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## The JIP-test as a tool for forestry in times of climate change

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### Abstract

Replicate common gardens of Italian and Greek provenances of *Quercus ilex*, *Q. pubescens*, and *Q. frainetto* seedlings were established in 2017 in Italy, Greece, and Germany (here: also *Q. robur*) to assess their potential for plantations for future climate scenarios. The JIP-test was applied on saplings (2016) and in the summers of 2017–2019 to assess the efficiency of the photosynthetic apparatus. Seedlings from the nurseries differed in the tested JIP parameters from older plants in the field. Further, considerable inter-annual and between-sites variability of the parameters occurred. *Q. pubescens* revealed the most stable behavior across time and site, with the Greek provenance usually showing higher values of performance index and the probability of electron transport beyond  $Q_A^-$  than the Italian provenance. In the German sites, Greek *Q. pubescens* revealed higher values of most of the tested JIP parameters than the local species, *Q. robur*.

*Additional key words:* assisted migration; Mediterranean oaks; selection.

### Introduction

Human-induced climate change represents a fundamental challenge for European forests. Vegetation dynamics models predict transformation of forests as the climate changes, meaning that new strategies will be required to maintain functional forests. Apart from the expected increase in average temperatures, a key stressor of European forests will be the increasing probability of extreme summer drought associated with extreme heat (Carnicer *et al.* 2011), like it occurred in Central Europe in 2003 (Bréda *et al.* 2006), 2018, or in Italy in 2017 (Puletti *et al.* 2019), and with an exceptional heatwave in early summer 2019. These stressful conditions are widely

anticipated to increase in the future for Central and Southern European forests, and many European tree species are expected to migrate towards the north-east and/or to regions of higher elevation (Attorre *et al.* 2011, Hickler *et al.* 2012). Changes in the environment may be so drastic that, if unmanaged, a declining forest tree species will not immediately be replaced by the establishment of a new species. Intermediate ecosystems including grass- or shrublands may develop and delay the establishment of new forests through competitive processes (Resco de Dios *et al.* 2007). Additionally, especially in the eastern Mediterranean countries, public ground with degrading forest cover is frequently used by the local population as grazing grounds for goats and sheep, leading to further

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*Abbreviations:* ChlF – chlorophyll fluorescence; DE – Germany; GR: – Greece; IT – Italy; OLY – Olympiada; PI<sub>abs</sub> – performance index on absorption basis; RC/ABS – density of active reaction centres; RIE – Frankfurt-Riedberg; SAN – Sant'Anatolia di Narco; SWA – Frankfurt-Schwanheim;  $\Delta V_{IP}$  – relative fluorescence increase between I and P step, *i.e.*, indicator for the relative contribution of electron transport from the intersystem carriers to the electron end acceptors of PSI;  $\phi_{Po}$  – quantum efficiency of reduction of  $Q_A^-$ ;  $\Psi_{Eo}$  – probability of electron transport beyond  $Q_A^-$ .

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degradation and erosion. Continuous maintenance of forest cover is therefore fundamental to protect these ecosystems.

To ameliorate the economic and ecological consequences of forest transformation in Western and Central Europe (Hanewinkel *et al.* 2013), the introduction of southern provenances of valuable timber species, including Mediterranean oak species to Central Europe (*cf.* Koller *et al.* 2014) have been suggested (for review see *e.g.*, Bussotti *et al.* 2015). This approach of latitudinal assisted migration may be extended to forest management strategies in mountain regions in Mediterranean countries in the form of altitudinal assisted migration as well.

Common garden trials are commonly used in forestry to test the suitability of certain species or population to be grown in different ecological conditions (Mátyás 1996), and now they are considered an essential tool to determine the phenotypic plasticity of selected species/provenances (Nicotra *et al.* 2010) for 'assisted migration' in view of climate change (Vitasse *et al.* 2009, Williams and Dumroese 2013, Koralewski *et al.* 2015).

In the present contribution, we report on experimental plantations of oak seedlings, *i.e.*, *Quercus pubescens* Willd., *Quercus frainetto* Ten., and *Quercus ilex* L. from Greek and Italian provenances in replicate common gardens under different, but typical growth conditions in Southern Germany, Central Italy, and Northeastern Greece. For comparative purposes, the German common gardens also contain the local dominant oak species, *Quercus robur* L. One central aim of the study is to compare the photosynthetic performance of (identical) provenances of these species under different macro- and microclimatic conditions, providing information for the establishment of oak plantations in these countries. One possible means to assess the physiological fitness of plants, especially under stress conditions, for selection purposes in the case of cultivated species or their close relatives is application of the JIP-test (*cf. e.g.*, Oukarroum *et al.* 2007, Stefanov *et al.* 2011, Jedmowski *et al.* 2014). The study presented here aims at evaluating the usefulness of the JIP-test in screening setups of genetically very heterogeneous, wind-pollinating trees under forestry field conditions, where plants are exposed to a wide (and mostly uncontrolled) variety of environmental factors including temperature regimes, water availability or soil properties.

## Materials and methods

**Plant material:** Seeds of *Q. pubescens*, *Q. frainetto*, and *Q. ilex* were collected in autumn 2015 in Central Italy and Northeastern Greece, respectively (Table 1S, *supplement*). They were shipped by air mail to tree nurseries in Germany, Italy, and Greece, and approximately 1,000 seeds per species/provenance were immediately sown in plastic trays (*Quickpot 15T/16* or *24/18*) in substrate according to the conditions developed regionally for optimum growth of oaks, which differed from country to country. In Greece, we used a 70% peat moss/30% black peat mixture with 12 kg m<sup>-3</sup> clay added (GR), in Italy a mixture of peat, loam, and volcanic lapilli (40/40/20) fertilized with 25 g l<sup>-1</sup> pelleted humified manure and 2 g l<sup>-1</sup> *Osmocote Exact*

(IT), and in Germany, we used a commercially available mixture of humus, different organic fibres, and volcanic lapilli (*FE Typ Bio Blumenerde #03010*, *HAWITA Co.*, Vechta, Germany). Overwintering occurred outdoors in Italy and Greece, and in a non-heated, but frost-free glasshouse in Germany. In spring 2016, 800 1-year-old seedlings of *Q. robur* (provenance 81707 'Oberheingraben', supported by *Darmstädter Forstbauschulen*, Darmstadt, Germany) were added to the ensemble in Germany. All plants were grown outdoors with regular watering in the tree nurseries in the pots during 2016. In Germany, plants were shaded with a net during autumn. In autumn 2016, all emerged saplings were marked with an ID number, evaluated, and scored for growth and physiological parameters as described below. In spring 2017, 420 (Germany) and 210 (Italy, Greece) of the best growing plants per species and provenance were selected and transferred to the plantation sites (210 per site, species, and provenance, except for *Q. frainetto* from Italy in the Greek nursery, where only 170 good plants were available). Seedlings were planted on 24 February–4 March 2017 (Greece) and on 15 April–1 May 2017 (Germany, Italy).

**Common garden experiments:** In Germany, one plantation is situated in a managed oak (*Q. robur*, *Quercus petraea* (Matt.) Liebl.) and *Pinus sylvestris* L. forest (*ca.* 50 trees/ha) on fluvial sand (Frankfurt-Schwanheim, 'SWA', 50°04'12.6"N, 08°33'42.2"E, 114 m a.s.l.). The site has traditionally been used as oak forest since medieval times, mostly for timber, firewood production, and hog feeding purposes. Due to drinking water wells, groundwater table is now at approximately 2 m below soil surface. The other German plantation was established in the north of Frankfurt (Riedberg, 'RIE', 50°10'12.8"N, 08°37'53.2"E, 130 m a.s.l.) on loamy soil, traditionally used for agriculture, where oak seedlings were irrigated during the first two years (2017, 2018) of the experiment when necessary. In Greece, one plantation has been established near Olympiada (Chalkidiki, 'OLY', 40°36'33.6"N, 23°45'05.0"E, 48 m a.s.l.) in a wide river valley. The site is mostly free of woody vegetation and has been used as pasture in natural *Q. ilex-Q. pubescens* forest stands. The Greek common garden site has loamy soil, without irrigation, resulting in soil water availability provided solely by precipitation.

In Italy, the common garden plantation was realized at Sant'Anatolia di Narco ('SAN'), Umbria, in the Central Apennine (42°44'18.4"N, 12°50'17.6"E, 290 m a.s.l.), in a river valley on calcareous soil. Soil water is available from the river Nera, which is only 20 m away from the site. In addition, the seedlings were watered during the first year of the experiment, when necessary. The natural vegetation close to the common garden site is a mixed (mostly oak) forest, with *Q. ilex-Q. pubescens* in the warm exposure, while a gallery forest, dominated by *Fraxinus angustifolia* Vahl. and *Alnus glutinosa* (L.) Gaertn., characterize the vegetation along the river.

Seedlings were planted in groups of 21 plants each, except for Italian *Q. frainetto* in the Greek plantation, where 17 plants were used, in a clustered arrangement

called ‘grouped’ scheme (Saha *et al.* 2012, 2016; Skiadaresis *et al.* 2016) at 1-m spacing in  $4 \times 4$  m plots, with 2-m spacing between seedlings from adjacent plots. The edge positions in the plots were not used, leading to rows of 3/5/5/5/3 plants per plot. This plantation scheme is more cost-effective than row plantation and well suited to obtain good tree quality in oaks, as evaluated in a meta-analysis by Saha *et al.* (2012).

Climate data were recorded in/or close to the field sites At SWA site the mean annual temperature ( $T_{\text{mean}}$ ) is 10.5°C and annual precipitation is 629 mm (1981–2010; <https://cdc.dwd.de/portal/201908021100/searchview>). Minimum and maximum temperatures recorded between 1981 and 2016 were –19.1 and 38.7°C, respectively. Years 2018 and 2019, however, deviated strongly from the mean values, with average annual temperature in Frankfurt city, 11 km NE of the common garden at SWA and 6 km SE of the RIE site of 12.9°C in 2018 (the highest average temperature ever recorded in Germany) and an all-time maximum temperature of 40.2°C in July 2019 (Deutscher Wetterdienst Offenbach, Germany). In 2018, SWA only received 375 mm rainfall. At the RIE site, temperature regimes are similar, but annual precipitation can be up to 200 mm higher due to its situation at the Taunus mountain range (personal observations). At Casteldilago (Perugia, 225 m a.s.l.), 18 km SSW of SAN, annual  $T_{\text{mean}}$  is 14.4°C and annual precipitation is 1,158 mm. At OLY, annual  $T_{\text{mean}}$  is 16.0°C and annual precipitation is 756 mm (2008–2017). Here, the minimum and maximum temperatures recorded between 2008 and 2016 were –6.0° and 38.5°C, respectively.

**Chlorophyll (Chl) fluorescence measurements:** The induction curve of the Chl fluorescence (ChlF) emitted from PSII during the first second of illumination of dark-adapted leaves (OJIP transient) was measured with a *Pocket PEA* chlorophyll fluorometer (Hansatech, King's Lynn, UK). All measurements were performed on recent-year, fully developed, and healthy leaves. Data were analyzed with *PEA Plus 1.0.0.1* (Hansatech) and with *Biolzyzer 4HP* (Bioenergetics Lab, Univ. of Geneva, Switzerland) software. In this contribution, we presented representative ChlF induction curves [double normalized for 50  $\mu\text{s}$  (0) and 1 s (1)], which were selected according to their performance index ( $PI_{\text{abs}}$ ), showing the curves of those plants whose  $PI_{\text{abs}}$  was the closest to the mean  $PI_{\text{abs}}$  of the plants of the same provenance.  $PI_{\text{abs}}$  is a parameter summarizing the effects of light trapping (RC/ABS), quantum efficiency of reduction of  $Q_A$  ( $\phi_{\text{P}_0}$ ) and efficiency of electron transport from  $Q_A^-$  to the intersystem carriers of the electron chain ( $\Psi_{\text{E}_0}$ ). Additionally, we calculated  $\Delta V_{\text{IP}}$  ( $= 1 - V_1$ ) as indicator for the relative contribution of the electron transport from the intersystem carriers to the electron end acceptors of PSI. For definitions and explanations of the physicochemical background of these parameters *cf.* Strasser *et al.* (2000, 2004) and Schansker *et al.* (2005).

Since JIP parameters may vary considerably in the course of time in oaks, especially with respect to leaf senescence in autumn, all measurements were performed

when leaves had attained their full photosynthetic capacity (*i.e.*, at earliest in June) and before significant senescence effects under normal weather conditions were to be expected (*i.e.*, at latest by mid-September). Furthermore, to be able to compare the data sets within a given plantation, all plants within one plantation were measured within a few days.

In general, measurements were performed on all plants of each provenance at night after a minimum of two hours of darkness to achieve the recovery from potential photoinhibition during daytime. The following exceptions from this rule had to be implemented for practical purposes: in Italy in 2018, one leaf from each of 50–75 plants per provenance were collected in the late afternoon, immediately placed in sealed plastic bags to prevent desiccation, and kept in the dark for a minimum of 2 h prior to measurements. In 2019, in the Italian site, 50 plants per provenance were measured in the field, *i.e.*, in general the five plants of the central row of each plot. The data of measurements are given in Table 2S (*supplement*) and extreme environmental conditions prior to the measurements are indicated.

**Chlorophyll content** was estimated using *SPAD-502 Plus* Chlorophyll Meter (Konica Minolta, Chiyoda, Japan).

**Data analysis:** All data sets presented in Figs. 2–6 were analyzed with the *PRISM 2.0* software ([www.graphpad.com](http://www.graphpad.com)) pairwise for each plantation and year for statistically significant differences between Greek and Italian provenances of oak species, by *Mann-Whitney's* test. In addition, on all seven data sets per year and plantation site a *Kruskal-Wallis* test with *Dunn's* post-hoc test was performed in the case of the German common gardens to identify possible significant differences between the local oak species *Q. robur* and the Mediterranean species.

## Results

**Environmental conditions:** For the seedlings grown in Germany, a notable late frost occurred in late April 2017, when part of the seedlings of the deciduous species had already developed leaves and were partially damaged. A similar phenomenon took place in SAN, with a minor late frost episode in late April that affected fully grown trees of different deciduous species in the SAN valley, including the late-flushing *Q. pubescens*. However, only minor damage (*i.e.*, frozen leaves or sprouting buds) was recorded in the seedlings. In Greece, very unusual, extreme frost occurred in winter 2016/17. On 8 January 2017, –9.5°C were recorded at OLY, where the seedlings overwintered outdoors in the nursery. With several additional strong frost nights occurring around this period, the *Q. ilex* seedlings were severely damaged which became evident only later after plantation. In Greece, heavy rain events occurred in the beginning of April and in July 2017, but most of the precipitation was runoff water and unavailable for the plants.

The summer 2018 was exceptionally dry and hot with all-time heat records in Central Europe, leading to severe

tree dieback both of adult trees and of recently planted or naturally propagated young trees in German forests. During winter 2018/19, water tablets in the soil did not recover due to limited precipitation, and summer 2019 again was extremely hot and, partially, dry with new all-time record heat events in June and again in July in Western and Central Europe. As a consequence, forestry damages observed in Western and Central Europe were enhanced and tree dieback of most present tree species in the forest site at SWA could be recorded continuing into August 2019. During these years, summer was relatively wet in Central Italy and hot with regular, but local heavy thunderstorms in Northern Greece.

**Seedling emergence and survival:** From the seeds collected and planted in pots, on average 68% germinated and gave rise to young plants. In general, the seedlings grown in the German plantations revealed satisfying survival rates in the first three years after plantation, *i.e.*, 72–98%, depending on species and site. When the two German sites were compared, the drier (sandy soil) site led to higher seedling mortality. In Italy, all provenances of *Q. ilex* and *Q. pubescens* showed good survival (75–82%) until 2019, while *Q. frainetto* revealed *ca.* 60% survival. In Greece, the conditions were obviously very challenging for the young trees, especially for *Q. ilex* with only 2% surviving until 2019 (both provenances). Here, *Q. pubescens* seedlings revealed the best survival rates (11 and 18% for the Italian and Greek provenances, respectively).

**Physiological fitness of the seedlings:** Table 2S shows the dates of the measuring campaigns and important environmental conditions previous to the measuring dates, which may influence JIP parameters. In summary, plants

developed very well at the two German and the Italian site. At the Greek site, plants were very stressed by abiotic conditions, including sharp frost prior to plantation, affecting *Q. ilex*, and several severe drought events. SPAD readings revealed that the plants growing at SWA had slightly higher median Chl contents (*i.e.*, SPAD values of 40 units) than those at RIE (35–38 units) (data not shown). In Italy, *Q. frainetto* revealed significantly lower SPAD readings (medians around 30 units) than the other species (medians around 40 units) (data not shown). Detailed biometric data of plant growth, including Chl contents, will be published elsewhere in due course.

Depending on species/provenance and site, the ChlF induction curves indicated how stressful the respective site conditions were for the plants. This is illustrated in the double normalized induction curves in Fig. 1. In three out of six cases, *i.e.*, *Q. ilex* from Greece and Italy and for *Q. pubescens* from Greece, the induction curves measured at the Greek site revealed higher fluorescence emission between 0.1 and 100 ms, indicating that electron flow in the electron transport chain was restricted in comparison to the respective provenance grown at the German or Italian sites (*cf.* ‘Discussion’). Thus, for these provenances, the plants at OLY appear to be more stressed than those at the other sites. A striking feature is that in many plants at OLY, the I-step (and to a lesser extent the J-step) was reached significantly earlier (*i.e.*, after 10 ms instead of 30 ms). However, these curves only represented individual leaves on individual plants, thus, for statistical purposes, we assessed the double normalized curves of all plants to calculate overall JIP parameters describing the density of reaction centers (10RC/ABS), the quantum efficiency of  $Q_A$  reduction ( $\phi_{P_0}$ ), the efficiency of electron flow into the intersystem carriers ( $\Psi_{E_0}$ ), and the relative contribution of electron flow to the PSI end acceptors ( $\Delta V_{IP}$ ), as well as

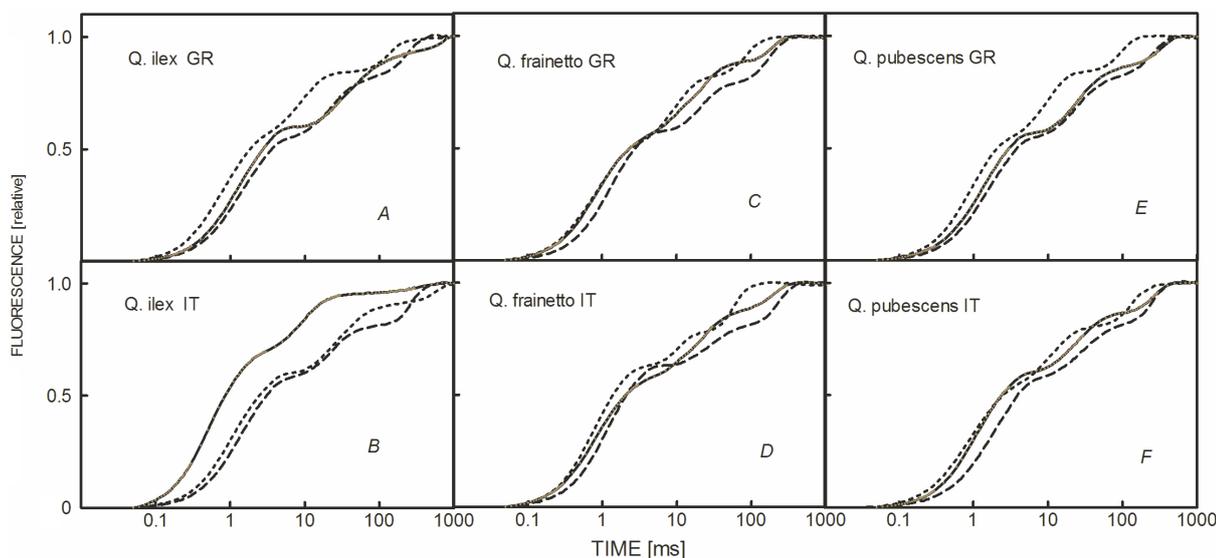


Fig. 1. Double normalized plots [ $F_0$  (50  $\mu$ s) = 0,  $F_m$  = 1] of the ChlF induction curves of oak seedlings at Frankfurt-Riedberg, Germany (*dotted line*), Sant'Anatolia di Narco, Italy (*dashed line*), and Olympiada, Greece (*solid line*) measured in 2017. The curves of those plants with the closest performance index on absorption basis ( $PI_{abs}$ ) value to the mean of the  $PI_{abs}$  values of the respective population (species  $\times$  provenance) are shown.

the widely used combination of the first three components, *i.e.*,  $PI_{abs}$ . The values for all these parameters at all sites and in each year are depicted in Figs. 2–6.

To summarize the results presented in Figs. 2–6, we state that despite the genetic heterogeneity of the seed material in many cases significant differences between Greek and Italian provenances within a given species could be observed. Secondly, in all three Mediterranean species, plants belonging to the same genetic group (species/provenance) derived from different tree nurseries – *i.e.*, seedlings grown under different conditions – showed strong differences of the ChlF parameters  $PI_{abs}$ , RC/ABS, and  $\Psi_{Eo}$ ; the seedlings grown in Germany (SWA 2016 and RIE 2016) under sheltered conditions revealed higher values of these parameters than those grown in Italy or Greece under outdoor conditions. In the following years,  $PI_{abs}$  values of the field-grown plants in Germany decreased to below 15 units in all species/provenances, with the highest values observed in *Q. pubescens* from Greece and a general tendency towards minimum values in 2018 in all species/provenances. In Italy and Greece, initial  $PI_{abs}$  values of the nursery plants in 2016 were lower than that

in Germany and remained (on average) below 5 or 10 units throughout the years. The lowest  $PI_{abs}$  values (around 1 unit) were recorded in the plants at OLY in 2018 pointing to very stressful abiotic conditions. In Italy, the lowest  $PI_{abs}$  values were also recorded in 2018 (Fig. 2). The observed differences in  $PI_{abs}$  were mainly due to differences in its components RC/ABS (Fig. 3), and to some extent also to  $\phi_{Po}$  (especially, at OLY; Fig. 4). Variability in  $\Psi_{Eo}$  appeared smaller, except for the plants from the German nursery (2016, very high values) and the plants at OLY in 2017 (very low values, Fig. 5). In addition to the site effects on  $PI_{abs}$  and its components, we observed site-specific effects on  $\Delta V_{IP}$  (Fig. 6), a parameter which correlates with the efficiency of electron transport to the acceptors of PSI. This parameter was always the lowest at OLY.

To summarize the differences in ChlF parameters observed between the Greek and Italian provenances (within each year and site), as shown in Figs. 2–6, their patterns (*i.e.*, the presence or absence of significant differences between them) and between *Q. robur* and the Mediterranean provenances is shown in Table 1.

In general, for  $PI_{abs}$  and its underlying components

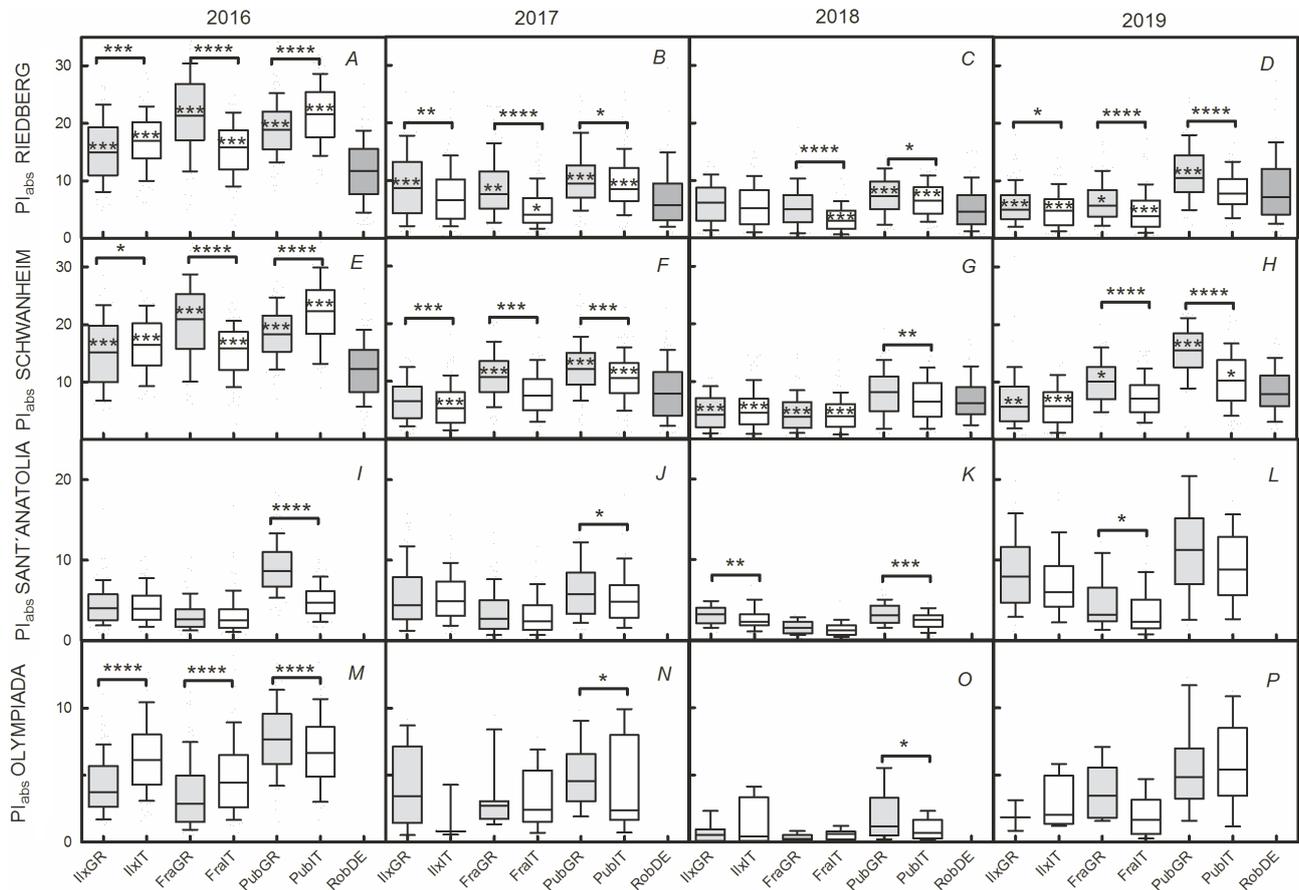


Fig. 2. Development of performance index on absorption basis ( $PI_{abs}$ ) from seedling stage (2016) to 2019 at the plantation sites. Data are grouped and compared species-wise and, in the German sites, to data observed in the local species, *Quercus robur*. Box plots (25–75 quartiles) with 10–90% whiskers are shown. Statistically significant differences between Greek and Italian provenances are shown by brackets and asterisks with significance levels at 5 (\*), 1 (\*\*), 0.1 (\*\*\*), and 0.01% (\*\*\*\*), respectively. Statistically significant differences of the Mediterranean provenances to *Q. robur* are indicated by asterisks in the respective boxes. Ilx – *Quercus ilex*; Fra – *Quercus frainetto*; Pub – *Quercus pubescens*; ROB – *Quercus robur*; GR – Greek provenance; IT – Italian provenance; DE – German provenance.

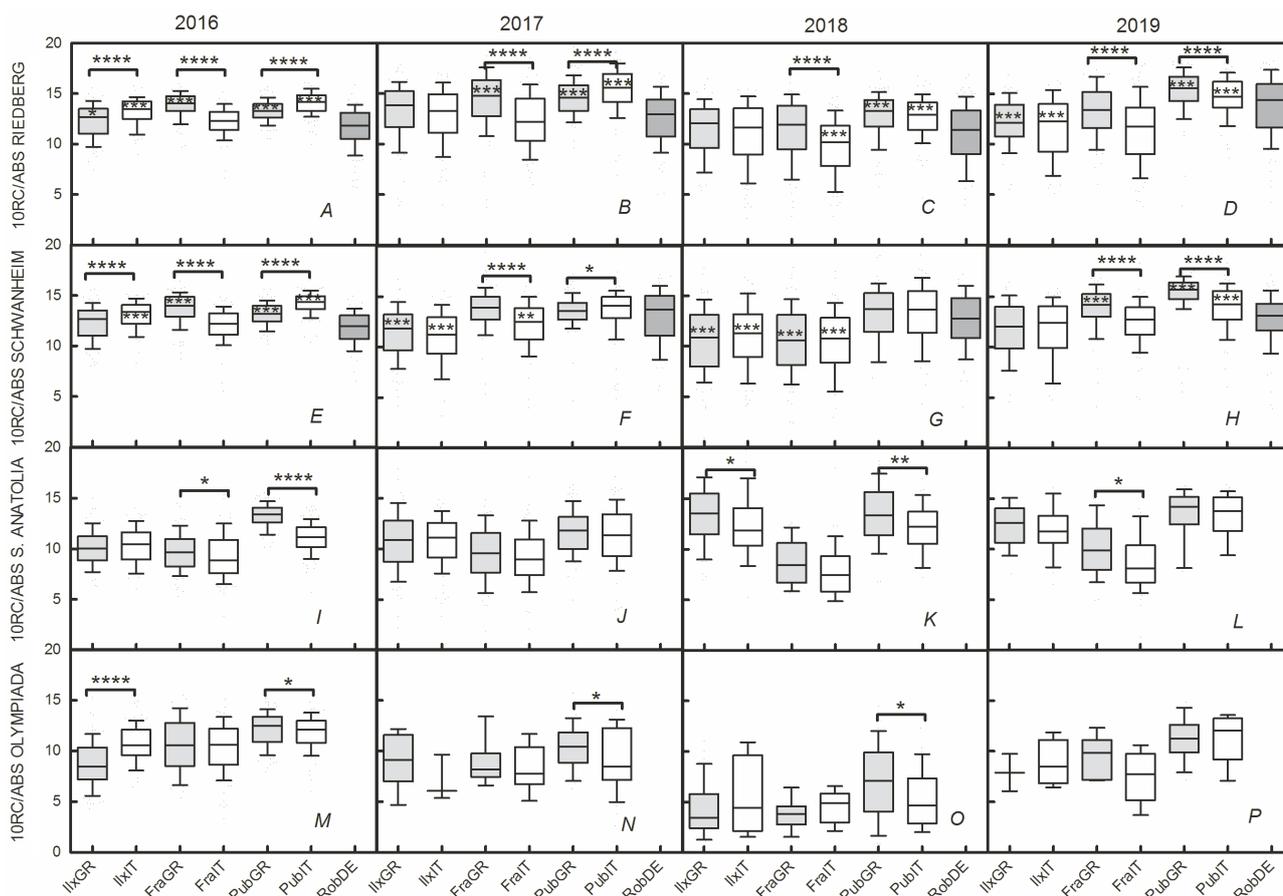


Fig. 3. Development of density of active reaction centres (10RC/ABS) from seedling stage (2016) to 2019 at the plantation sites. Data are grouped and compared species-wise and, in the German sites, to data observed in the local species, *Quercus robur*. Box plots (25–75 quartiles) with 10–90% whiskers are shown. Statistically significant differences between Greek and Italian provenances are shown by brackets and asterisks with significance levels at 5 (\*), 1 (\*\*), 0.1 (\*\*\*), and 0.01% (\*\*\*\*), respectively. Statistically significant differences of the Mediterranean provenances to *Q. robur* are indicated by asterisks in the respective boxes. Ilx – *Quercus ilex*; Fra – *Quercus frainetto*; Pub – *Quercus pubescens*; ROB – *Quercus robur*; GR – Greek provenance; IT – Italian provenance; DE – German provenance.

RC/ABS and  $\phi_{p0}$ , the Italian provenances tended to show higher values in the seedlings from the German and Greek nurseries, while  $\Delta V_{IP}$  was higher in the Greek provenance of *Q. ilex* in all nurseries. *Q. frainetto* and *Q. pubescens* seedlings showed different behaviors under different nursery conditions. In the plants in the field sites,  $PI_{abs}$  of Greek *Q. pubescens* was higher than that in its Italian provenance under most conditions (site/year); the higher values for the Greek provenances were also recorded in most *Q. frainetto* at the two German sites. Whenever one of the  $PI_{abs}$  or one of its components RC/ABS,  $\phi_{p0}$  or  $\Psi_{E0}$  revealed significant differences in the Greek vs. the Italian provenances in the field sites in 2017–2019, the Greek provenance showed the higher values with only one exception (*Q. pubescens*, RC/ABS in 2017). For  $\Delta V_{IP}$ , a different pattern occurred: in some combinations of species/site/year, the Greek provenance revealed higher values than that of the Italian one, in others, it was *vice versa*. Considering the relative values of the JIP parameters of *Q. robur* in the German plantations as compared to the Mediterranean species, we often (in

9 out of 12 combinations of provenance, site, and year) found higher values of  $PI_{abs}$  in *Q. pubescens*, especially in its Greek provenance (5 out of 6 combinations of site and year; Fig. 3, Table 1).

### Discussion

#### Abiotic factors influencing seedlings establishment:

While seedling emergence and survival at the German and Italian sites was satisfactory, problems occurred at the very dry Greek site in OLY. Here, the evergreen *Q. ilex* plants suffered severely from the extreme frost period in early January 2017 in the nursery, an effect also described for other broadleaf seedlings (Radoglou and Raftoyannis 2001). In addition, precipitation was low in March/early April, especially at OLY, and since the plants could not develop new root systems fast enough, many of them died of drought before the first stronger rainfalls occurred again in later April (*see* Bréda *et al.* 2006). The better survival of *Q. pubescens* under identical conditions than *Q. frainetto* confirmed earlier findings for drought-stressed seedlings

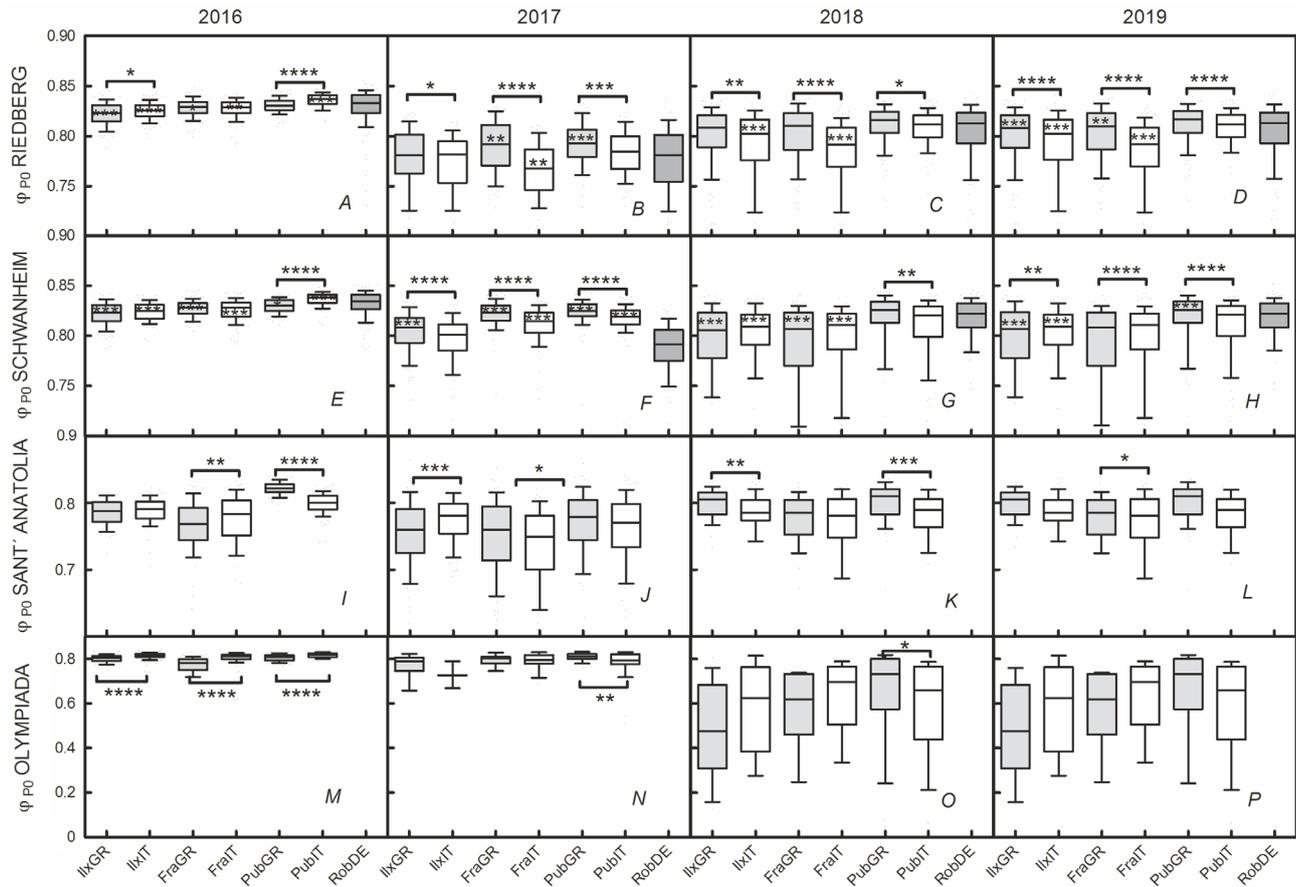


Fig. 4. Development of quantum efficiency of reduction of  $Q_A$  ( $\phi_{Po}$ ) from seedling stage (2016) to 2019 at the plantation sites. Data are grouped and compared species-wise and, in the German sites, to data observed in the local species, *Quercus robur*. Box plots (25–75 quartiles) with 10–90% whiskers are shown. Statistically significant differences between Greek and Italian provenances are shown by brackets and asterisks with significance levels at 5 (\*), 1 (\*\*), 0.1 (\*\*\*), and 0.01% (\*\*\*\*), respectively. Statistically significant differences of the Mediterranean provenances to *Q. robur* are indicated by asterisks in the respective boxes. Ilx – *Quercus ilex*; Fra – *Quercus frainetto*; Pub – *Quercus pubescens*; ROB – *Quercus robur*; GR – Greek provenance; IT – Italian provenance; DE – German provenance.

of the two species in northern Greece (Siam *et al.* 2009).

#### Comparison of the growth conditions at the common garden sites as reflected in physiological parameters at species level:

With respect to the physiological fitness of the plants, it became evident that plants at both the Greek and the Italian sites appeared to be more stressed in summer 2017 than those at the German sites, with lower  $PI_{abs}$  across all species and provenances (Fig. 2). A similar finding with a north-south gradient of  $PI_{abs}$  across different sets of forest trees has been observed in a comprehensive field study with mature oaks, other deciduous species and conifers (Pollastrini *et al.* 2016). In 2018, plants at the German sites also showed lower values than in 2017, probably a consequence of the combined drought/heat stress to which they had been exposed, since at least for *Q. frainetto* and *Q. pubescens*, the  $PI_{abs}$  values recovered in 2019, when they were measured before the onset of the first heat wave at the end of June. In Italy,  $PI_{abs}$  values in 2018 were low for all six provenances, with low variability, when compared to the other years. The low variability can be explained by the different measuring technique in this particular year:

at daytime, leaves were collected and placed in plastic bags in the dark. During this procedure, healthy leaves were collected, thus avoiding damaged (by insect attack), infected (by pathogens) or early senescing leaves. This selection of healthy leaves could not be realized in all other measuring campaigns, where leaves were measured *in situ* in the dark; the experimenter being equipped with a weak green light to find the leaves on the trees. We cannot exclude the possibility that the cutting of the leaves and their storage in the plastic bags until measurements (2–3 h) led to a decrease in the absolute values of  $PI_{abs}$ ; this should be followed up in an independent experimental setup. Nevertheless, the cut and stored leaves may well be suited to identify significant differences between genetically different provenances of the same species, as demonstrated for *Q. ilex* and *Q. pubescens*.

The observed differences in  $PI_{abs}$  were mainly connected to differences in its component RC/ABS and, less so with respect to  $\phi_{Po}$  (Figs. 3–5). Consistently lower RC/ABS values for *Q. frainetto* at SAN in 2017–2019, as compared to the other species, which were not observed in the nursery plants in 2016, can be explained by strong

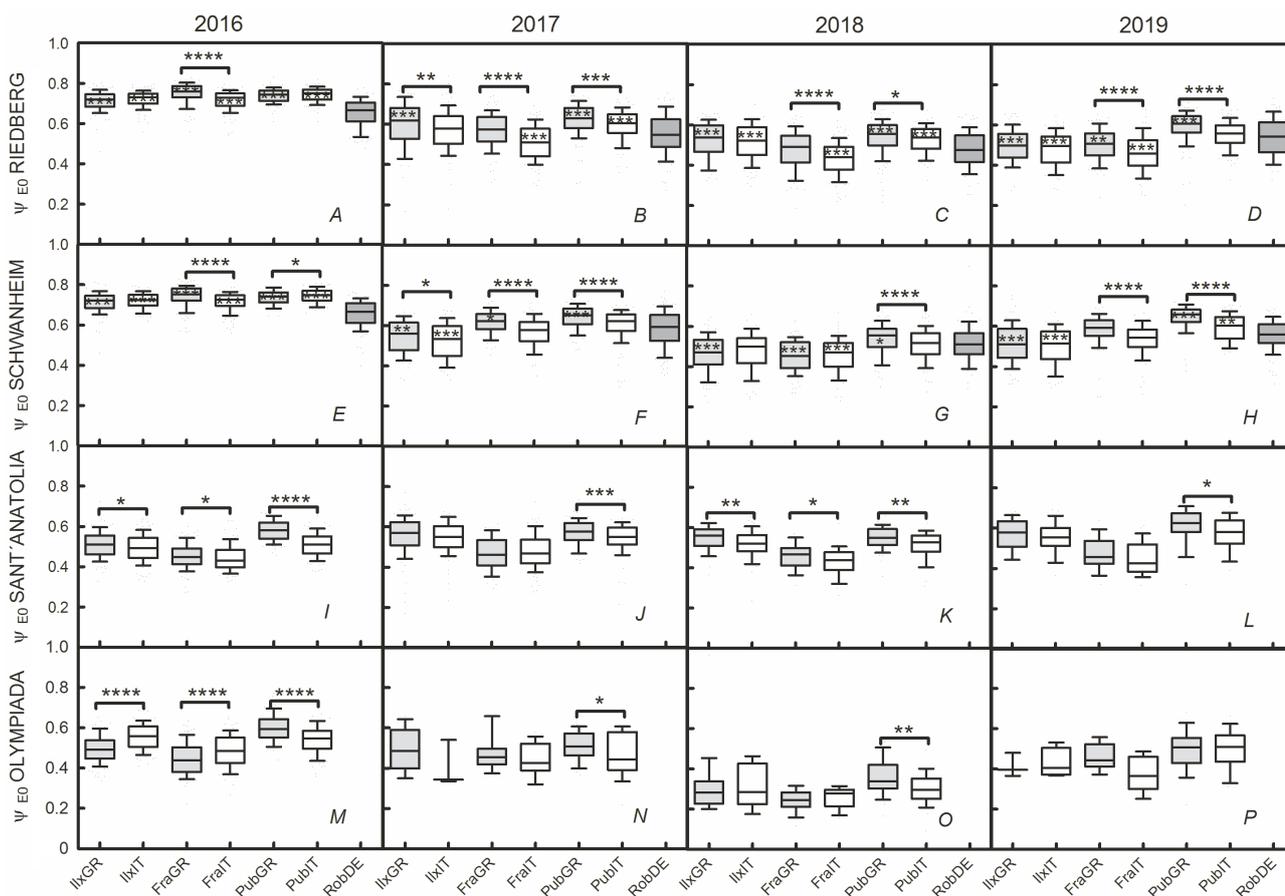


Fig. 5. Development of probability of electron transport beyond  $Q_A^-$  ( $\Psi_{Eo}$ ) from seedling stage (2016) to 2019 at the plantation sites. Data are grouped and compared species-wise and, in the German sites, to data observed in the local species, *Quercus robur*: Box plots (25–75 quartiles) with 10–90% whiskers are shown. Statistically significant differences between Greek and Italian provenances are shown by brackets and asterisks with significance levels at 5 (\*), 1 (\*\*), 0.1 (\*\*\*), and 0.01% (\*\*\*\*), respectively. Statistically significant differences of the Mediterranean provenances to *Q. robur* are indicated by asterisks in the respective boxes. Ilx – *Quercus ilex*; Fra – *Quercus frainetto*; Pub – *Quercus pubescens*; ROB – *Quercus robur*; GR – Greek provenance; IT – Italian provenance; DE – German provenance.

shading by adjacent, uncut vegetation of weeds at SAN, whereas plants at RIE and at OLY grew in full, and at SWA in partial sunlight. The strong shading of *Q. frainetto* at SAN probably resulted in an earlier onset of (partial) leaf yellowing (senescence) already in September, as reflected in lower Chl contents (data not shown). Decreasing RC/ABS ratios have also been reported during leaf senescence in oaks, with *Q. frainetto* showing stronger and much earlier effects than *Q. pubescens* (Holland *et al.* 2014).

Shading results not only in acclimation of leaf structure (Daas-Ghrib *et al.* 2011) in oaks, but also of the photosynthetic apparatus with an increase of the antenna size (Valladares *et al.* 2000, Valladares and Niinemets 2008), resulting in increasing ratios of Chl to RC and, consequently, decreasing RC/ABS ratios. This is in accordance with the slightly higher Chl contents in the plants growing at SWA as compared to those at RIE (not shown). (Reversible) inactivation of reaction centers (silent RCs *sensu* Strasser *et al.* 2004) will have the same effect on RC/ABS (Krause and Weis 1991, Strasser *et al.* 2004), and this may occur as a result of overexcitation of the

photosynthetic apparatus, when excess light energy cannot appropriately be transferred to photosynthetic electron transport, *e.g.*, due to closed stomata under water shortage conditions. The latter effect could explain the low RC/ABS values consistently observed at the Greek site in 2018–2019.

The quantum yield of the primary photochemistry process, *i.e.*, the capacity for  $Q_A$  reduction ( $\phi_{P_0}$ ), was the highest at the German sites and showed the lowest values at the Greek site, indicating a recognizable latitudinal gradient with a reduction of  $\phi_{P_0}$  southward. This behaviour, together with the reduction of reaction centres per Chl unit (RC/ABS) may be caused by photoinhibition due to higher solar radiation also in concurrency with higher air temperatures and lower water availability at the southernmost site (Pollastrini *et al.* 2016), which would enhance dissipation processes, especially if combined with drought.

Sun-exposed leaves have a lower capacity to trap electrons and reduce  $Q_A$  ( $\phi_{P_0}$ ) and a greater capacity to reduce the final electron acceptors, beyond PSI (Cascio

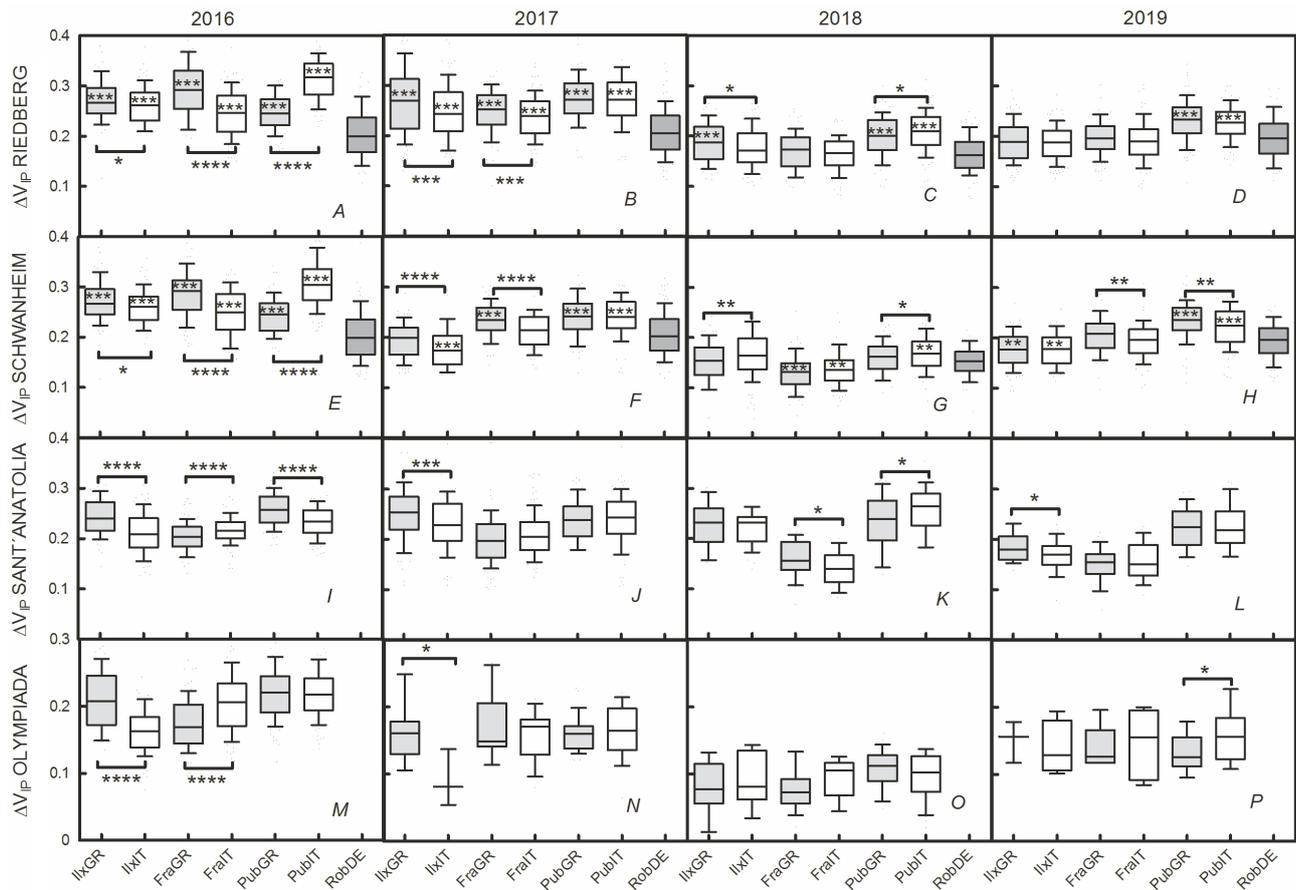


Fig. 6. Development of relative fluorescence increase between I- and P-step ( $\Delta V_{IP}$ ) from seedling stage (2016) to 2019 at the plantation sites. Data are grouped and compared species-wise and, in the German sites, to data observed in the local species, *Quercus robur*. Box plots (25–75 quartiles) with 10–90% whiskers are shown. Statistically significant differences between Greek and Italian provenances are shown by brackets and asterisks with significance levels at 5 (\*), 1 (\*\*), 0.1 (\*\*\*), and 0.01% (\*\*\*\*), respectively. Statistically significant differences of the Mediterranean provenances to *Q. robur* are indicated by asterisks in the respective boxes. Ilx – *Quercus ilex*; Fra – *Quercus frainetto*; Pub – *Quercus pubescens*; ROB – *Quercus robur*; GR – Greek provenance; IT – Italian provenance; DE – German provenance.

*et al.* 2010, Desotgiu *et al.* 2012, Pollastrini *et al.* 2017). The function of these adjustments is to enhance controlled energy dissipation ways, thus reducing the pressure from overexcitation (photochemical and nonphotochemical deexcitation pathways). The decrease of trapping and primary photochemistry capacity (measured with  $\phi_{p_0}$ ) in full sunlight leaves is induced by protein D1 degradation in PSII RC (photoinhibition; Ohira *et al.* 2004), and can be considered as a downregulation mechanism (Štroch *et al.* 2008). In fact, it reduces the electron flow within the electron transport chain when net photosynthesis is limited and the reductant potential cannot be utilized for metabolism (Lu *et al.* 2001, Baraldi *et al.* 2008). On the other hand, electrons flow quicker in the electron transport chain so enhancing the reduction of Rubisco by the electron acceptors beyond PSI.

All plants growing outdoors in Germany and Italy showed  $\Psi_{E_0}$  values around 0.5–0.6, apparently with genetically fixed differences between Greek and Italian *Q. pubescens*. A tendency towards lower values was visi-

ble in the shaded *Q. frainetto* plants in Italy. In Greece, very low values of  $\Psi_{E_0}$  were recorded, especially in 2018.  $V_j$  plays a crucial role in the calculation of  $\Psi_{E_0}$ , and a contemporary depression of the J- and I-steps has been reported in severely drought stressed poplar leaves (Pollastrini *et al.* 2014). These two parameters indicate the capacity to transport electrons through electron transport chain (ETC). This capacity can be inhibited by the inactivation of the targets beyond the ETC, like Rubisco (Flexas and Medrano 2002).

The lowest values of the electron transport rates at the J- and I-steps ( $\Psi_{E_0}$  and  $\Delta V_{IP}$ ) observed at OLY may be connected to drought stress. Several papers connected the depression of the IP-phase with drought conditions (*e.g.*, Oukarroum *et al.* 2009). Drought inhibits Rubisco to receive the electron flow from NADPH, mainly because of  $CO_2$  shortage, so the depression of the IP-phase (*i.e.*, the increase in  $V_j$ ) indicates the ‘traffic jam’ of electrons at the acceptor side of PSI caused by the decreased activity of ferredoxin NADP<sup>+</sup>-reductase (Schansker *et al.* 2005).



**JIP parameters as potential selection criteria in inter- and intraspecific comparisons:** In previous studies, OJIP induction curves and the derived parameters have been suggested as potential selection indicators for stress resistance in crops and even weeds, with special emphasis on heat, salt and drought resistance (e.g., Oukarroum *et al.* 2007, 2009; Jedmowski *et al.* 2014, Sharma *et al.* 2014, Chen *et al.* 2016, Kalaji *et al.* 2018). For woody plants, less examples, mostly with an emphasis on cold and salt tolerance, have been published [e.g., Percival and Fraser (2001) on *Crataegus* spp., Percival (2005) on *Quercus robur* and *Q. ilex*, Percival *et al.* (2003) on *Acer* genotypes, Percival *et al.* (2006) on *Fraxinus* genotypes]. In general, these studies were performed either with genetically homogeneous crop populations or, in the case of tree species, under controlled stress conditions, thus limiting data variability and skewing. A large dataset analysis of prompt fluorescence parameters across plant species, environments, and plant community characteristics (e.g., species composition) indicated, that the parameters  $\phi_{Po}$  and  $\Delta V_{IP}$ , connected to photon capture and first photochemical events and to the efficiency of electron transport around PSI, respectively, explain most of the variability of the fluorescence induction curves found across species and environmental conditions (Bussotti *et al.* 2020). In a study carried out in field conditions (Salvatori *et al.* 2016), environmental effects on the physiological status of *Quercus cerris* L. and *Fraxinus ornus* L. could better be monitored using  $PI_{tot}$  (including  $PI_{abs}$  plus the effects on the I–P phase) than by  $PI_{abs}$  alone.

The present study combined the approach to use JIP parameters as selection criteria for relative stress tolerance of different oak species provenances in field conditions in forests. It was conducted on seedling populations derived from seeds (acorns) collected in free wind-pollinating populations and therefore with genetic heterogeneity, potentially leading to variability in the structure and efficiency of the photosynthetic apparatus of plants. By means of JIP parameters, we evaluated the different photosynthetic performance of the seedlings, considering also minor heterogeneities of microclimatic conditions and soil within the common gardens. A comparative analysis of the selected JIP parameters among species, sites, and years with respect to the Greek or Italian provenances, was carried out (Table 1). In many cases, significant differences between Greek and Italian provenances within a given species were observed. However, these relative differences were not always constant over time, especially when growth conditions changed from the sheltered, optimized growth conditions in the nursery to the field. Therefore, JIP parameters measured on seedlings derived from nurseries as predictors for their further physiological performance under field conditions should be used with caution. As an example, we may look at the relative performance of the seedlings grown in Germany and Greece in 2016 and their performance in 2017–2019: while, except for *Q. pubescens* in OLY, the Italian provenances showed higher  $PI_{abs}$  values in the nursery plants in 2016 (i.e., under optimized growth conditions), in the following years, in the field, this tendency reversed. In

the case of  $\Delta V_{IP}$ , the pattern was inconsistent in all species. In other words,  $PI_{abs}$  or  $\Delta V_{IP}$ , appear not to be robust and effective parameters to identify and select young, nursery-grown plants on the basis of their photosynthetic efficiency. However, once growing under field conditions, the relative order between Greek vs. Italian provenances in terms of  $PI_{abs}$  and especially of its component  $\Psi_{Eo}$  was very consistent in *Q. pubescens* across all years and sites: in 10 ( $PI_{abs}$ ) and 11 ( $\Psi_{Eo}$ ) out of 12 possible combinations (year/site), the Greek provenance showed higher values for these parameters than the Italian one, indicating that electron transport beyond  $Q_A^-$  functions better in the Greek provenance under many different field conditions. Similarly, Greek *Q. frainetto* developed higher  $PI_{abs}$ ,  $\phi_{Po}$ , and  $\Psi_{Eo}$  values than their Italian counterpart under several conditions (5 to 7 out of 9 possible combinations of year and site in Germany and Italy).

While modifications of electron transport around PSI seem to play an important role in tree responses to environmental stressors, as visualized in the variability of the I–P phase (e.g., Bussotti *et al.* 2011, 2020; Salvatori *et al.* 2013), the  $\Delta V_{IP}$  parameter appears to be of only limited value for selection purposes in the assessment of plant performance under field conditions. In *Q. pubescens*, the relative values of  $\Delta V_{IP}$  of Italian vs. Greek provenances were highly variable across sites and years. Only in *Q. ilex*, in 6 out of 12 year/site combinations the Greek provenance revealed a higher  $\Delta V_{IP}$  than the Italian provenance, suggesting that in this species this parameter might be useful for selection purposes in field-grown material.

A statistical analysis of the data set in OLY is hampered by the low numbers of survivors in *Q. ilex* and *Q. frainetto*, but for *Q. pubescens*, the trend observed in the other countries [i.e., that the Greek provenance showed higher values of  $PI_{abs}$  (and also of RC/ABS,  $\phi_{Po}$ , and  $\Psi_{Eo}$ )] than the Italian one could be confirmed in two years in the field (2017, 2018).

To assess whether the growth conditions at SWA and RIE would favor the plantation of the local species, *Q. robur*, over the Mediterranean species, we tested, as a preliminary indication,  $PI_{abs}$  of *Q. robur* (and the other ChlF parameters analyzed in this study) in a *Kruskal-Wallis* test against the respective values of the other provenances. In general, we observed consistently higher values of  $PI_{abs}$  in the Greek *Q. pubescens* than in *Q. robur* in all years and on both sites. While an interspecific comparison of  $PI_{abs}$  and other ChlF parameters must be considered with care due to species-specific morphological and physiological properties, which influence these values, we can at least conclude that the growth conditions at RIE and SWA appear to be favorable for the physiological performance, evaluated in terms of photosynthetic efficiency, of the (Greek) *Q. pubescens*, especially since they also produced higher  $PI_{abs}$  values in the German sites than in the Mediterranean sites.

In consequence, we conclude that the JIP-test can be a helpful tool in selection procedures of tree provenances under field conditions, despite high genetic variability in many European tree species. However, the robustness of its parameters across time, site conditions, and stressful

growth situations should be tested for each species before application in large-scale field trials. It should further be noted that the present study is based on the comparison of average values in populations of trees from different provenances, not on the performance of individual trees within these populations. From our experience, we suggest to use the JIP-test-derived parameters as effective tools to assess the physiological suitability of a given provenance for populating forest sites, together with measures of basic growth and morphometric data.

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