

Seedlings from most populations reached a greater height in the growth chamber under irrigation scheme A1, but this trend was statistically significant only for populations E1 and D3 during the first two years of the study (2014 and 2015) and for E3 for the first year (2014) only (Table 4). Population E4 demonstrated a reverse trend, where seedlings became higher under the frequent irrigation scheme A2 during all years of the study, but these differences were not significant. No significant difference in seedling height was observed between the two irrigation schemes after the third year of the study (2016). In general, height growth was almost cut in half each year for most plants (Figure 6). Seedlings belonging to different populations demonstrated differences only under irrigation scheme A2. Among all populations, E4 had on average higher and E1 and D3 smaller seedlings in 2014, while in 2015, seedlings of E3 and E4 were significantly higher and D3 was less high. In 2016, no statistically significant differences were observed among populations in seedling height (Table 4).

Seedling height in the growth chamber was not influenced significantly by population or provenance, according to the HLM model comparison, except for a significant influence of provenance on height growth between 2015 and 2016 (Table 5), due to D2 demonstrating higher average values than D3 and D4 and subsequently provenance Drama having lower height growth as compared to Evros during the same period (Supplement 3). The irrigation scheme significantly influenced seedling height in 2015 and growth between 2014 - 2015 and 2015 - 2016 (Table 5). According to the HLM model comparison, A1 had a positive influence on seedling height in the second year (2015) and the height growth between 2014-2015 and a negative influence on the height growth between 2015 - 2016 (Supplement 3). A large proportion of the total variance in height and growth traits was explained by the irrigation schemes after the first (6.5%) and mainly after the second year (24.4%), but not after the third year of the study. Provenances and populations explained smaller proportions of the total variance in 2014 (3% and 5%, respectively), had no effect in 2015 and explained a higher proportion of the phenotypic variance in seedling height in 2016 (6.6% and 9.3%, respectively). Provenances and populations had no influence on seedling growth between 2014 - 2015 but accounted for a larger fraction of variance in height growth between 2015 - 2016 (10.1% and 15%, respectively) (Figure 5).

Table 4. Total seedling height under irrigation schemes for the years of study (Mean values±SE).

Population	**Seedling height (cm)***					
	2014		**2015**		**2016**	
	A1	**A2**	**A1**	**A2**	**A1**	**A2**
E1	10.71 $^{\mathbf{A,a}}$ (±0.48)	9.00 $^{\mathbf{A,b}}$ (±0.41)	15.52 $^{\mathbf{A,a}}$ (±0.79)	12.87 $^{\mathbf{AB,b}}$ (±0.68)	17.27 ^{A,a} (±0.92)	15.42 ^{A,a} (±0.62)
E2	10.27 $^{\mathbf{A,a}}$ (±0.61)	9.63 $^{\mathbf{AB,a}}$ (±0.72)	14.59 $^{\mathbf{A,a}}$ (±0.63)	12.85 $^{\mathbf{AB,a}}$ (±1.14)	15.81 ^{A,a} (±1.08)	14.32 ^{A,a} (±0.68)
E3	12.13 $^{\mathbf{A,a}}$ (±0.60)	10.73 $^{\mathbf{AB,b}}$ (±0.27)	15.46 $^{\mathbf{A,a}}$ (±0.80)	14.75 $^{\mathbf{B,a}}$ (±0.94)	17.56 ^{A,a} (±0.98)	16.92 ^{A,a} (±0.74)
E4	10.70 $^{\mathbf{A,a}}$ (±0.58)	11.08 $^{\mathbf{B,a}}$ (±0.53)	14.36 $^{\mathbf{A,a}}$ (±0.83)	14.69 $^{\mathbf{B,a}}$ (±0.76)	16.42 ^{A,a} (±0.52)	17.17 ^{A,a} (±0.73)
D2	11.19 $^{\mathbf{A,a}}$ (±0.78)	10.44 $^{\mathbf{AB,a}}$ (±0.72)	14.93 $^{\mathbf{A,a}}$ (±1.27)	13.70 $^{\mathbf{AB,a}}$ (±0.63)	17.92 ^{A,a} (±1.26)	17.08 ^{A,a} (±1.22)
D3	10.58 $^{\mathbf{A,a}}$ (±0.99)	8.98 $^{\mathbf{A,b}}$ (±0.94)	15.06 $^{\mathbf{A,a}}$ (±1.01)	12.02 $^{\mathbf{A,b}}$ (±0.85)	17.50 ^{A,a} (±2.13)	15.83 ^{A,a} (±1.56)
D4	10.92 $^{\mathbf{A,a}}$ (±1.03)	10.60 $^{\mathbf{AB,a}}$ (±0.55)	14.90 $^{\mathbf{A,a}}$ (±1.46)	13.41 $^{\mathbf{AB,a}}$ (±0.72)	16.06 $^{A, a}$ (±1.31)	16.55 ^{A,a} (±0.83)

** Values within columns followed by the same capital letter do not differ among populations for each irrigation scheme and year of study. Values within rows that followed by the same small letter do not differ between irrigation schemes per population for each year of stud separately.*

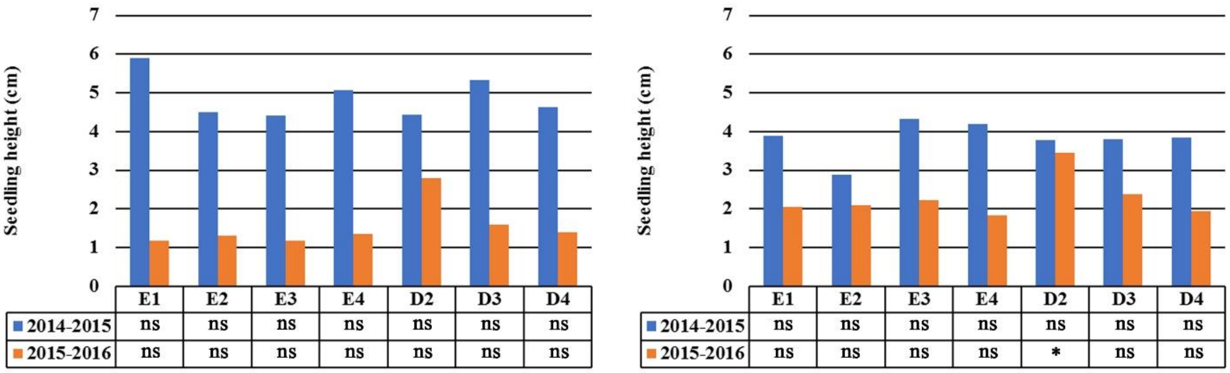

Figure 6. Plasticity in seedling height between populations under A1 irrigation scheme (left) and under A2 (right) between consecutive years of the experiment.

3.3 Leaf phenological traits

In the spring of 2014, bud burst occurred significantly earlier under irrigation scheme A2, in comparison to A1, for seedlings belonging to all populations besides E1 and E4 (Table 6). However, in 2015, only populations E2 and E3 continued to have significant differences in bud burst between A1 and A2, while in 2016, no significant differences could be observed. A similar trend was observed for leaf senescence, as far as differences between A1 and A2 are concerned. Significant differences between the irrigation schemes were observed in all populations of Drama in 2014 and in some populations in 2015 (D3 and D4), but not in 2016 (Table 6). In all cases for which significant differences were observed, leaf senescence under A2 occurred later than under A1. Significant differences in the length of the growing period between A1 and A2 schemes were present for all populations besides E1 and E4 in 2014 (Table 6). In 2015, only population E3 did not differ significantly in leaf senescence between A1 and A2 and finally in 2016 no difference between the two schemes was detected. In all populations and during all years, irrigation scheme A2 was associated with a longer growing period than A1.

Table 5. Model comparisons of the HLM models for the complete data via LRT (D: likelihood ratio statistic; P: p-value of the statistical significance of LRT test).

Dependent variables	**Model 1 (Null Model)**	**Model 2 (Population effects)**		**Model 3 (Model 2 + Provenance effects)**		**Model 4 (Model 3 + Irrigation scheme effects)**	
	D	***D***	***P***	***D***	***P***	***D***	***P***
BB 2014	1247.2	1242.2	n.s.	1240.7	n.s.	1232.5	**
BB 2015	1241.8	1212.8	**	1191.0	**	1183.9	**
BB 2016	1267.4	1230.3	**	1207.3	**	1204.2	n.s.
LS 2014	923.05	912.1	n.s.	900.56	**	893.7	**
LS 2015	1068.8	1060.9	n.s.	1059.2	n.s.	1052.2	**
LS 2016	1028.3	1014.7	*	1014.6	n.s.	1014	n.s.
VP 2014	1267.4	1261.5	n.s.	1261.5	n.s.	1252.7	**
VP 2015	1281.3	1256.3	**	1233.7	**	1225.2	**
VP 2016	1302.3	1270.0	**	1251.5	**	1248.1	n.s.
SH 2014	680.42	673.39	n.s.	673.24	n.s.	672.74	n.s.
SH 2015	771.03	763.74	n.s.	763.43	n.s.	758.45	*
SH 2016	772.97	766.08	n.s.	765.00	n.s.	762.97	n.s.
GD 2014-20105	690.65	687.61	n.s.	686.32	n.s.	681.12	*
GD 2014-2015	527.75	521.75	n.s.	506.35	**	500.70	*

¥Significant at *p<0.05, **p<0.01

Symbols: BB (Bud burst), LS (Leaf senescence), VP (Growing period), SH (Seedling height), GD (Height difference)

Under irrigation scheme A1, differences in bud burst among populations were recorded in all years of the experiment. Under A2, differences among populations were observed in 2015 and 2016, but not in 2014 (Table6). In the first year of the experiment (2014) in the growth chamber, under A1, population E1 had the earliest bud burst and D4 the latest. This pattern changed in 2015, with E1 and E4 demonstrating the latest bud burst, while the populations of Drama showed the earliest bud burst. In 2016, bud burst occurred later than in 2015 for all populations, but the same trend as observed in 2015 remained with populations of Drama having the earliest bud burst. Under A2, differences were recorded mainly at the provenance level, in 2015 and 2016, again with populations of Drama having the earliest bud burst. Within provenance Evros, E1 and E4 had the latest bud burst in 2015.

For leaf senescence, differences among populations in 2014 were found only under the A2 scheme, in contrast to bud burst. No difference was recorded in 2015 for both schemes, while minor differences among populations were recorded in 2016 (Table 6). Under irrigation scheme A1, differences among populations occurred in the third year of the experiment (2016), with seedlings belonging to population E3 demonstrating a later leaf senescence than the remaining populations. Under A2, leaf senescence occurred the earliest in population E2 and the latest in D2 and D3 during 2014, while in 2016 only population D3 showed a significantly later leaf senescence.

Table 6. Phenological trait values of seedlings under irrigation schemes for the years of study (Mean values±SE).

Population	Bud Burst Days*					
	2014		2015		2016	
	A1	A2	A1	A2	A1	A2
E1	101 ^{A,a} (±4.21)	98 ^{A,a} (±3.26)	120 ^{A,a} (±2.83)	111 ^{A,a} (±2.57)	120 ^{AC,a} (±2.94)	119 ^{A,a} (±4.60)
E2	114 ^{BC,a} (±3.90)	97 ^{A,b} (±2.64)	114 ^{AB,a} (±2.48)	103 ^{AB,b} (±4.01)	122 ^{A,a} (±2.72)	120 ^{A,a} (±2.28)
E3	117 ^{BC,a} (±4.71)	94 ^{A,b} (±4.22)	113 ^{AC,a} (±5.20)	100 ^{AB,b} (±5.33)	119 ^{AC,a} (±3.81)	119 ^{A,a} (±5.38)
E4	107 ^{AB,a} (±4.91)	100 ^{A,b} (±3.01)	119 ^{A,a} (±4.37)	111 ^{A,a} (±3.01)	123 ^{A,a} (±3.09)	120 ^{A,a} (±2.87)
D2	115 ^{ABC,a} (±3.27)	95 ^{A,b} (±2.24)	105 ^{BCD,a} (±6.07)	93 ^{B,a} (±2.24)	108 ^{BC,a} (±5.17)	97 ^{B,a} (±3.27)
D3	116 ^{BC,a} (±6.27)	99 ^{A,b} (±3.04)	96 ^{D,a} (±4.19)	92 ^{B,a} (±3.04)	101 ^{B,a} (±4.15)	96 ^{B,a} (±6.01)
D4	125 ^{C,a} (±1.71)	94 ^{A,b} (±2.09)	105 ^{CD,a} (±4.92)	94 ^{B,a} (±2.09)	110 ^{AB,a} (±5.82)	101 ^{B,a} (±4.50)

Population	Leaf Senescence Days*					
	2014		2015		2016	
	A1	A2	A1	A2	A1	A2
E1	293 ^{A,a} (±0.72)	295 ^{AB,a} (±1.45)	278 ^{A,a} (±1.22)	286 ^{A,b} (±2.36)	298 ^{A,a} (±2.44)	302 ^{A,a} (±2.35)
E2	294 ^{A,a} (±0.93)	293 ^{A,a} (±0.81)	285 ^{A,a} (±2.05)	290 ^{A,a} (±2.23)	303 ^{A,a} (±1.49)	302 ^{A,a} (±2.78)
E3	292 ^{A,a} (±0.10)	297 ^{AB,a} (±2.69)	286 ^{A,a} (±2.47)	288 ^{A,a} (±3.69)	307 ^{B,a} (±1.82)	300 ^{A,a} (±2.20)
E4	293 ^{A,a} (±0.70)	296 ^{AB,a} (±0.99)	282 ^{A,a} (±3.01)	288 ^{A,a} (±1.54)	302 ^{A,a} (±1.09)	302 ^{A,a} (±1.27)
D2	295 ^{A,a} (±2.18)	302 ^{B,b} (±1.62)	281 ^{A,a} (±3.00)	288 ^{A,a} (±2.26)	299 ^{A,a} (±1.35)	301 ^{A,a} (±1.90)
D3	292 ^{A,a} (±0.10)	304 ^{B,b} (±2.13)	280 ^{A,a} (±4.05)	288 ^{A,b} (±0.10)	304 ^{AB,a} (±4.30)	310 ^{B,a} (±0.10)
D4	294 ^{A,a} (±1.64)	299 ^{AB,b} (±1.80)	283 ^{A,a} (±2.26)	289 ^{A,b} (±1.83)	299 ^{A,a} (±1.07)	300 ^{A,a} (±1.33)

Population	Vegetative Period Days*					
	2014		2015		2016	
	A1	A2	A1	A2	A1	A2
E1	191 ^{A,a} (±4.24)	197 ^{A,a} (±3.23)	157 ^{A,a} (±3.15)	174 ^{A,b} (±3.70)	178 ^{A,a} (±4.25)	182 ^{A,a} (±4.05)
E2	179 ^{BC,a} (±3.71)	196 ^{A,b} (±2.87)	170 ^{B,a} (±2.88)	186 ^{AC,b} (±4.68)	180 ^{A,a} (±2.93)	182 ^{A,a} (±3.66)
E3	174 ^{BC,a} (±4.71)	202 ^{AC,b} (±5.75)	172 ^{BC,a} (±5.56)	186 ^{AC,a} (±6.17)	188 ^{AB,a} (±4.22)	181 ^{A,a} (±6.04)
E4	185 ^{AB,a} (±4.64)	194 ^{AD,a} (±3.37)	162 ^{AB,a} (±4.15)	177 ^{A,b} (±3.45)	179 ^{A,a} (±3.14)	182 ^{A,a} (±3.10)
D2	179 ^{ABC,a}	205 ^{BC,b}	175 ^{BC,a}	194 ^{BC,b}	191 ^{AB,a}	205 ^{B,a}

	(±4.70)	(±4.17)	(±7.49)	(±3.40)	(±6.11)	(±4.18)
D3	175 ^{BC,a} (±6.27)	204 ^{AB,b} (±5.88)	184 ^{C,a} (±1.56)	195 ^{BC,b} (±3.04)	203 ^{B,a} (±8.10)	214 ^{C,a} (±6.01)
D4	168 ^{C,a} (±2.69)	204 ^{BCD,b} (±3.81)	177 ^{BC,a} (±6.01)	194 ^{BC,b} (±2.99)	189 ^{AB,a} (±5.87)	199 ^{BC,a} (±5.02)

** Values of phenological traits within columns followed by the same capital letter do not differ among populations for each irrigation scheme and year of study. Values of phenological traits within rows that followed by the same small letter do not differ between irrigation schemes per population for each year of stud separately.*

Populations demonstrated significant differences among each other in the duration of the growing period for both irrigation schemes and for all years (Table 6). Under scheme A1, populations E1 and E4, contrary to E2 and E3, had the longest vegetation period in 2014, but the shortest in 2015 and 2016. On the contrary, populations D3 and D4 had the shorted growing period in 2014, which gradually increased in 2015 and 2016, as compared to D2. Differences were most apparent between provenances under irrigation scheme A2, especially during 2016. Populations belonging to Drama provenance had a longer growing period during all years under A2.

Differences in bud burst between years (plasticity) were found for each irrigation scheme (Figure 7). The greatest delay in bud burst between years 2014 - 2015 was found for population E1 (+19 days), while seedlings belonging to D3 and D4 flushed much earlier in the second year of the study (-21 and -20 days, respectively), under A1. Under irrigation scheme A2, populations E2 and E4 had a significant delayed leaf flush in 2016 as compared to 2015. For leaf senescence, all populations showed a similar plasticity trend for both irrigation schemes (Figure 7). Seedlings presented an earlier leaf senescence in 2015 as compared to 2014, and a later leaf senescence in 2016 as compared to 2015. Results were more pronounced under scheme A1 and the populations showing the largest plasticity were E1, D2 and D3. Finally, the two provenances had different plasticity reactions for the length of the growing period. The largest plasticity was observed under A1, where the populations of Evros and D2 had a shorter growing period in 2015, while D3 and D4 had a longer one for the same year. In 2016, all populations demonstrated an increased growing period under A1. An opposite trend was observed under irrigation scheme A2, with all populations having a shorter growing period in 2015 and a longer one in 2016 (Figure 7).

The comparison of the HLM models revealed a similar trend for the timing of bud burst, leaf senescence and the length of the growing period, during the three years of the experiment (Table 5). The irrigation scheme had a significant influence on all traits in the first two years of the experiment (2014 and 2015), but not for the last year (2016). An opposite trend was observed for population and provenance that had significant effects on bud burst and growing period in 2015 and 2016, but not in 2014. Provenance showed a significant influence on the time of leaf senescence only in the first year and population only in the last year of the study. Provenance Evros had a significantly earlier bud burst and a longer growing period than Drama in 2015 and 2016, while Drama had an earlier leaf senescence in 2014 (Supplement 3). At the level of populations within the Evros provenance, E1 and E4 demonstrated a later bud burst and an earlier leaf senescence and thus a shorter growing period during the second year of the study (2015). In 2016, E3 showed a delay in leaf senescence in comparison to the other populations of Evros. Within Drama, the only significant difference among populations was observed in 2016, when leaf senescence in D3 occurred later than in the other populations. Compared to A1, the irrigation scheme A2 caused earlier bud burst, delayed leaf senescence and a longer growing period. Finally, there was a decreasing influence with time of the irrigation scheme on the total variance among seedlings for all phenology traits and the opposite trend was observed for population and provenance (Figure 5).

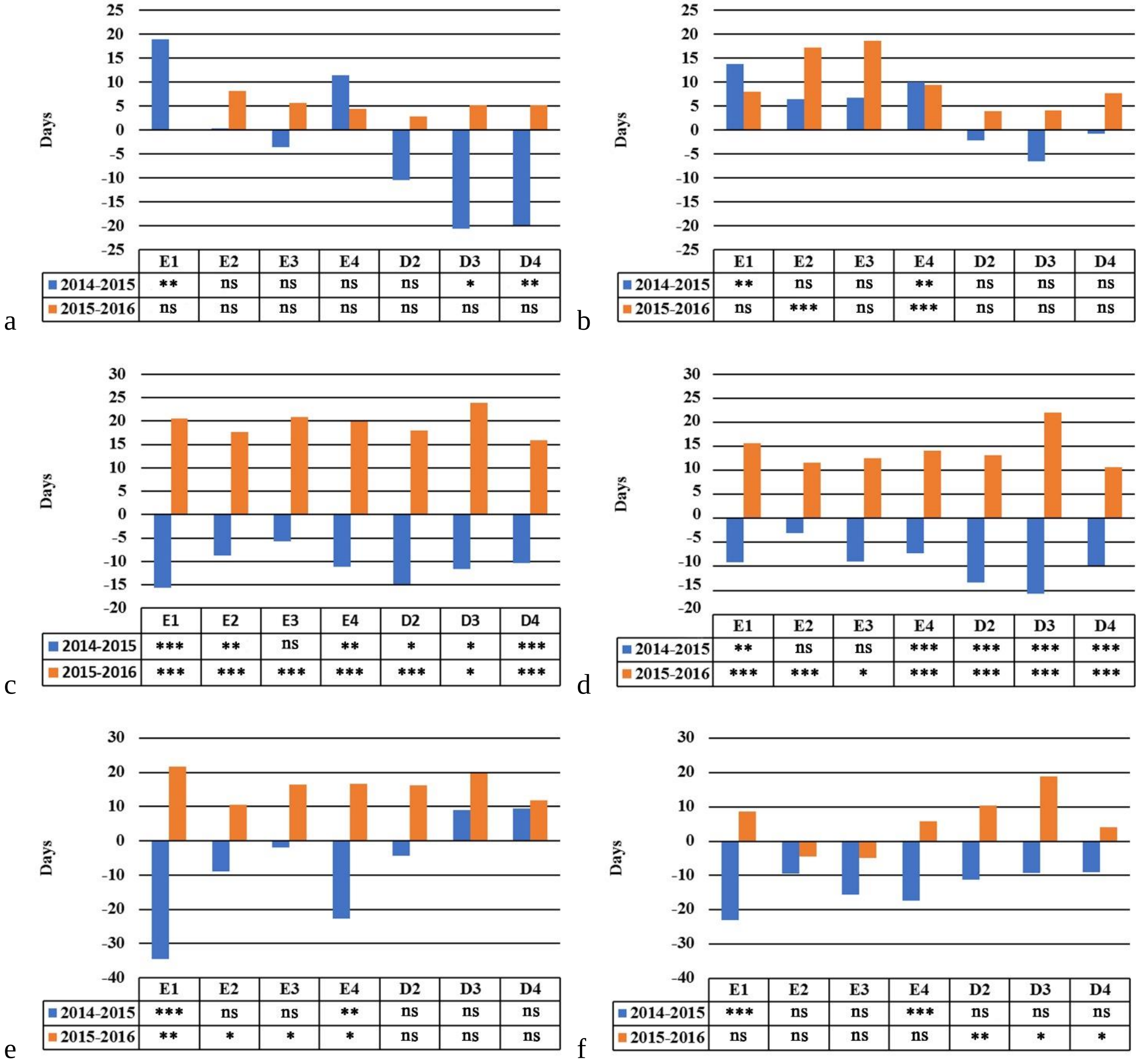

Figure 7. Plasticity values for populations under A1 irrigation scheme for bud burst (a) and under A2 (b), for leaf senescence (c under A1 and d under A2) and for the length of the growing period (e under A1 and f under A2) between consecutive years of the experiment.

Irrigation scheme was the only fixed effect explaining a significant proportion of variance in 2014 for all traits, while in 2016, only (or mostly) provenance and population explained part of the variance. Provenance explained a larger proportion of variance (28.6%) than population (22.8%) for bud burst in the third year of the study, while an opposite trend was observed for leaf senescence (14.5% for population and 11.3% for provenance) and the growing period (28.3% for population and 18.6% for provenance).

4 Discussion

4.1 Seedling survival and height growth

In the growth chamber, seedling mortality for all populations and provenances was observed during the first experimental year only. Seedlings survived better in the growth chamber than in the field, possibly due to their imminent exposure to winter conditions in the mountainous location of Agios Georgios, where selection pressure was potentially larger. In the protected environment of the growth chamber, irrigation frequency proved to be an important factor for seedling survival, since mortality increased when irrigation occurred after long intervals of drought (A1 scheme). Differences in survival among populations and provenances were more pronounced under the less favorable conditions, in the growth chamber due to drought stress imposed by non-frequent irrigation (scheme A1) and in the field due to the exposure to winter conditions. This trend, where the largest differences in survival among populations occur when conditions are unfavorable, is common in provenance tests for beech (e.g. Banach et al., 2015). In the growth chamber, when irrigation occurred less frequently (A1), seedlings from provenance Evros generally presented higher survival, indicating a possible adaptation to prolonged drought intervals, since summer drought in Evros lasts longer and temperatures are generally higher than in Drama.

Among the Evros populations, E4 was the only one demonstrating significantly lower seedling survival under less frequent irrigation. It is possible that population E4, which is located in an isolated microenvironment in Evros, is more sensitive to longer periods without rain. Indeed, population E4 is close to an area covered with beech forests characterized as an “island” of oceanic climate. This is emphasized by the frequent occurrence of the plant species *Galium rotundifolium*, a good indicator of oceanic climate (Ellenberg et al., 1992), which is distributed in northeast Greece along the mountains facing the Aegean Sea and absent in the whole area of Rodopi, except the area surrounding population E4. High interpopulation variability in seedling survival and its response to irrigation frequency was also observed in the Drama provenance. Seedling survival of population D3 was relatively low under both irrigation schemes and did not increase with more frequent irrigation, unlike for populations D2 and D4. This trend possibly indicates a different local adaptation of beech to the microenvironment of D3.

In the field trial, plant loss occurred during the winter, indicating that low temperatures were the limiting factor for seedling survival. This further suggests the existence of a strong local adaptation pattern to these factors in beech (see Kreyling et al., 2012, 2014). Populations E1 and E2, that demonstrated the highest survival rates under drought conditions in the climate chamber, had the highest mortality rates under field conditions. The highest survival rate was observed for the two populations with the closest geographical origin to the location of the test site (D3 and D4). In a similar way, local provenances and populations were superior in survival in other beech common garden trials (Banach et al., 2015), but also the opposite trend has been reported (e.g. Sulkowska 2004, Hofmann et al., 2015; Müller and Finkeldey 2017). It is worth noting that adaptive differentiation to the colder conditions in the field experiment, as expressed in survival rates, was observed mainly among populations within provenances. Thus, seedlings from the distant populations E3 and E4 survived as well as some populations of the local Drama provenance, indicating that environmental heterogeneity at a smaller geographical scale can create significant adaptive differentiation.

Under both irrigation schemes in the growth chamber, provenance and population were generally not differentiating factors for seedling height. The only exception was observed in height growth in the third year of the experiment, which was faster for provenance Evros than for Drama and for populations

D1 and D2 within Drama in comparison to D3 and D4. Nevertheless, at the end of the third year all seedlings achieved similar heights without differences among populations and provenances. In contrast to seedling survival, differences in growth and height among populations were observed only under frequent irrigation (A2 scheme) in the first two years of the study, while no differences were found under longer drought intervals (A1). Similarly, Harter et al., (2015) did not find any differences in seedling height between two beech provenances under water deficit for 60 days.

Various studies report that non-frequent irrigation leads to lower shoot height in seedlings (Arend et al., 2011, Thiel et al., 2014). However, in the current study seedling height was generally larger under irrigation scheme A1 rather than A2. Thus, it seems that seedlings invest more in increasing shoot height under longer drought intervals as compared with a more frequent irrigation pattern, since A1 and A2 differ in the distribution pattern of the same water amount per month and not in the precipitation height itself, indicating that in this case the distribution pattern was more critical than the absolute amount of water received by the plant. It seems that fewer significant rain events may yield higher biomass increase than more frequent minor events. Since this kind of irrigation pattern comparison has not yet been performed in any other common environment study for forest trees, the growth behavior of beech seedlings in this experiment needs to be further investigated using physiological and anatomical traits. In any case, these responses to irrigation frequency seem to be temporary and, after a short time, reversible, as shown in our study at the end of the third year, revealing the existence of possible trade-offs between different traits as part of a complex adaptive strategy aiming at the best possible use of the available water.

4.2 Leaf phenological traits

In our experiment, the two irrigation frequency schemes produced different leaf phenological responses at all traits during the first two years of the study. In the third year, the differences in leaf phenological traits were influenced mainly by provenance and population. Considering the adaptive significance of the timing of bud burst and leaf senescence that define the length of the growing period, we assume that seedlings probably needed a certain period of time until they were able to physiologically adapt to the growth chamber conditions and irrigation schemes. The results of our study imply that the duration of the growing period in beech seedlings was mostly determined by the timing of bud burst, while the differences between populations, provenances and irrigation schemes in leaf senescence were less pronounced.

Bud burst occurred earlier and leaves matured later under frequent irrigation (A2) especially during the first years of the study, a trend that was consistent for all populations. Thus, the existence of longer drought intervals between irrigation events (A1) has shrunk the growing period of plants in the chamber, an expected response to stressful conditions. However, this shortened growing period of seedlings growing in the growth chamber under irrigation scheme A1 showed an unexpected increase in height growth, as explained above, in comparison to irrigation scheme A2.

In our experiment, differences in bud burst timing were observed in beech seedlings originating from different provenances, under less frequent irrigation (A1) during all years and under frequent irrigation (A2) during the last two years of the study. Bud burst is considered to be under strong genetic control (Dittmar and Elling 2006; Doi et al., 2010; Abbott et al., 2015; Gömöry et al., 2015; Müller et al., 2017) and provenances often show adaptive differences in this trait in common environment experiments, that correspond to specific environmental conditions at the sites of origin (von Wehlisch et al., 1995; Schüler et al., 2012; Kramer et al., 2017). Several studies based on provenance tests report specific geographical and climatic trends for leaf phenology in beech provenances. These trends include clines in bud burst defined by longitude, latitude and altitude in European beech provenances (e.g. Nørgård Nielsen et al., 2003; Robson et al., 2011; Gömöry and Paule 2011; Schüler et al., 2012; Petkova et al., 2017), with the southeastern ones demonstrating earlier bud burst (Ivankovic et al., 2011; Ballian et al., 2015). Provenance tests all over the continent describe a general trend of populations growing in warmer and less continental climates to have an earlier bud burst than populations from colder climates (Robson et al., 2011). Extrapolating this trend to the provenances and populations of our study, we would expect provenance Evros to have an earlier bud burst than Drama. While this was indeed the case for the first year of the study in the growth chamber, the phenological trend observed reversed during the next two years. Thus, in the first year of the growth chamber experiment, provenance Evros had an earlier bud burst and a longer growing period than Drama, while in the next years, bud burst had shifted to a later date for Evros and to an earlier date for Drama. These findings show that provenances initially demonstrated the expected genetic response matching the environmental conditions at their sites of origin, with Drama having a cooler and more temperate climate than Evros. However, the projected conditions of 2050 under climate change applied in the growth chamber, probably stimulated a differentiated plastic reaction of both provenances. We assume that the same environmental signals that trigger bud flush in plants, such as day length, temperature, humidity, had a different effect on the provenances in this study.

Our findings reveal two different strategies of beech seedling plasticity among the populations and provenances studied. These strategies differ mainly in bud burst timing in spring and less significantly in the timing of leaf senescence in autumn. Plants that follow a “defensive” strategy, flush later in the spring of the second year and further delay bud burst in the spring of the third year. A more “aggressive” strategy occurs when seedlings flush earlier in the spring of the second year and then delay bud burst in the spring of the third year. In both strategies, leaf senescence occurs earlier in the autumn of the second year and later in the autumn of the third year. As a result, seedlings following the defensive strategy reduce their growth period in the second year in order to be less exposed to the climatic conditions of the growth chamber, while the ones following the aggressive strategy maintain more or less the same length of their growing period by pushing it earlier in the spring of the second year. The defensive strategy is probably connected with genotypes better adapted to longer intervals of summer droughts, such as populations E1 and E4 under non-frequent irrigation and all Evros populations under frequent irrigation. In contrast, the aggressive plasticity strategy in leaf phenology is followed by seedlings that are better adapted to temperate sites, such as the ones belonging to provenance Drama under both irrigation schemes and E3 under non-frequent irrigation.

Most reports from provenance trials in the field for beech show that, unlike in our study, bud burst timing remains constant and the order of provenances in this regard remains unchanged during years, without a strong interaction between provenance and test site (Robson et al., 2011). We assume that stressful environmental conditions, such as the ones simulated in the growth chamber, may trigger unexpected physiological responses, where trees alter their plant physiological profile in order to survive. Since phenological traits are complex in nature and in their underlying mechanisms (Vitasse et al., 2010; Fu et al., 2012; Basler and Körner 2014), further studies of provenances and families of trees utilizing functional and anatomical traits are needed to understand these adaptation strategies.

4.3 Adaptive and phylogenetic differentiation patterns

The results of our study reveal the existence of high genetic diversity in adaptive traits in the beech forests of N.E. Greece. These adaptive differences occur at multiple spatial levels, among distant and neighboring populations. There is a clear geographical and environmental trend in adaptation to climate. On the eastern side of the study area (provenance Evros), beech populations are better adapted to dry climatic conditions with longer intervals of drought during the summer and low probability of late frosts in the spring. As a result, seedlings from Evros demonstrate higher survival and earlier bud burst in the first year of the study than seedlings from Drama, under simulated climate change conditions, especially when irrigation is not frequent. At the same time, most of the Evros populations show a specific phenotypic plasticity pattern, as a response to the simulated climate change conditions in the growth chamber, with a shortening of the growth period during the second year of the study. Respectively, populations located on the western side of the study area (provenance Drama) seem to demonstrate adaptations to more temperate conditions, characterized mainly by long and cold winters and more humid summers. Seedlings originating from Drama showed lower survival under long drought intervals during the summer in the growth chamber and a late bud burst in the first year, but then shifted their growing season earlier and flushed earlier than the Evros seedlings in the second year of the study.

Besides the different environmental conditions that may have caused adaptive genetic differentiation between the two provenances of this study, another reason for the differences in adaptive traits that exist between Evros and Drama may be the presence of different levels of a possible admixture between two beech species, *F. sylvatica* and *F. orientalis* that presumably form a contact zone in the southeastern part of Europe (e.g. Paule 1995, Tsiripidis and Athanasiadis, 2003; Papageorgiou et al., 2008; Govaerts et al., 2013). In N.E, Greece, several authors suggest an increasing admixture trend towards the east (Moulopoulos 1965; Tsiripidis and Athanasiadis 2003; Papageorgiou et al., 2008; Hatziskakis et al., 2011), with provenance Evros being genetically and morphologically closer to *F. orientalis* and provenance Drama to *F. sylvatica.* Since the former species grows in warmer and drier climates than the latter, adaptive differentiation may exist between them, as suggested by earlier studies (Atalay, 1992; Tsiripidis and Athanasiadis, 2003; Papageorgiou et al., 2008).

Adaptive differences were recorded within provenances as well, indicating that beech populations that belong to the same geographic region and are located within a small distance exhibit large genetic differences in adaptive traits. Differences in altitude, aspect and topographical connectivity between populations probably define an environmental mosaic with semi-isolated patches of beech forests, where natural selection can locally cause well adapted ecotypes that differ at a small spatial scale. In Evros, population E1 was proven to be best adapted to warmer conditions and summers with prolonged periods without rain, as shown by the high survival rate of E1 in the growth chamber, the early bud burst in the first year of the study and the defensive phenological plasticity pattern in the next years. Indeed, E1 shows adaptive differences from the neighboring population E2 (only 682 m. apart), probably because the environmental conditions at these sites are critically different. Population E1 is a marginal beech stand located on a south facing slope, while E2 is a dense forest on the north facing slope of the mountain, growing under much more favorable conditions. Furthermore, population E4 showed a different adaptive pattern in seedling survival than E1, which can be attributed to the more oceanic microclimate of the specific location, as explained above.

Despite the differences in the survival rate between E1 and E4, these two populations demonstrated a similar defensive phenological strategy, much different to E2 and E3 in the Evros provenance. Besides the adaptation to environmental heterogeneity at a small scale, another possible explanation may exist for the adaptive similarities between E1 and E4. According to a fine scale genetic analysis of chloroplast DNA haplotypes in beech populations in the region (Manolis et al., 2017), both E1 and E4 are monomorphic for the same haplotype, which is connected with a local postglacial lineage. This means that these populations probably derive from the postglacial expansion of the same beech population in a local refugium. There is a possibility that populations E1 and E4 have a common postglacial origin from a close by refugium where beech survived during multiple glacial cycles and

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probably developed effective adaptive strategies that include high phenotypic plasticity. The origin of a local glacial refugium has been suggested as a possible explanation for late bud burst of the Slovenian beech provenance Idrija-II/2 in European field trials in, as a possible adaptation to long cold winters during glaciation (Brus, 2010; Robson et al., 2011). Thus, adaptive differences between distant or close-by populations may derive from selective evolutionary responses to environmental conditions of past refugia, in parallel with the ongoing processes of adaptation to current environmental conditions.

4.4 Conclusions

Beech seedlings deriving from populations of N.E. Greece were in general able to survive well under climate change conditions, simulated and applied in the growth chamber. Plants showed adaptive differences that allowed them to avoid high levels of mortality in the growth chamber. Furthermore, beech genotypes demonstrated an impressive phenotypic plasticity as a response to different environmental conditions and precipitation frequency in particular. These plastic responses allow beech seedlings to alter the duration of their growing season as a response to environmental signals, allowing them to avoid environmental stress and high selection pressure. In our study we were able to describe different adaptation strategies, that relate to the distribution patterns of specific environmental factors, rather than the average annual or monthly values of these measures. Indeed, fluctuations in temperature and precipitation within each year seem to be crucial for survival and growth, as well as the duration of the growing season. For this reason, provenance Evros is considered to be well adapted to a less temperate climate, due to the low rainfall during summer, despite the high annual precipitation that occurs mainly during the winter in this region. Furthermore, our study proved the adaptive significance of the distribution of precipitation at a small temporal scale, since different adaptive strategies appeared among beech seedlings when the same amount of water was distributed differently within each month. This indicates that the physiological response mechanisms of beech individuals are very complex and depend on several interacting parameters that are difficult to study in total. For this reason, conclusions about the suitability of provenances for translocation and use in afforestation or reforestation projects should consider the small scale ecotypic diversity of the species and view multiple environmental and climatic parameters in connection to each other.

Despite the existence of adaptive diversity among the populations of beech in N.E. Greece, the survival of beech and other temperate forest tree species in the future remains unknown, since the speed, the uniformity and the intensity of climate change are different in different climate models. We expect severe climate fluctuation in the near future, with an increased intensity in the forests of the Mediterranean ecoregion being most at risk. Beech populations in the rear edge of the distribution of the species have a large adaptive potential and their persistence seems to be of major importance for forests and forestry all over Europe, pressing for an adjustment of forest management and conservation policies.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

GV, ACP and OG developed the original idea of the research, GV, ACP, TM and IT(1) planned the experiments in the chamber and the field, GV, AM and IT(2) planned sampling and collected the seed, GV, TM,AM and IT(1) supervised the experiment in the growth chambers, GV, ACP, OG and IT analysed the data, CM and ACP developed the models, GV developed the first draft of the manuscript and all authors contributed in preparing the manuscript in its final form.

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