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Temporal Response to Drought Stress in Several *Prunus* Rootstocks and Wild Species

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Abstract: *Prunus* species are important crops in temperate regions. In these regions, drought periods are predicted to occur more frequently due to climate change. In this sense, to reduce the impact of climate warming, obtaining new tolerant/resistant cultivars and rootstocks is a mandatory goal in *Prunus* breeding. Therefore, the current study assembled three *Prunus* species including almond, (*P. dulcis* Mill D.A. Webb), apricot (*P. armeniaca* L.) and peach (*P. persica* L.) to model the temporal effects of drought. A hybrid peach × almond and a wild almond-relative species *Prunus webbii* were also included in the study. Physiological traits associated with photosynthetic activity, leaf water status, and chlorophyll content were assessed under three watering treatments. Results showed that effects of time, genotype, and treatment interact significantly in all traits. In addition, results confirmed that *P. webbii* have a greater tolerance to drought than commercial rootstocks. However, "Real Fino" apricot showed the fastest recovery after re-irrigation while being one of the most affected cultivars. In addition, from the better response to these watering treatments by the almond genotypes, two different trends were observed after re-irrigation treatment that clearly differentiate the response of the almond cultivar "Garrigue" from the rest of *Prunus* genotypes. A better characterization of the short-term drought response in *Prunus*, an accurate and more efficient evaluation of the genotype effect was obtained from the use of mixed models considering appropriate variance–covariance structures. Although the advantages of these approaches are rarely used in *Prunus* breeding, these methodologies should be undertaken in the future by breeders to increase efficiency in developing new breeding materials.

Keywords: drought stress; *Prunus*; mixed model; breeding; recovery

1. Introduction

The scarcity of water resources and high irradiance and the temperature during the summer are characteristics of different regions of the world, such as the Mediterranean area [1] or California area [2].

These areas are considered centers of agriculture in their respective countries and produce a very significant portion of the agricultural national production. It has been proved that effects of drought have a huge impact on the morphology, physiology and biochemistry of fruit trees [3–6]. Within the actual climate warming, drought periods in arid and semi-arid regions worldwide are predicted to occur more frequently [1]. The *Prunus* genus englobes important fruit trees (peaches, cherries, apricots, plums) and also important nut trees, such as almond, are cultivated worldwide. At the same time, these species represent the most economically important fruit of these regions, for example, almond generated USD 5.60 billion in cash receipts in California, [7].

In the cultivated peach (*P. persica* L.) and its relative species, a suite of morphological and physiological adaptations to drought have been described, allowing them to survive a water stress situation [8–12]. The degree of adaptation to drought may vary considerably among and within species. In dry regions, such as the Mediterranean area, the choice of proper rootstocks with multiple tolerances to stresses is crucial to preventing future problems in orchards and to reducing management costs [13,14]. Due to this fact, classical breeding programs tried to develop new rootstocks as part of their breeding goals to improve resistant to drought [15]. Suitable rootstocks could increase adaptation to drought, heavy soils, waterlogging, alkalinity, vigor control and soil fungal diseases [16].

To study drought response, several parameters and their relations such as stomatal conductance (G_S), net photosynthetic rate (P_N), water distribution between symplast and apoplast, leaf turgor, transpiration rate (E), synthesis of abscisic acid, intercellular CO_2 concentration (Ci), electron transport rate, carboxylation efficiency, intrinsic water-use efficiency have been studied in fruit trees [6,17–20]. Under drought stress conditions, all metabolic processes, including photosynthesis, are negatively affected [20,21]. In addition, Rieger and Duemmel [8] concluded that shoot characteristics measured via carbon assimilation rate and leaf conductance were more closely associated with drought adaptation than root characteristics in six *Prunus* species from divergent habitats. From all measured parameters, only a fraction of root biomass in fibrous roots was correlated with drought resistance. According to the variation in leaf characteristics, the authors assumed that genetic improvement of drought resistance of stone fruits may be achieved by incorporating xerophytic leaf characteristics into scion cultivars [8].

Torrecillas et al. [22] used the two almond (*P. dulcis*) cultivars "Ramillete" and "Garrigues" to study water stress mechanism on almonds. The authors evaluated osmotic adjustment and turgor maintenance, leaf turgor and stomatal conductance. According to the results, different mechanisms to resist water stress seem to have been developed by these cultivars. These differences could be associated to differences in cell wall thickness or in cell wall structure [23]. In addition, higher leaf conductance values for plants grafted onto "Garrigues" were observed by Alarcón et al. [24] after a short and long time of water deficit exposure, in comparison with plants grafted onto "GF 677". The authors suggested that "Garrigues" seedlings, as a rootstock, are less drought tolerant than "GF 677", because the latter avoided the loss of water via transpiration and maintained the leaf water content under stress [24].

Four Prunus rootstocks ("GF 677" (*P. persica* × *P. dulcis*), "Cadaman" (*P. davidiana* × *P. persica*), "ROOTPAC® 20" (*P. cersífera* × *P. dulcis*) and "ROOTPAC® R" (*P. cersífera* × *P. dulcis*)) were submitted to drought stress to study physiological, biochemical and molecular parameters and to improve water-use efficiency (WUE) in stone fruit [4]. According to the results, a lower net photosynthesis rate, stomatal conductance and transpiration rate, and higher intrinsic leaf WUE (A_N/G_S) were observed in the stressed plants. In addition, these results showed that accumulation of sorbitol and raffinose, proline, and the increase in expression of the *Δ-1-pyrroline-carboxylate synthase* (P5SC) gene could be used as markers of drought tolerance in peach cultivars grafted on *Prunus* rootstocks [4].

More recently, Bielsa et al. [25] studied a collection of wild-relative species and cultivated hybrid rootstocks of *Prunus* to estimate their water use efficiency (WUE) and genetic variation in two drought-induction genes. Results showed that almond and peach wild-relative species had the highest WUE in comparison with hybrid genotypes, with almond wild-relative species being the best candidates to develop new cultivars with drought tolerance. Moreover, few differences were

observed in the promoter regions of the studied genes, which could be also used to improve *Prunus* rootstock germplasm.

In general, a common pattern between these agronomic experiments studying response to drought (and other abiotic stresses) is that the collected data have been based on repeated measurements on the same plant (either across time or across space). It is often plausible that two measurements taken closer in time on the same unit (e.g., plant) are likely to be stronger correlated than measurements taken further apart in time. In this sense, an appropriate statistical methodology is essential for an efficient and reliable analysis of these agronomics experiments which are often expensive and time-consuming [26]. However, studies about modelling the temporal physiological response of the most important *Prunus* species under drought stress via mixed model are still scarce.

The purpose of the present study was to analyze several physiological parameters in different *Prunus* genotypes using a mixed model approach to determine if these *Prunus* rootstocks and wild species respond similarly to drought stress and recover afterwards or, contrarily, if different patterns of response can be observed. In addition, the study tries to answer the question of which parameters best describe the drought and recovery pattern.

2. Materials and Methods

2.1. Plant Material

The plant material used represents commercial rootstocks from the most important *Prunus* species, including the apricot (*P. armeniaca* L.) "Real Fino", the peach cultivar "Montclar", the hybrid peach × almond "GF 677" and the almond "Garrigues". In addition, the wild Mediterranean almond species, *Prunus webbii* [27], was also included in the study. "GF 677" (Paramount® in the USA) is a natural hybrid selected by INRA. It is a very vigorous rootstock (10–15%) more vigorous than peach seedlings [28] with a well-developed root system that ensures good anchorage. "Montclar®" was selected at INRA in 1960. It has high seed production and increased vigor in scion cultivars [29]. It also exhibits great resistance to iron-induced chlorosis and has a good uptake of iron and magnesium from the soil. "Real Fino" is a traditional apricot seedling rootstock. It is specially adapted to light soils containing limestone due to its resistance to iron chlorosis [30]. "Garrigues" is the most common almond rootstock in Spain. Seedlings from this cultivar are uniform with a strong, deep, root system. Finally, *P. webbii*, is a wild almond species grown in a wide range of countries, from Balkan peninsula to Italy or Spain [31]. This small and very thorny bush species with small leaves has been used as a source of new genes for improving important traits, such as self-compatibility or tolerance to adverse environment in almond [32]. All plants from "Real Fino", "GF 677", "Garrigues", "Montclar" and *P. webbii*, were open pollinated seedlings collected from each individual mother tree located in the germplasm collection of the experimental field of CEBAS-CSIC at Santomera and Cieza (Murcia, Spain). Seedlings were germinated and planted in individual pots at the same time. At the beginning of the experiment, the development of the plants was similar across individuals. Note that we used the terms, species, cultivars and hybrid for different genotypes till now, as they were partly species while others were genotypes. To simplify presentation, we used the term "genotype" globally to describe differences between the five genotypes used.

2.2. Experimental Design

The experiment was performed in controlled greenhouse conditions in the experimental field of CEBAS-CSIC at Santomera (Murcia, Spain). Hourly means of temperature and relative humidity in greenhouse conditions were recorded to assure constant conditions during the experiment (Figure S1). The greenhouse experiment was conducted to test the influence of three watering treatments (described below) on the above-described material. A total of nine tables were used within the greenhouse. Within a table, five zones were defined. Genotypes were randomly allocated to these zones. Within a zone, there existed eight pots planted with the same genotype and with one plant per pot. Thus, a

total of 360 pots and plants existed. Pot size was 15 × 15 × 19.5 cm (3.5 L). Watering treatments were randomly allocated to tables (three tables for each treatment) according to a randomized complete block design. Furthermore, genotypes were randomly allocated to zones according to a randomized complete block design. The complete experimental design can be denoted as split-plot design with eight plants per zone.

At each time point, a random sample of plants per zone were measured. On each plant selected at one time point all measures described below were performed, these measures resulted in a loss of eight leaves in each sampled plant. Note that a loss of leaves each second day can result in damaged plants in the long term. Therefore, sampled plants varied between time points to avoid or reduce effects of repeated destructive measures (destructive effect) on the same plant. Further to note is that this is the main reason for planting more plants per genotype than plants sampled per time point. In addition, natural leaf drop-off should be expected as a result of management and the different treatments. To conclude, the experimental design can be denoted as split-plot design with repeated measures within a zone and across time.

2.3. Watering Treatments

The three different watering treatments included drought, recovery and well-watered (control). All plants were irrigated to full water capacity at the beginning of the experiment at day 0. Afterwards, the experiment and measurements started, as has been commented below. In the well-watered control treatment, plants were irrigated daily to full water capacity. The recovery treatment consists of exposing the plants to a period of drought until an established day and after that the plants are watered again (to full water capacity). The drought treatment consists of not watering plants at all. The established day of the watering day in the recovery treatment was selected according to the previous observed tolerance to drought of these genotypes, which is described below.

2.4. Time Points and Duration of Experiment

Measurements of the physiological parameters (described below) were taken repeatedly every second day in the selected plants, between 9 and 11 am (GMT). The duration of the experiment was 21 days for "Garrigues" and *P. webbii* and 17 days for the rest of the genotypes. The re-watering point was established at day 17 of the experiment for "Garrigues" and *P. webbii* (because of their drought tolerance) and day 13 in the case of the rest of the genotype. To facilitate interpretation of data, each time point was denoted with the word "Time" and the number of measures. As per example, day 1 (first measurement) is Time1, day 13 is Time7, and day 21 of the experiment is Time11.

At Time8 the LI-COR machine used during all the experiments was not available to the authors. To avoid loss of data, authors decided to use a different one for this day. As the machine effect cannot be separated from time point effects here (the second machine is used for time point 8 only), for these traits (net photosynthesis rate (P_N), stomatal conductance (G_S), and transpiration rate (E)) data from Time8 were dropped prior to analysis.

2.5. Physiological Parameters Measurement

A destructive (leaf water potential (ψ_w)) and five non-destructive (net photosynthesis rate (P_N)), stomatal conductance (G_S), and transpiration rate (E), chlorophyll content (ChC) and maximal photochemical efficiency of photosystem II (Fv/Fm) traits were conducted in this experiment and have been described in detail below.

The assessment of leaf ChC was realized on one fully developed leaf of the selected plants by means of a portable device SPAD-502 (Minolta, Kyoto, Japan). The SPAD-502 m is used extensively in research and agricultural settings as a rapid, inexpensive, and non-destructive method [33]. The SPAD-502 m consists of two light-emitting diodes (LEDs) and a silicon photodiode receptor. It measures leaf transmittance in the red region (650 nm) and infrared region (940 nm) of the electromagnetic spectrum. A relative SPAD-502 m value (ranging from 0 to 99) is derived from the transmittance values, which is

proportional to the chlorophyll content in the sample [34,35]. Three measurements were taken in different areas of the leaf, and the average value was obtained.

The chlorophyll fluorescence was analyzed on a second leaf by means of a portable Chlorophyll Fluorometer (Opti-Sciences, Hudson, NH, USA). This equipment obtains $F_v/F_m = (F_m - F_0)/F_m$ and thus is the ratio between the variable fluorescence ($F_m - F_0$) and the maximal fluorescence. F_m is the maximal fluorescence intensity in a leaf adapted to darkness during 30 min, induced by a far red light excitation source (3000 µmol m^{-2} s^{-1}) during 0.8 s. F_0 is the minimal fluorescence intensity due to the exposition of a leaf to an actinic light source (400 µmol m^{-2} s^{-1}).

The measurements of P_N, G_S, and E were all taken from the third leaf. The fourth unfolded leaf was selected and a portable LI-COR meter (LI-6400XT) was used following the method of Haider et al. [36].

Finally, another leaf was used to measure ψ_w using a pressure chamber, following the recommendations of Turner [37] to prevent leaf water loss during measurements. Leaves were selected at random from the middle third of the shoots. Note that due to the special leaf morphology of *Prunus webbii*, only SPAD and F_v/F_m measures were evaluated for this genotype. The remaining (four) sampled leaves were stored for molecular analysis (data not used in the current study).

2.6. Modelling Analysis

Each trait was evaluated across the whole study period (from day 1 to day 21). The analysis was based on a mixed model approach using the following model:

$$\boldsymbol{y}_{ijkl} = \boldsymbol{\mu} + \boldsymbol{r}_k + \boldsymbol{t}_{kj} + \boldsymbol{z}_{kij} + \boldsymbol{\tau}_i + \boldsymbol{\varphi}_j + (\boldsymbol{\tau}\boldsymbol{\varphi})_{ij} + \boldsymbol{e}_{ijkl},$$

where $\boldsymbol{r}_k$, $\boldsymbol{t}_{kj}$, and $\boldsymbol{z}_{kij}$ are the vectors of fixed effects of the *k*th replicate for each time point, the vector of random effects of the *j*th main-plot error within the *k*th replicate (which corresponds to table effects for each time point) and the *i*th zone within table *kj* for each time point. Note that these are vectors with an effect for each time point. $\boldsymbol{e}_{ijkl}$ is the vector of residual errors of the observation vector $\boldsymbol{y}_{ijkl}$. Note that residual error effects are the confounded effects of plot error and the plant effects. $\boldsymbol{\tau}_i$ is the vector of fixed effect of the *i*th genotype, $\boldsymbol{\varphi}_j$ is the vector of effect of the *j*th watering treatment, and $(\boldsymbol{\tau}\boldsymbol{\varphi})_{ij}$ is the vector of interaction effects between the *i*th cultivar and the *j*th watering treatment. Each vector includes effects for up to eleven time points. As time points cannot be randomized, a first order autoregressive variance–covariance structure was fitted to all random effects. This accounts for correlations due to the repeated measures structure of the data. For the residual error effects, a homogeneous and heterogeneous first order autoregressive variance–covariance structure was fitted. The latter means that the model allows for time-point specific variances. The model with the smaller Akaike information criterion (AIC) [38,39] was chosen. Residuals were checked graphically for deviations from normal distribution and homogeneous (or the fitted heterogeneous) variance. For the traits E and G_S, data have been logarithmical transformed prior to analysis. For the trait Fv/Fm, data were logit transformed prior to analysis. Means were back transformed for presentation purpose only. Back-transformed values represent the median of the distribution on the original scale. Standard errors were back transformed using the delta method. A letter display based on Fisher's Least Significant Difference (LSD) multiple comparison tests were used in case of finding significant effects via F-test. Note that data were unbalanced at time point 10 and 11. Thus, genotype-by-treatment means across time were not estimable. Therefore, means across the first nine time points were estimated. Additionally, specific contrasts were estimated to account for the fact that drought took four days longer for drought-tolerant genotypes. All analyses were performed using the procedure PROC MIXED within SAS [40].

3. Results

The hourly mean values of the temperature and relative humidity collected between 9 a.m. and 11 a.m., varied during the study period (19th June to 9th July), within the ranges 25.8–35.4 °C and 17.5–70.2%, respectively (Figure S1). A punctual high value of relative humidity was recorded at

9.00 a.m. on the 20 June. Significance for effects from the mixed model analysis is summarized in Table 1. There were significant interaction effects between time, genotype and treatment for all traits. The three-way interactions were significant for Fv/Fm only. No interactions between genotype and treatment were found for P_N, ψ_w, and SPAD.

Table 1. Summary of linear mixed model analysis of effects of water treatment and time on measured parameters over the entire experimental period. Significant F-tests are shown in bold ($p < 0.05$). E: transpiration rate; G_S: stomatal conductance; P_N: net photosynthesis rate; ψ_w: leaf water potential; SPAD: chlorophyll content; Fv/Fm: maximal photochemical efficiency of photosystem II.

	E	**G_S**	**P_N**	**ψ_w**	**SPAD**	**Fv/Fm**
Effect	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Time	<0.0001	<0.0001	<0.0001	<0.0001	0.0525	<0.0001
Genotype	<0.0001	<0.0001	0.0046	0.0002	<0.0001	<0.0001
Treatment	0.00029	0.0037	<0.0001	<0.0001	0.0591	<0.0001
Treatment × genotype	0.0484	0.0372	0.0245	0.1029	0.3116	<0.0001
Genotype × time	0.004	0.0007	0.0011	0.0009	0.0084	<0.0001
Treatment × time	0.0023	0.0035	<0.0001	0.0023	0.6165	<0.0001
Treatment × genotype × time	0.2321	0.1341	0.0711	0.1846	0.5548	<0.0001
Time × rep	-	-	-	-	-	0.0147

3.1. Response of Genotype-By-Treatment Combinations to Drought

The results showed that significant differences between cultivars and treatments were observed only for the three traits E and G_S and P_N (Table 2). In the control treatment, cultivar "Montclar" showed the lowest significant values for E, G_S and P_N (0.43, 15.28, 11.97 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively) in comparison with the other three cultivars. For the other two treatments, recovery and drought, "Garrigues" was the cultivar with the highest significant values for E, G_S and P_N (as in the case of G_S, 16.27 and 11.52 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively). The control treatment had larger values for all traits and genotypes. In general, the lowest values were observed for the drought treatment.

Table 2. Mean values for the traits studied. Means with at least one identical lowercase letter within a column of one trait are not significantly different from each other cultivars. Means with at least one identical uppercase letter within a row are not significantly different from each other between treatment ($p < 0.05$). E: transpiration rate; G_S: stomatal conductance; P_N: net photosynthesis rate.

Trait		**Garrigues**			**GF 677**			**Montclar**			**Real Fino**			***P. webbii***
E	Control	0.99	a	A	0.70	a	A	0.43	b	A	1.00	a	A	–
	Recovery	0.52	a	B	0.31	b	B	0.23	b	B	0.23	b	B	--
	Drought	0.39	a	B	0.23	bc	B	0.17	c	B	0.26	ab	B	--
G_S	Control	36.39	a	A	24.39	b	A	15.28	c	A	38.63	a	A	--
	Recovery	16.27	a	B	8.77	b	B	6.89	b	B	7.15	b	B	--
	Drought	11.52	a	B	6.53	b	B	5.47	b	B	7.79	b	B	--
P_N	Control	32.33	a	A	19.29	b	A	11.97	c	A	30.04	ab	A	--
	Recovery	15.20	a	B	7.53	b	B	5.60	b	B	5.49	b	B	--
	Drought	9.89	a	B	5.90	bc	B	4.18	c	B	6.60	b	B	--

3.2. Temporal Response of Genotypes to Drought

In general, genotype and treatment differences vary across time for all traits studied here except Fv/Fm (Table 1). For the latter, genotype-by-treatment differences vary across time. Only in the case of SPAD, treatment × time interactions were not significant.

3.2.1. Genotype × Time

Regarding the behavior of genotypes across time for E, significant differences were observed since Time1 (Figure S2). Plants from cultivar "Garrigues" showed significantly higher values of E compared to all other genotypes across the majority of time points between Time2 and Time10. At Time10 the value of E (0.92 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) significantly increased, and no significant differences were observed between Time10 and Time2. In the case of "Real Fino" and "GF 677", Time9 values (0.43 and 0.42 mmol H_2O $m^{-2} \cdot s^{-1}$, respectively) were not significantly different from earlier time points such as Time4 and Time5, respectively. The peach cultivar "Montclar" showed the smallest values for E and values at Time9 were not significantly different from Time5 and Time6 (Table 3).

Regarding G_S, the cultivars "Real Fino" and "Montclar" showed a constant significant decrease in G_S values across time starting from Time1 (Figure S3). In comparison, "GF 677" decrease more slowly. At Time9, G_S values were higher for "GF 677" and "Real Fino" compared to previous time points but were not different from values at Time 4. In contrast, "Montclar" remained small and at a value similar to the one observed at Time4. G_S values for "Garrigues" showed a similar decrease but in general higher G_S values across time points, except at Time7 and Time9 (3.26 and 5.35 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively), in comparison with the others three genotypes. At Time10, this cultivar showed similar values of G_S than at Time3 and Time4 (Table 3).

Regarding P_N, a significant and constant decrease was observed for all the genotypes until Time6 (Figure S4). At this time point, the P_N values for three genotypes "GF 677", "Montclar" and "Real Fino" reached negative values not observed before. At Time7, a punctual significant increment of the values of this trait was observed for all genotypes, in comparison with previous Time6 but similar to Time5. At Time7 and at Time9, there were no significant differences between genotypes. Despite the lack of significant differences between genotypes at Time9, P_N values were higher for "GF 677" and "Real Fino" compared to the other two genotypes. Both genotypes were not significantly different from each other after Time4. For "Montclar" these values were lower, but again not significantly different from values at Time4. In the case of cultivar "Garrigues", at Time10 similar values (2.98 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) with Time4 and Time5 were observed (Table 3).

Regarding ψ_w, significant differences between genotypes started at Time3. In general, the three non-almond genotypes showed a constant increase in ψ_w across time points until Time7. After this time point, values of ψ_w were significantly lower from values at Time7 (Figure S5). In this sense, at Time9, values of ψ_w for "GF 677" (−3.00 MPa) and "Montclar" (−2.26 MPa) were not significantly different to Time5 (−3.19 and −2.19 MPa); in the case of "Real Fino", the final values (−2.51 MPa) were similar to Time3 and Time4 (−2.28 and −2.83 MPa, respectively). In the case of "Garrigues", a constant increase in ψ_w across time points until Time9 can be observed. Afterwards, values decreased to values found at Time5 (Table 3).

Regarding SPAD, the results showed that values fluctuated for each genotype across time points. In general, lower values were observed in the last time points of the experiment for all genotypes compared to *P. webbii* (25.09). As can be observed in Table 3, the observed differences were significant only at some time points (Figure S5).

Table 3. Mean values for the different traits studied. Means with at least one identical lowercase letter within a column are not significantly different from each other time points. Means with at least one identical uppercase letter within a row of one trait are not significantly different from each other between genotypes ($p < 0.05$). E: transpiration rate; G_S: stomatal conductance; P_N: net photosynthesis rate; ψ_w: leaf water potential; SPAD: chlorophyll content.

Trait	Genotype	Time1			Time2			Time3			Time4			Time5			Time6			Time7			Time8			Time9			Time10			Time11		
E	Garrigues	1.65	a	A	0.97	abc	A	0.73	bd	A	1.12	ab	A	0.52	d	A	0.59	cd	A	0.16	e	AB				0.24	e	B	0.92	ad		--		
	GF 677	0.70	a	BC	0.64	a	A	0.46	ab	AB	0.44	ab	B	0.29	bc	B	0.19	c	B	0.17	c	AB				0.42	ab	A	--			--		
	Montclar	0.56	a	C	0.56	a	A	0.27	bc	B	0.41	ab	B	0.21	cd	B	0.14	de	B	0.12	e	B			A	0.18	ce	B	--			--		
	Real Fino	1.16	a	AB	0.77	ab	A	0.32	cd	B	0.45	c	B	0.18	e	B	0.22	de	B	0.25	ce	A				0.43	bc	A	--			--		
G_S	Garrigues	49.33	a	A	35.52	ab	A	24.10	bc	A	38.87	ab	A	14.86	c	A	16.45	c	A	3.26	d	B				5.35	d	B	27.60	ac		--		
	GF 677	18.63	a	BC	20.25	a	AB	13.33	a	AB	12.45	ab	B	6.91	bc	B	3.89	c	B	3.64	c	AB				10.22	ab	A	--			--		
	Montclar	15.58	a	C	17.58	a	B	7.44	bc	B	12.00	ab	B	5.32	cd	BC	3.10	de	B	2.27	e	B				4.04	ce	B	--			--		
	Real Fino	33.75	a	AB	25.58	a	AB	9.14	bd	B	12.48	b	B	3.81	e	C	4.73	de	B	6.41	cde	A				10.83	bc	A	--			--		
P_N	Garrigues	8.58	a	A	4.95	b	AB	4.77	bc	A	4.46	bc	A	3.15	bc	A	0.93	d	A	1.05	d	AB				1.04	d	A	2.98	cd		--		
	GF 677	5.72	a	BC	5.00	a	AB	4.11	ab	AB	1.86	bc	B	1.96	c	AB	−2.66	d	B	1.69	c	AB				2.18	bc	A	--			--		
	Montclar	3.58	a	C	3.32	a	B	2.35	ab	B	0.85	b	B	1.16	b	AB	−1.77	c	B	1.06	b	B				1.20	b	A	--			--		
	Real Fino	7.18	a	AB	5.85	a	A	2.20	b	B	1.22	bc	B	1.11	bc	B	−0.72	c	AB	3.02	b	A				2.69	b	A	--			--		
ψ_w	Garrigues	−1.45	de	A	−1.04	e	A	−1.39	e	B	−1.16	e	B	−2.17	cd	B	−2.81	ac	B	−3.36	a	A	−2.96	ab	AB	−3.14	a	A	−2.28	bc	A	--		
	GF 677	−1.51	c	A	−1.26	c	A	−1.92	c	AB	−2.65	b	A	−3.19	ab	A	−2.84	ab	AB	−3.59	a	A	−3.33	ab	A	−3.00	ab	A	--			--		
	Montclar	−1.48	d	A	−1.12	d	A	−1.71	cd	AB	−2.72	b	A	−2.19	bc	B	−3.51	a	A	−3.71	a	A	−2.52	b	B	−2.26	bc	B	--			--		
	Real Fino	−1.47	d	A	−1.22	d	A	−2.28	c	A	−2.83	ac	A	−3.22	ab	A	−3.13	ab	AB	−3.30	a	A	−2.97	ac	AB	−2.51	bc	AB	--			--		
SPAD	Garrigues	33.82	a	B	30.89	a	C	31.46	a	B	33.88	a	BC	35.96	a	B	34.34	a	B	20.33	b	C	31.87	a	B	19.69	b	C	14.16	b	B	--		
	GF 677	42.45	ab	A	44.22	ab	A	41.85	ab	A	42.15	ab	A	46.20	a	A	38.95	bc	AB	43.76	ab	A	41.74	ac	A	31.63	c	AB	--			--		
	Montclar	43.41	ab	A	39.98	ab	AB	37.29	b	AB	43.89	a	A	43.09	ab	A	44.51	a	A	43.55	ab	A	37.21	ab	AB	36.77	ab	A	--			--		
	Real Fino	31.66	bc	B	32.57	bc	C	37.35	ab	AB	29.76	c	C	36.02	ab	B	40.85	a	AB	37.50	ab	AB	34.54	ac	AB	38.27	ac	A	--			--		
	P. webbii	40.27	a	A	36.68	ab	BC	34.19	ac	B	38.73	a	AB	32.14	bcd	B	35.70	ab	B	36.13	ab	B	35.64	ab	AB	25.61	cd	BC	31.91	ad	A	25.09	d	A

3.2.2. Treatment × Time

For E, G_S, and P_N significant differences between treatments started after Time4. In the case of ψ_w, significant differences appeared at Time3 (Table 4). In the case of the parameter associated with the maximal photochemical efficiency of photosystem II (Fv/Fm), a genotype specific response was observed, as will be commented below.

In addition, the comparison of the specific time points (7/9 vs. 9/10) via contrast, due to the different time points of recovery, showed a significant effect of the recovery treatment for the majority of the traits. Only in the case of SPAD and Fv/Fm were non-significant differences observed ($p = 0.4223$ and $p = 0.1603$, respectively) (Supplemental Table S1). After contrast comparison between Time1 and Time 9/10, significant differences were observed only for P_N and SPAD. The observed positive estimates (values from Time1 were larger than values from Time 9/10) can be associated with an incomplete recovery for these two traits. The rest of the traits showed non-significant differences between both time points (Supplemental Table S2).

3.2.3. Genotype × Treatment × Time

Only in the case of Fv/Fm, the three-way interactions were significant. Significant differences between genotype were observed across time points for this trait (Table 5, Figure S6). Two genotypes "GF 677" and *P. webbii* showed a punctual difference between treatments at Time4. However, in general, "Garrigues" and *P. webbii* showed significant differences between treatments at the last time points, Time9, Time10 and Time11. At Time5, "Real Fino" started to show significant differences between treatments. For "GF 677" and "Montclar", significant differences started at Time7 and Time9, respectively (Table 5).

For the control treatments, Fv/Fm values were constant and no significant differences were observed between genotypes at all time points. In the case of recovery treatment, the first significant differences between genotypes were observed at Time6. For Time8 and Time9, there were significant differences between genotypes. Significant lower values were observed for "Real Fino" at Time9 after recovery and drought (0.19 and 0.03, respectively) treatment (Table 6). At Time10, cultivar "Real Fino" showed lower significant values in comparison with "Garrigues" and *P. webbii* (Table 6) after recovery.

Table 4. Mean values for the different treatment used. Means with at least one identical lowercase letter within a column are not significantly different from each other time points. Means with at least one identical uppercase letter within a row of one trait are not significantly different from each other between treatments ($p < 0.05$). E: transpiration rate; G_S: stomatal conductance; P_N: net photosynthesis rate; ψ_w: leaf water potential.

		Time1			**Time2**			**Time3**			**Time4**			**Time5**			**Time6**			**Time7**			**Time8**			**Time9**			**Tme10**		
E	Control	0.91	a	A	0.81	a	A	0.46	b	A	0.87	a	A	0.69	ab	A	0.89	a	A	0.68	ab	A				0.80	ab	A	1.10	a	A
	Recovery	0.84	a	A	0.81	a	A	0.39	bc	A	0.48	ab	AB	0.20	de	B	0.12	ef	B	0.09	f	B				0.26	cd	B	0.62	a	B
	Drought	1.01	a	A	0.56	ab	A	0.38	b	A	0.39	b	B	0.15	c	B	0.13	c	B	0.07	d	B				0.13	c	C	--		
G_S	Control	26.40	ab	A	27.26	a	A	14.16	b	A	26.54	a	A	19.99	ab	A	25.72	ab	A	17.55	ab	A				22.50	ab	A	35.66	a	A
	Recovery	23.40	ab	A	27.87	a	A	11.81	bc	A	14.71	b	AB	4.50	d	B	2.44	e	B	1.79	e	B				6.09	cd	B	19.19	a	B
	Drought	29.67	a	A	17.86	ab	A	10.75	b	A	11.32	b	B	3.43	c	B	2.70	cd	B	1.52	d	B				2.50	cd	C	--		
P_N	Control	5.26	a	B	5.39	a	A	2.86	b	A	4.62	ab	A	3.91	ab	A	3.74	ab	A	5.29	a	A				4.85	a	A			
	Recovery	5.54	a	B	5.56	a	A	3.85	ab	A	1.82	bc	B	1.57	c	B	−3.25	d	B	0.14	c	B				0.72	c	B			
	Drought	7.99	a	A	3.39	b	B	3.38	b	A	−0.15	c	C	0.05	c	B	−3.65	d	B	−0.31	c	B				−0.23	c	B			
ψ_w	Control	−1.47	bc	A	−1.16	c	A	−1.25	bc	B	−1.57	bc	C	−1.52	bc	B	−1.92	b	B	−2.63	a	B	−1.63	bc	B	−1.54	bc	C			
	Recovery	−1.43	bc	A	−1.26	c	A	−2.15	bc	A	−2.36	bc	B	−3.13	bc	A	−3.79	b	A	−3.94	a	A	−3.53	bc	A	−2.65	bc	B			
	Drought	−1.53	cd	A	−1.06	d	A	−2.08	c	A	−3.10	b	A	−3.41	ab	A	−3.51	ab	A	−3.91	a	A	−3.67	ab	A	−3.99	a	A			

Table 5. Mean values for the Fv/Fm. Means with at least one identical lowercase letter within a column are not significantly different from each other time points. Means with at least one identical uppercase letter within a row of one genotype are not significantly different from each other between treatments ($p < 0.05$).

Genotype	Treatment	Time1			Time2			Time3			Time4			Time5			Time6			Time7			Time8			Time9			Time10			Time11		
Garrigues	Control	0.83	a	A	0.81	ab	A	0.81	ab	A	0.82	ab	A	0.82	ab	A	0.82	a	A	0.77	b	A	0.83	a	A	0.78	ab	A	0.82	ab	A	0.82	ab	A
	Recovery	0.82	b	A	0.82	ab	A	0.84	a	A	0.82	ab	A	0.82	ab	A	0.81	abc	A	0.69	abc	A	0.75	abc	A	0.36	c	AB	0.80	b	A	0.81	ab	A
	Drought	0.83	a	A	0.80	a	A	0.83	a	A	0.82	a	A	0.80	a	A	0.79	ab	A	0.67	ab	A	0.70	ab	A	0.23	bc	B	0.11	c	B	--		
GF 677	Control	0.81	a	A	0.80	a	A	0.80	a	A	0.82	a	A	0.79	a	A	0.80	a	A	0.77	a	A	0.82	a	A	0.78	a	A	--			--		
	Recovery	0.81	a	A	0.82	ab	A	0.84	b	A	0.79	a	A	0.81	ab	A	0.67	ab	A	0.26	c	B	0.67	abc	AB	0.68	abc	A	--			--		
	Drought	0.78	bc	A	0.80	ac	A	0.83	a	A	0.70	b	B	0.68	ab	A	0.61	ab	A	0.22	bcd	AB	0.03	d	B	0.37	bcd	A	--			--		
Montclar	Control	0.77	bc	A	0.81	ab	A	0.81	ab	A	0.81	ab	A	0.79	ab	A	0.81	a	A	0.74	c	A	0.80	ac	A	0.79	ac	A	--			--		
	Recovery	0.80	a	A	0.79	a	A	0.80	a	A	0.77	a	A	0.79	a	A	0.59	a	A	0.62	a	A	0.66	a	A	0.70	a	AB	--			--		
	Drought	0.81	a	A	0.80	a	A	0.84	a	A	0.79	a	A	0.75	a	A	0.66	ab	A	0.47	ab	A	0.45	ab	A	0.18	b	B	--			--		
Real Fino	Control	0.83	a	A	0.81	a	A	0.81	ab	A	0.79	ab	A	0.81	a	A	0.80	ab	A	0.75	b	A	0.82	a	A	0.83	ab	A	0.84	a	A	--		
	Recovery	0.82	a	A	0.81	ab	A	0.82	a	A	0.82	a	A	0.67	d	B	0.32	e	B	0.58	cde	A	0.29	de	B	0.19	e	B	0.76	bc	B	--		
	Drought	0.82	a	A	0.80	a	A	0.79	a	A	0.82	a	A	0.27	b	C	0.07	b	B	0.06	b	B	0.16	b	B	0.03	b	B	--			--		
P. webbii	Control	0.81	bc	A	0.80	bcd	A	0.86	a	A	0.77	ce	B	0.80	cd	A	0.81	bc	A	0.73	e	A	0.81	bcd	A	0.67	de	A	0.82	ac	A	0.86	ab	A
	Recovery	0.82	a	A	0.79	b	A	0.82	ab	A	0.83	a	A	0.79	ab	A	0.79	ab	A	0.76	ab	A	0.78	ab	A	0.83	ab	A	0.82	ab	A	0.65	ab	AB
	Drought	0.82	a	A	0.81	a	A	0.82	a	A	0.84	a	AB	0.79	a	A	0.81	a	A	0.76	ab	A	0.76	ab	A	0.53	ab	A	0.35	b	B	0.27	b	B

Table 6. Mean values for the Fv/Fm. Means with at least one identical lowercase letter within a column are not significantly different from each other between genotypes. Means with at least one identical uppercase letter within a row of each time point are not significantly different from each other between treatments ($p < 0.05$).

		Garrigues			**GF 677**			**Montclar**			**Real Fino**			***P. webbii***		
Time1	Control	0.83	a	A	0.81	a	A	0.77	b	A	0.83	a	A	0.81	a	A
	Recovery	0.82	ab	A	0.81	ab	A	0.80	b	A	0.82	ab	A	0.82	a	A
	Drought	0.83	a	A	0.78	a	A	0.81	a	A	0.82	a	A	0.82	a	A
Time2	Control	0.81	a	A	0.8	a	A	0.81	a	A	0.81	a	A	0.80	a	A
	Recovery	0.82	a	A	0.82	a	A	0.79	a	A	0.81	a	A	0.79	a	A
	Drought	0.80	a	A	0.80	a	A	0.80	a	A	0.8	a	A	0.81	a	A
Time3	Control	0.81	ab	A	0.80	b	A	0.81	ab	A	0.81	ab	A	0.86	a	A
	Recovery	0.84	a	A	0.84	ab	A	0.8	b	A	0.82	ab	A	0.82	ab	A
	Drought	0.83	a	A	0.83	ab	A	0.84	a	A	0.79	b	A	0.82	ab	A
Time4	Control	0.82	a	A	0.82	a	A	0.81	a	A	0.79	a	A	0.77	a	B
	Recovery	0.82	ab	A	0.79	bc	A	0.77	c	A	0.82	ab	A	0.83	a	A
	Drought	0.82	a	A	0.7	b	B	0.79	ab	A	0.82	a	A	0.84	a	AB
Time5	Control	0.82	a	A	0.79	a	A	0.79	a	A	0.81	a	A	0.80	a	A
	Recovery	0.82	a	A	0.81	a	A	0.79	a	A	0.67	b	B	0.79	a	A
	Drought	0.80	a	A	0.68	ab	A	0.75	a	A	0.27	b	C	0.79	a	A
Time6	Control	0.82	a	A	0.80	a	A	0.81	a	A	0.8	a	A	0.81	a	A
	Recovery	0.81	a	A	0.67	ab	A	0.59	ab	A	0.32	b	B	0.79	a	A
	Drought	0.79	a	A	0.61	a	A	0.66	a	A	0.07	b	B	0.81	a	A
Time7	Control	0.77	a	A	0.77	a	A	0.74	a	A	0.75	a	A	0.73	a	A
	Recovery	0.69	a	A	0.26	b	B	0.62	a	A	0.58	ab	A	0.76	a	A
	Drought	0.67	ab	A	0.22	ab	AB	0.47	ab	A	0.06	b	B	0.76	a	A
Time8	Control	0.83	a	A	0.82	a	A	0.8	a	A	0.82	a	A	0.81	a	A
	Recovery	0.75	a	A	0.67	a	AB	0.66	a	A	0.29	a	B	0.78	a	A
	Drought	0.70	a	A	0.03	b	B	0.45	ab	A	0.16	ab	B	0.76	a	A
Time9	Control	0.78	a	A	0.78	a	A	0.79	a	A	0.83	a	A	0.67	a	A
	Recovery	0.36	a	AB	0.68	a	A	0.70	a	AB	0.19	a	B	0.83	a	A
	Drought	0.23	ab	B	0.37	ab	A	0.18	ab	B	0.03	b	B	0.53	a	A
Time10	Control	0.82	a	A	--			--			0.84	a	A	0.82	a	A
	Recovery	0.80	a	A	--			--			0.76	b	B	0.82	a	A
	Drought	0.11	b	B	--			--			--			0.35	a	B
Time11	Control	0.82	a	A	--			--			--			0.86	a	A
	Recovery	0.81	a	A	--			--			--			0.65	a	AB
	Drought	--			--			--			--			0.26	a	B

4. Discussion

4.1. Modelling Temporal Physiological Response to Drought

The monitoring of a short-term response to drought in four *Prunus* species and one inter-specific hybrid was successfully carried out in greenhouse conditions for this study. Traditionally, monitoring experiments have been described as destructive and time-consuming approaches [41]. Clearly, it was mandatory for some traits in our design. However, sampling of a subset of one to three plants out of eight per time point tried to reduce the effect of repeated destructive measures (destructive effect) on the same plant. It was possible by the randomization on the selection of each plant to

measure and avoid the use of the same plant in two consecutive time points. A mixed model was used to consider the experimental design with repeated measures in space and time. The latter was modelled with a first-order autoregressive variance–covariance structure. It allowed us to estimate differences between treatment means at each time point and differences between means at different times for the same treatment providing a more efficient estimate of the effects. According to Piepho and Edmondson [26], in agronomic experiments a proper statistical design and effective control of the variability and the use of covariates are key elements to improving the precision and accuracy of the experiment. In this sense, traditionally agronomic experiments with repeat measures in *Prunus* have failed on these requirements [4,12,24,42], opting to ignore the covariance structure. Ignoring the covariance structure may result in erroneous and inefficient inference [43]. From a statistical standpoint, repeated measurements taken on the same subject, for instance a plot or plant, invalidate the basic assumption of independent error terms, which is at the basis of the traditional least squares method [44].

At Time8, the LI-COR machine used during all the experiments was not available to the authors and unfortunately, the machine effect cannot be separated from time point effects as the second machine is used for time point 8 only, hence, data from Time8 were dropped prior to analysis. According to Casler [45], biological experiments are a creative series of decisions that are meant to solve one or more problems. In this sense, the authors here modified their previous decision-making process because of this unexpected logistic problem.

In addition, some plant to plant variation could be expected in our experiment due to the fact that the majority of plants used from the same genotype were seedlings (not genetically identical). The use of rootstock seedlings is a normal practice in commercial orchards of fruit trees, as in the case of peach cultivation [46]. For the current experiment, the fitted correlation across observations from the same plant can be seen as the plant effect and thus can be separated from the error effects.

4.2. Physiological Response to Drought

In general, the constant and similarly high values for all traits studied here in the control plants during the experiment indicated that plants were adequately irrigated and indicating a normal development of each plant. In our study, all physiological traits associated with photosynthetic activity, leaf water status and chlorophyll content were negatively affected by drought. These negative effects of drought have been deeply reviewed by Chaves et al. [21]. The pattern of these negative effects seems to be trait-specific, with a clear difference between recovery and drought treatments after re-watering.

In general, all traits were impaired by drought but after the rehydration stage, the values of all traits were recovered almost until the initial level, as has been confirmed by our contrast results. Similar behavior was previously observed for values of leaf osmotic potential in "Garrigues" during recovery period by Torrecillas et al. [22], however, this behavior seems not be common in other almond cultivars, such as "Ramillete".

In the case of E and G_S, the recovery values showed an open "U" pattern (Figures S1 and S2); values for these two traits presented a slow continuous decrease, until a point where these values were constant, and with a final moderate increase. In the case of the net photosynthetic rate (P_N), two different patterns, genotype-specific, were observed; one was a "U" pattern for "Garrigues" and another one was a "V" pattern for the rest of genotypes evaluated (Figure S4). For ψ_w, a "U" pattern was observed for the recovery values (Figure S5). The values showed a steep increase after Time2, with constant values during several time points and after that, a steep decrease.

For SPAD, unclear patterns were observed (Figure S5). For Fv/Fm, an exponential decrease shape was observed after Time4 and a steep increase after re-watering for all genotypes. In the case of drought treatment, a steep increase was observed for ψ_w and an exponential decrease shape was observed for Fv/Fm (Figure S5); the rest of traits showed a constant decrease, more marked for G_S. The observed plummet in G_S at earlier time points, has been previously observed in young apple tree leaves [20].

This reduction pattern in stomatal conductance seems to have protective effects, allowing the plant to save water and to improve its efficient use [47].

At Time5, significant differences between treatments were clearly observed in comparison with earlier time points for E and G_S. Indeed, a positive correlation has been observed between these two parameters in apple trees under stress conditions [20]. In addition, this reduction pattern in stomatal conductance seems to be acting in our *Prunus* species as protective barrier to drought effects. The consequence of this stomatal limitation is followed by a decrease in photosynthesis [48]. In our experiment, this cascade response seems to be occurring, since values of P_N were significantly decreasing at Time6.

Clearly, the observed temporal response statistically confirmed that the first trait affected by drought treatment was ψ_w. After Time3, significant differences were observed between time points. The response started to be more significant at Time4 and Time5, which is correlated with previous traits in our experiment. In apples, correlations between ψ_w and the previous traits have been observed in drought conditions [20].

Graphical comparison between G_S and ψ_w has been used to classify species into isohydric or anisohydric species [49,50]. In our experiment, the majority of the species showed lower values of ψ_w after Time4, while minimum values of G_S were observed after this time point. This interpretation could be aligned with an anisohydric behavior. Anisohydric plants allow their leaf and stem water potential to decrease during drought by sustaining relatively high Gs (and thus C assimilation) [51,52], being more productive under optimal and mild-to-moderate drought conditions [53]. However, isohydric behaviors (where stomatal regulation maintains a fairly consistent minimum Ψ_w from day to day [54]) have been also observed in several cultivars of *Prunus persica* [55]. Clearly, it is not an easy task to classify a species in such categories, which seems to depend on the cultivar in question. Furthermore, for specific species this classification seems to be a continuum, rather than dichotomy between isohydric and anisohydric, as has been highlighted by [56]. Additional studies should be carried out to increase the knowledge about the specific behavior of this important *Prunus* species.

4.3. Genotype Response to Drought

The different morphology between genotypes, such as the small leaves showed by *P. webbii*, prevented the collection of all data for this genotype. In this study, at Time2, drought effects started to appear in all genotypes, except in "Garrigues" and *P. webbii*. For these two genotypes, the negative effects of drought seemed to be delayed in comparison with the rest. Two possible explanations could be that the transpiring leaf mass is lower in the two genotypes, however, leaf morphology is clearly different between both, and a great drop of leaves in both, which was not observed. These results are in contrast with previous results obtained by Alarcon et al. [24], where "GF 677" seems to be more drought tolerant than "Garrigues" after two different cycles of drought.

P. webbii seems to be the most resistant genotype studied here. Indeed, wild-relative species of almond seems to be more resistant to drought than other fruit tree species [25]. This wild-relative species has been utilized for straightforward rootstock development or to create interspecific hybrids, for introgression of resistance to abiotic and biotic stresses [25,32,57].

One important reason for this resistance could be the shoot morphology of this species. In other *Prunus* species, shoot characteristics were more closely associated with drought adaptation than root characteristics [8]. The mechanisms of tolerance to drought seem to be genotype specific. The resistant mechanism of "Garrigues" and other almond cultivars have been historically studied by several authors [22,58]. In the case of "Garrigues", the developed osmotic adjustment during the greater part of the stress period, seem to help this cultivar to survive in drought conditions [22].

In addition, some degree of osmotic adjustment together with avoidance mechanisms has been observed in apricot plants submitted to short-term water stress periods [59]. In our experiment, plants from cultivar "Real Fino" suffered greater defoliation in comparison with other cultivars (data not shown). Partial defoliations have been already observed in young apricot plants under severe water

stress conditions ($\psi_w < -1.75$ MPa), which seem to be a tolerance mechanism [60]. After re-irrigation, a faster recovery was observed for this cultivar, which is evident in the analysis, allowing us to evaluate plant recovery for this cultivar longer than others. In addition, the values of G_S were the highest at Time9. One explanation is that the remaining leaves were young leaves, which have higher values of G_S than mature leaves [60].

Clearly, this short-term study in greenhouse has tried to reduce the enormous complexity associated with this kind of study [61]. In this sense, to deal with the stress level, plant morphology, limitations of the methodologies used (sensitivity and repeatability) or the different adaptive capacity of the responses (genetic, physiological, anatomical and metabolic) in a single experiment requires well-replicated measurements [62]. The obtained results with a correct statistical procedure suggested that effects of a severe dehydration were reached. It is clear that some parameters seem to be more affected than others and can be a more valuable technique for these particular conditions than others, such as in the case of P_N and ψ_w, whilst contrarily SPAD seems not be useful under these conditions. Within this scenario, chlorophyll fluorescence seems to be altered at the end of the experiment in the majority of the cultivars. This study has tried to bring a more classical view of plant physiology in *Prunus* and will help to develop a stronger partnership between practical and fundamental research in the future, something that needs to be re-established in an intimate and meaningful way [61].

5. Conclusions

The temporal drought response in several *Prunus* species was successfully monitored. The results can contribute to a more complete understanding of drought response in *Prunus* and to classify these species according to their response. In general, in a short time period, physiological parameters, such as P_N and ψ_w, are good and quick parameters to detect water deficit in *Prunus* species; however, both parameters cannot be measured in species with small leaves, such as *P. webbii*. Contrarily, SPAD and Fv/Fm seem to be unaffected or to have a late response in the same time period. Clearly, almond species, such as *P. webbii*, with a special morphology and historical tolerance, and "Garrigues" almond, showed the best drought tolerance across time points in comparison with other *Prunus* species such as apricot and peach. The future implementation of omics approaches across time points will help the dissection of this response in *Prunus* species, determining when genes express or switch off in *Prunus* response to drought.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1383/s1, Figure S1: Hourly temperature and relative humidity recorded during June and July in greenhouse. Black line represents mean temperature and relative humidity by day. Two-dash line represents upper temperature and relative humidity by day. Dotted line represents lower temperature and relative humidity by day. Green boxplots show the distributions of meteorological data (9:00 a.m. to 11:00 a.m.) of the respective variables on the measuring days. Figure S2: Lsmeans for E across time point and treatments, for each genotype. Figure S3: Lsmeans for G_S across time point and treatments, for each genotype. Figure S4: Lsmeans for P_N across time point and treatments, for each genotype. Figure S5: Lsmeans for ψ_w and SPAD across time point and treatments, for each genotype. Figure S6: Lsmeans for Fv/Fm across time point and treatments, for each genotype. Table S1: Specific contrasts between Time7 vs Time9 ('GF 677', 'Real Fino', 'Montclar') and Time9 vs. Time10 ('Garrigues', *Prunus webbii*), Table S2. Contrast between Time1 and Time 9/10 for all genotypes used.

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