

## Article

# Delayed Inhibition of Photosynthetic Performance—Three Linden Species in an Urban Environment

Monika Czaja <sup>1,\*</sup>, Anna Kołton <sup>2</sup> and Piotr Muras <sup>1</sup>

<sup>1</sup> Department of Ornamental Plants and Garden Art, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 31-425 Kraków, Poland; piotr.muras@urk.edu.pl

<sup>2</sup> Department of Botany, Physiology and Plant Protection, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, 31-425 Kraków, Poland; anna.kolton@urk.edu.pl

\* Correspondence: monika.czaja@urk.edu.pl or mon.czaja@gmail.com

**Abstract:** The presented study concerns the delayed response of photosynthetic performance to summer drought. Increasing drought events are thought to be one of the most detrimental and expensive natural disasters. For that reason, we studied the condition of urban trees, which are especially susceptible to water deficit. Three species of linden trees (*T. tomentosa*, *T. × europaea* and *T. cordata*) growing in the city area of Krakow, Poland, were investigated for two consecutive years to compare their response to a precipitation deficit. For the first time, the physiological traits of linden urban trees were compared in years with and without a natural summer drought. For evaluation of the photosynthetic performance, we used measurements of the chlorophyll *a* fluorescence concerning the OJIP transients and derived parameters. The contents of photosynthetic pigments, such as chlorophylls and carotenoids, were analysed, and stress-sensible parameter ratios, such as chl *a/b* and chl/carot, were calculated. The most common method of research conduction assumes examination close to the presence of a stressor. We chose an innovative method of prolonged investigation after relief from the stress conditions. While there was no evident reaction of PSII to meteorological drought during the stress occurrence, all of the investigated species of linden revealed delayed reaction of PSII to precipitation deficit in summer. Changes in PSII were connected with the decrease of active reaction centres (RCs), which was evident in the increase of the ABS/RC, DI<sub>0</sub>/RC and TR<sub>0</sub>/RC, and the decrease of ET<sub>0</sub>/RC. *T. × europaea* demonstrated a more intense decrease of certain parameters during late senescence compared with other species. Despite the close relation of *T. cordata* and *T. × europaea*, species showed different physiological traits during senescence after a summer drought. Our results underline the need for monitoring tree conditions not only during stress but also in the wider perspective.

**Keywords:** *Tilia* sp.; urban stress; urban trees; drought; chlorophyll *a* fluorescence; OJIP



**Citation:** Czaja, M.; Kołton, A.; Muras, P. Delayed Inhibition of Photosynthetic Performance—Three Linden Species in an Urban Environment. *Forests* **2021**, *12*, 761. <https://doi.org/10.3390/f12060761>

Academic Editors: Piotr Robakowski and Emilia Pers-Kamczyc

Received: 22 April 2021

Accepted: 6 June 2021

Published: 9 June 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Drought has a broad range of definitions, which includes its drivers, sequence, and consequences of such events. ‘Meteorological drought’ is connected with the lack of precipitation, ‘hydrological drought’ with poor soil moisture, ‘agroecological drought’ concerns lower crop productivity and ‘socioeconomic drought’ is an effect of combined hydrological and agroecological drought [1,2]. Due to its complexity and lack of a unique definition, it is difficult to establish the exact beginning of a drought event and, thus, its duration [3]. This matter is highly important in many fields of study as increasing drought events are considered to be the most detrimental and expensive of natural disasters [4].

Due to climate change and global warming, droughts are predicted to be a more frequent and severe phenomenon connected with both widespread warming and precipitation decline [5]. The severity and frequency of drought are, therefore, linked with progressive climate change and also with these specific features of a particular environment [6]. One of the specific environments where drought occurs with high intensity is urban areas. The

urbanisation of natural and rural environments is a phenomenon that increases every year. It is estimated that 66% of the whole population will live in cities by the year 2050, while in 1950, it was only 30% [7].

Such transformations influence the soil properties, rainwater availability, air quality and the inflow of solar energy. This, in turn, changes the tree growth environments [8]. Dense buildings as well as intense and heavy transport influence the soil structure and functioning, leading to its physical degradation. In consequence, urban soils are compacted, with diminished porosity, which reduces water and air accumulation [9,10]. The degradation of the soil structure leads to a disturbed water uptake and mechanical resistance for root growth but also induces surface runoff (which limits the rainwater availability in urban areas) [10,11].

The aforementioned modifications increase the risk of drought events in the urban environment. In these specific conditions, the growth and physiology of trees are disturbed [12,13]. Therefore, long-term experiments concerning the reaction of urban trees to drought are of high importance. Knowledge in this field is crucial for designing valuable greenery in urban areas [14].

Under drought, plants avoid stress effects by increasing their root water uptake and stomatal closure and adjusting their osmotic processes. The production of stress-protectant metabolites, hormones and anti-oxidant systems is also activated if the water shortage lasts for a long time [13]. Photosynthesis, as a complex process, can be disturbed by stress at different levels. To avoid leaf dehydration, the first plant reaction to drought stress is stomatal closure. The uptake of CO<sub>2</sub> essential for the carbon reactions is then mechanically limited, and therefore a photosynthesis decline is observed [15].

A deficit of water disturbs the course of the light reactions as a limiting factor; moreover, under stressful conditions, such as drought, the oxygen-evolving complex (OEC) is inhibited, and electron transport from water splitting is blocked [16]. Meteorological summer drought is often connected with high light doses and temperatures. Under the circumstances of water deficit and light quanta excess, the photosynthetic redox reaction chain is disturbed. As a consequence, there is an overproduction of reactive oxygen species (ROS), which leads to photooxidation [17]. Protection of the photosynthetic apparatus from the excess of energy includes its dissipation to avoid critical damage.

Excessive light quanta can be dissipated as heat, non-photochemical quenching or chlorophyll *a* fluorescence (ChlF). Measurements of ChlF inform about the functioning of both the acceptor and donor side of Photosystem II [18]. Photosystems I and II (PSI and PSII) are responsible for light absorption and, therefore, the initiation of redox chain reactions. Those structures and their functioning measurements are, therefore, good indicators of the photosynthetic apparatus performance.

PSI is known to be more sensitive to drought, while PSII disruption is observed under severe stress [16,19]. The chlorophyll fluorescence curve, called OJIP transient, as well as OJIP-test calculated parameters are considered to be good stress indicators. Different parameters can illustrate stress-induced disturbances at specific stages of the electron transport chain and, therefore, show a broad picture of this phenomenon [18].

The growth and development as well as photosynthetic activity of the leaves change during the growing season. Changes in the photosynthetic activity of white oak (*Quercus alba* L.) and red maple (*Acer rubrum* L.) during the growing season were assessed [20]. An increase in the photosynthetic activity during leaf unfolding in spring and a decrease in the photosynthetic activity during aging was shown. These changes occurred quickly within only 2 to 3 weeks. Changes in the photosynthetic activity were associated with fluctuations in assimilatory pigments (chlorophylls and carotenoids). Similar seasonal dynamics of the maximum quantum yield of PSII or the electron transport rate was also observed.

Comparable observations of the photosynthetic activity and the content of assimilation pigments during the growing season were recorded for a mixed hardwood stand with red oak (*Quercus rubra*), red maple (*Acer rubrum* L.) and American beech (*Fagus grandifolia* Ehrh.) [21]. Moreover, the authors showed that the leaves reached their final size (width

and length) around day 160 of the year (June), and they are fully mature from that moment. In our work, the leaves collected in June were treated as fully developed.

From mid-September (around the 260th day of the year), changes related to aging were noticed. While the main leaf fall was observed from mid to late October, single leaves fell even at the beginning of November [21]. At this time, the disappearance of chlorophyll pigments and carotenoids were observed, with the former decomposing faster. For this reason, the car/chl ratio increased towards the end of the season. With reference to this information, in the presented research, the leaves collected in September were described as early senescence and those collected in October as late senescence.

In addition, changes in the chlorophyll *a* fluorescence were found to be noticeable during the growing season [22]. Five oak species showed an increase in the maximum quantum yield of the primary photochemistry of PSII and the performance index on an absorption basis at the beginning of the growing season, followed by stabilisation of these parameters and their reduction in October. The shape of the fluorescence transient curve also changed both at the beginning of leaf development and during senescence.

Somewhat differently, in the case of *Fagus sylvatica* L., the authors did not record changes in the maximum quantum yield of PSII (Fv/Fm) during the growing season [23]. The influence of artificial drought on this parameter was also not demonstrated. Interestingly, withholding irrigation decreased the PI<sub>total</sub> parameter, but after a few weeks of the duration of drought and more than 3 weeks later, the reduction in the intensity of photosynthesis started; the drought started in June and a decrease in the PI parameter was recorded in mid-August.

The information presented shows that the photosynthetic activity and chlorophyll *a* fluorescence of the leaves change during the growing season. Therefore, in the presented work, we demonstrate the dynamics of changes in the year with average precipitation and in the year with precipitation deficit.

The presented study concerns urban trees, which, as quoted, are particularly exposed to drought stress. It is known that linden trees show an anisohydric reaction to drought. Such behaviour maintains relatively high transpiration rates and carbon assimilation under water deficit in contrast to isohydric reactions. Such adaptation results in delayed growth reduction, evident in the year following drought events [24]. Such a reaction would not limit the carbon reactions themselves, while the CO<sub>2</sub> would be assimilated through stomata.

However, due to observed precipitation limitation (see results) and high temperatures, we assumed the disturbance of photosynthesis light reactions. As presented by Gillner et al. [25] anisohydric species, such as *Corylus* or *Tilia*, can maintain high efficiency of photosystem II during drought. While there was no evident reaction of PSII to meteorological drought in summer, we decided to evaluate if there were any further consequences of a summer drought event for PSII functioning. The study aimed to investigate how a precipitation deficit in summer affects the photosynthetic performance at two stages of senescence. To meet this objective, we chose an innovative method of prolonged investigation after the relief of the stress conditions. We formulated two main hypotheses:

1. The PSII reaction can be delayed and revealed few weeks after the stress factor appearance.
2. The species differ in the course of aging during normal and drought years.

## 2. Materials and Methods

### 2.1. Study Sites and Objects

The study was performed in Krakow (19°57' E, 50°03' N), the second-largest city in Poland, where intensive urbanisation has occurred in the last 50 years. Due to rapid city development, an increase in the impermeable surface coverage, which contributes to surface runoff and the worsening of water management, has been observed [26]. The experiment was conducted for three years (2013–2015) in ten different locations (two consecutive years for every location). The investigated trees were located within the urban area of Krakow. During the years of the experiment, the examined trees were not irrigated,

and the Municipal Management of Urban Green Areas in Krakow did not conduct any irrigation program.

The soil  $\text{pH}_{\text{KCl}}$  measured for every location ranged from 5.5 to 7.3 in the spring and from 4.9 to 7.2 in the autumn (data not published). The electrical conductivity of the soil in the investigated location ranged from 160 to 483  $\mu\text{S}\cdot\text{cm}^{-1}$  in the spring and from 82 to 402  $\mu\text{S}\cdot\text{cm}^{-1}$  in the autumn of 2014 (data not published). Taking into account the results of the soil analysis, we assumed that there was no excessive salinity or excessively high pH, which could be separate stress factors. However, climatic and growth environment features in urban areas are highly variable.

For that reason, we chose strict criteria for the selection of the studied trees. To ensure the credibility of the obtained results, we assumed three selection criteria. All of the examined trees were from (i) one genus—to avoid inter-generic differences; (ii) a sufficient number of trees in one location (3–23 individuals, planted at the same time)—to ensure a representative research sample; (iii) with a similar age of individuals—to avoid differences between young and mature trees (all selected plants were up to 15 years, planted on study sites no less than 2 years before the study).

The investigated trees were located along roads or in the near vicinity, planted as a strip with trees of the same age and species. Our research included 93 trees from the *Tilia* genus: 16 individuals of *T. cordata* (Mill.), 36 of *T. tomentosa* (Moench.) and 41 of *T. × europaea* (for more details see Table S1 and Figure S1 from Supplementary Materials). All of the investigated species have similar distributions. *T. cordata* and *T. tomentosa* have European origins; however *T. tomentosa* originates from drier regions. *T. × europaea* is a hybrid species of *T. cordata* and *T. platyphyllos*, which are both native in Poland.

The morphology of the species leaves is different, concerning mostly the size, thickness and hair coverage. Among the investigated species, *T. cordata* has the smallest leaf blades, and they are also less hairy. *T. tomentosa* has the largest leaf blades with dense hairs below [27]. Due to those morphological features, and its origin in relatively dry regions, *T. tomentosa* is thought to be better suited for urban areas compared with other species. However, while *T. cordata* is widely planted in European cities its suitability, as well as its hybrids is more often perceived [28,29].

## 2.2. Climatic Data

Detailed climatic data (temperature) were collected from the climatic computer located outside of the University of Agriculture's glasshouse. Precipitation measurements for Krakow and data comparing each month to precipitation norm based on measurements from the 1971–2000 period were gathered from Bulletins of the Hydrological and Meteorological Service [30–32]. The temperature and precipitation are presented in a Gaussen–Walter diagram.

This kind of climatic data presentation allows the estimation of a precipitation deficit or its excess, according to the specific presentation of the temperature and precipitation record. In the original presentation of this diagram, 10 °C corresponds to 20 mm of precipitation. However, as reported by Treder et al. [33] for the climatic conditions of Poland, 40 mm (or even 45 mm) would be more appropriate to counterbalance the temperature of 10 °C. For that reason, we applied the modifications proposed by Łukasiewicz [34] with a ratio of 1 °C to 4 mm between the mean temperature of a particular month and the amount of precipitation.

## 2.3. Leaves Collection and Analysis

Plant material was collected in the morning hours, from the outside part of the crown, and the eastern side of the tree. Such procedures eliminated the differences connected with non-uniform illumination of different parts of the crown [35]. Leaf samples were gathered from five branches parts from each tree (about 25–50 leaves from one individual). Leaves were carefully protected from drying and mechanical damage and immediately transported to the laboratory for further analysis.



All parameters were compared in three terms of collection. Leaves collected in June were described as “full development” (according to Lichtentaler and Babani [36]); leaves collected in September were “early senescence”; and, in October, the leaves were “late senescence”. Only leaves visually recognised as healthy (without chlorosis, necrosis or foraging pests), were taken for further investigation. In early senescence, only green leaves were chosen for analysis, where possible. In the late senescence stage, leaves have visible symptoms of natural senescence, such as discoloration and marginal necrosis.

The parameters of a given species in a selected period of a dry or normal year were considered as a treatment (three species  $\times$  three developmental phases  $\times$  2 years with different precipitation).

### 2.3.1. Chlorophyll *a* Fluorescence

For every location of tree growth, fluorescence measurements were made with the light intensity of  $3000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , for 10 replications, in each of the studied months. Prior to measurement, the leaves were dark-adapted for 30 min. All fluorescence measurements were obtained using Handy PEA (Plant Efficiency Analyser), Hansatech (UK). We analysed the OJIP fluorescence transient, called the JIP-test, where O is the fluorescence origin (first measured minimal level), J and I are intermediates and P is the maximum level of the fluorescence curve. We also calculated some of the JIP-test parameters, which are connected with a particular phase of electron transport.

All calculations were made according to formulas from Stirbet and Govindjee [37] and Stirbet et al. [38]:  $F_0$  (the minimum chlorophyll *a* fluorescence),  $F_m$  (the maximum chlorophyll *a* fluorescence after dark adaptation),  $F_v/F_0$  (the ratio of the photochemical and non-photochemical processes in photosystem II (PSII) (the maximum efficiency of the photochemical processes of PSII),  $F_v/F_m$  (the maximum quantum yield of PSII photochemistry),  $T_{fm}$  (the time to reach the maximum chlorophyll fluorescence), Area (the area above the OJIP transient and  $F_m$  line), PI total (the performance index for energy conservation from absorption to the reduction of PSI acceptors), RC/ABS (the PSII reaction centre per absorbed photon flux) ABS/RC (the absorbed photon flux per PSII reaction centre (RC) or apparent antenna size of an active PSII),  $TR_0/RC$  (the maximum trapped exciton flux per active PSII),  $ET_0/RC$  (the flux of electrons transferred from the primary electron acceptor (QA) per active PSII reaction centre) and  $DI_0/RC$  (the flux of energy dissipated in processes other than trapping per active PSII reaction centre). We evaluated the parameters concerning specific energy fluxes expressed per active PSII reactive centre. All of the measurements concerning the OJIP-test were taken for 10 replications for the mixed leaf sample from one location (for more details see Table S1 from Supplementary Materials).

### 2.3.2. Chlorophylls and Carotenoids Content

Assimilation pigment contents were analysed using chemical procedures. Pigment extraction proceeded with 80% acetone, according to procedures described by Wellburn [39]. Analyses were made in four replications for every location of tree growth in each of the studied months (for more details see Table S2 from Supplementary Materials). The measurements were taken on a Hitachi U-2900 (Tokyo, Japan) spectrometer with 663, 646, and 470 nm wavelengths. In addition to the chlorophyll sum, we also calculated the parameters of the chlorophyll *a* to *b* ratio (chl *a/b*) and chlorophyll sum to carotenoids (chl/carot), which are considered drought-stress-sensitive indicators [40,41].

### 2.4. Statistical Analysis

All statistical analyses were performed using Statistica v13.1, StatSoft software (TIBCO Software Inc., 2017, Palo Alto, CA, USA) from Statistica (data analysis software system, version 13.). The statistical differences were performed with the Kruskal–Wallis method and Dunn test. The test was performed separately to distinguish differences between

(I) early senescence and full development and (II) late senescence and full development. Significant differences were established with  $p < 0.05$ .

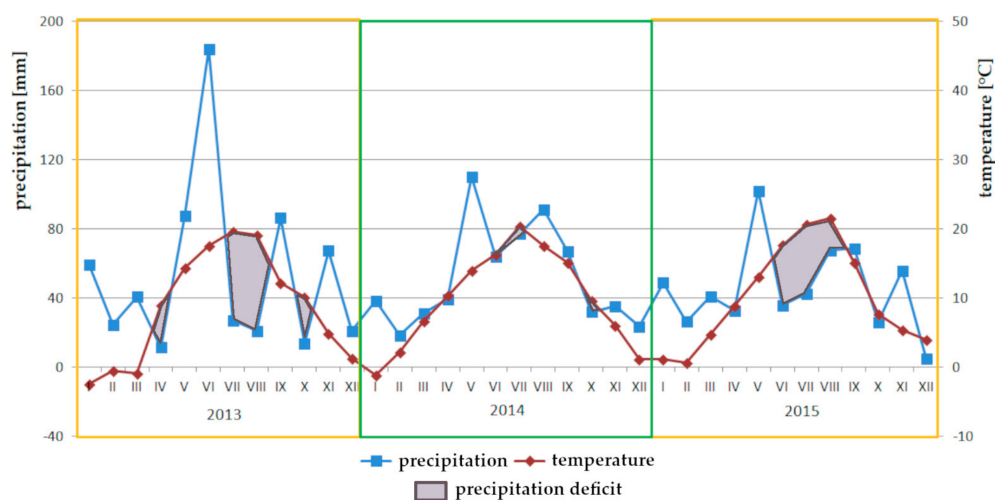
The method of cluster analysis was also used to group the examined treatments based on the measured features. Prior to analysis, all data were standardised. The Ward's method and Euclidean distance were used to group treatments. The dendrogram was created, and a line marked the cut-off point that allowed for the identification of the clusters. The treatments with similar characteristics were classified into one cluster.

### 3. Results

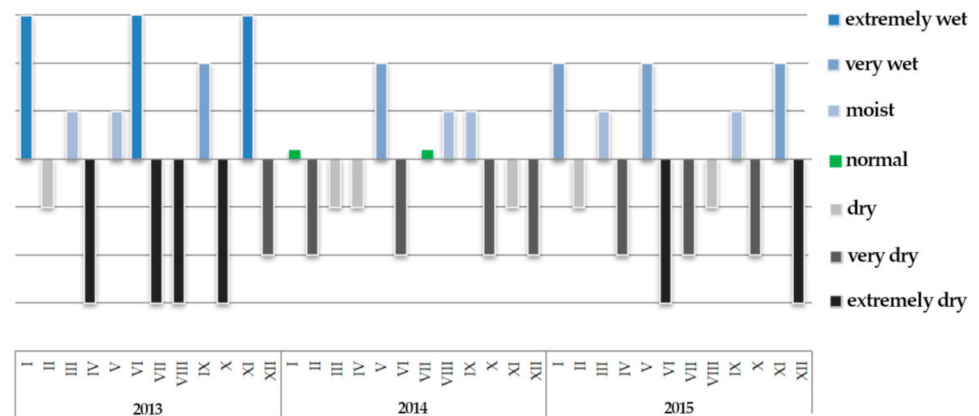
#### 3.1. Climatic Conditions

While the temperature course in studied years is similar, the precipitation distribution in the same month differs greatly between years (Figure 1). In 2013, precipitation deficits occurred in April, July and August and later in October. In 2014, from June to July, there was only a minor precipitation deficit according to the Gaussem–Walter diagram, which we recognised as negligible. In the third year of study, the months from June to August were characterised by insufficient precipitation. A similar classification of consecutive months in the years of study was observed in relation to the precipitation norm for Poland based on measurements from the 1971–2000 period (Figure 2).

The years 2013 and 2015 can be characterised as years with summer precipitation deficits, and 2014 can be classified as normal according to Figures 1 and 2. For that reason, the measured leaf parameters are presented in groups for each species of linden trees, comparing the results from years with a precipitation deficit (D) and with precipitation in the summer described as normal (N).



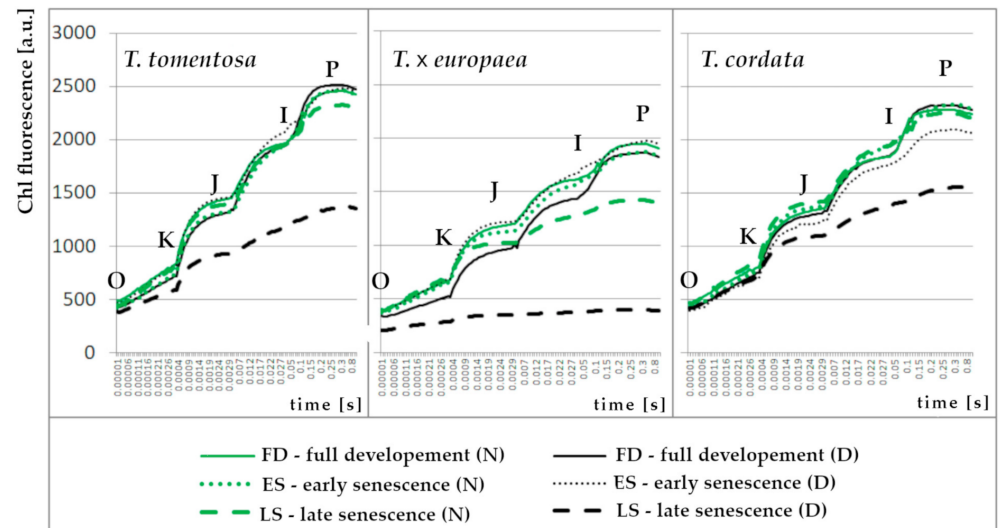
**Figure 1.** A Gaussem–Walter climatic diagram with Łukasiewicz [34] modification presenting conditions during three consecutive years of study. The values of precipitation and temperature are presented as the monthly sum and mean, respectively. The parts of the graph where the precipitation line is below the temperature are considered as precipitation deficit events.



**Figure 2.** Classification of consecutive months in the years of study according to the monthly precipitation sum and precipitation norm from 1971 to 2000. Based on data from Bulletins of the Hydrological and Meteorological Service [30–32].

### 3.2. OJIP-Transient

All of the investigated species showed a lower fluorescence transient during late senescence in the years with precipitation deficit in comparison to other terms of analysis (Figure 3). Meanwhile, for *T. cordata*, while the O-K phases were similar to other terms of analysis, the J-I and I-P phases were lower in all of the investigated species. In *T. cordata*, the early senescence (D) fluorescence transient (especially the J-I and I-P phases) were lower than in other terms.



**Figure 3.** OJIP chlorophyll *a* fluorescence transient (O–0.00005 s, K–0.003 s, J–0.002 s, I–0.03 s, and P = Fm) for investigated *Tilia* species measured for fully developed leaves, early and late senescence in seasons with a deficit of precipitation (D) and normal (N) summers.

For *T. × europaea*, the late senescence (N) curve was also lower than other terms in a normal year, which was not observed for other species. In addition, the chlorophyll *a* fluorescence transient for full development (D) in *T. × europaea* was lower than in a normal year. In general, the F<sub>p</sub> (F<sub>m</sub>) was lower for *T. × europaea*, reaching a maximum of about 2000, while for *T. cordata* and *T. tomentosa*, it was about 2300 and 2500, respectively. What is interesting for *T. tomentosa* is that, apart from late senescence (D), all OJIP curves had a similar course.

### 3.3. OJIP Test and Photosynthetic Pigment Parameters during the Normal Year

As presented for all investigated species, during the year without a precipitation deficit, the parameters in early senescence did not differ drastically from those in full development.  $F_0$  and the chl *a/b* ratio were significantly lower in all species during early senescence, and  $DI_0/RC$  was lower for both *T. tomentosa* and *T. cordata*. The  $Fv/F_0$ ,  $Fv/Fm$  and chl/carot ratios were significantly higher during early senescence than in the full development stage for *T. cordata* and *T. tomentosa*. In the late senescence Area,  $PI_{total}$  and chl *a/b* were lower than in the full development stage for all species, and  $TR_0/RC$  and  $ABS/RC$  were higher for *T. cordata* and *T. × europaea*. An increase in  $DI_0/RC$  at the late senescence stage was observed only in *T. × europaea*. The chlorophyll content did not differ significantly in the investigated terms for all of the investigated species.

### 3.4. OJIP Test and Photosynthetic Pigment Parameters during the Year with a Precipitation Deficit

The differences between parameters in the year with a precipitation deficit were much more evident for both stages of senescence.  $PI_{total}$  was the only parameter that decreased in early senescence for all of the investigated species. The  $Fv/F_0$ ,  $Fv/Fm$ ,  $RC/ABS$ ,  $ET_0/RC$  and chl/carot ratios decreased in early senescence in comparison to full development for *T. × europaea* and *T. tomentosa*. For all of the investigated species,  $Tfm$  was higher during early senescence than in full development, and  $F_0$ ,  $ABS/RC$ ,  $TR_0/RC$  and  $DI_0/RC$  were higher in *T. × europaea* and *T. tomentosa*.

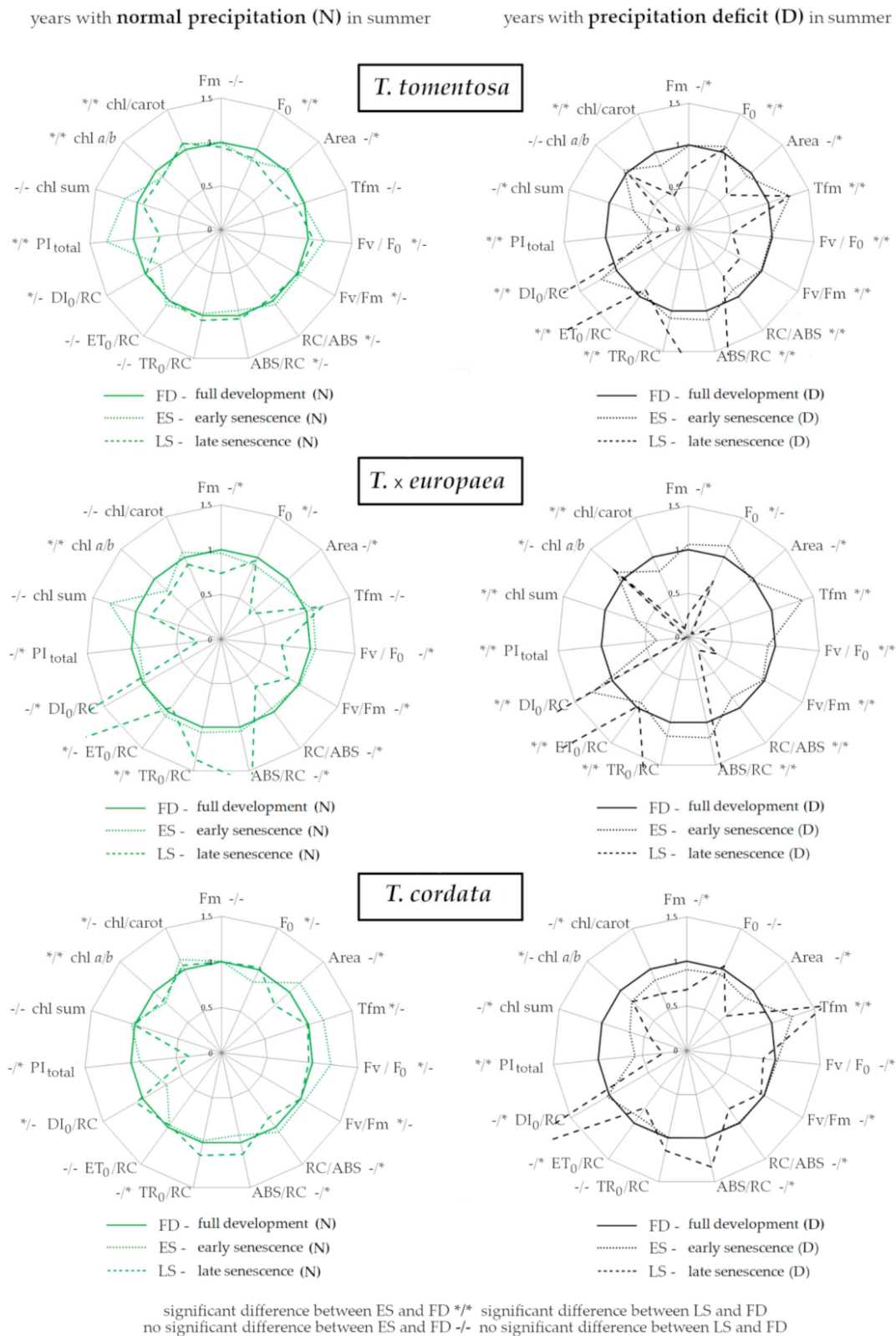
In late senescence, the  $Fm$ , Area,  $Fv/F_0$ ,  $Fv/Fm$ ,  $RC/ABS$ ,  $ET_0/RC$ ,  $PI_{total}$ , chl sum and chl/carot ratios decreased in all taxons. The  $DI_0/RC$  increased greatly in late senescence in comparison to full development for all of the investigated taxons. The  $Tfm$  parameter was higher in late senescence for *T. cordata* and *T. tomentosa* but lower in *T. × europaea* in comparison to full development (Figure 4).

### 3.5. Cluster Analysis

A cluster analysis made on the basis of all tested parameters separated as 18 treatments (three species × three developmental phases with two categories of precipitation deficit N and D) into four different clusters with cut-off point of a Euclidean distance 7.5 (Figure 5). Cluster 1 included all species analysed during full development and early senescence from category D (precipitation deficit). The second cluster contained all combinations with category N except *T. × europaea*\_LS\_N. This treatment was classified into cluster 3 along with *T. cordata*\_LS\_D and *T. tomentosa*\_LS\_D. One treatment was assigned to the last fourth cluster (*T. × europaea*\_LS\_D).

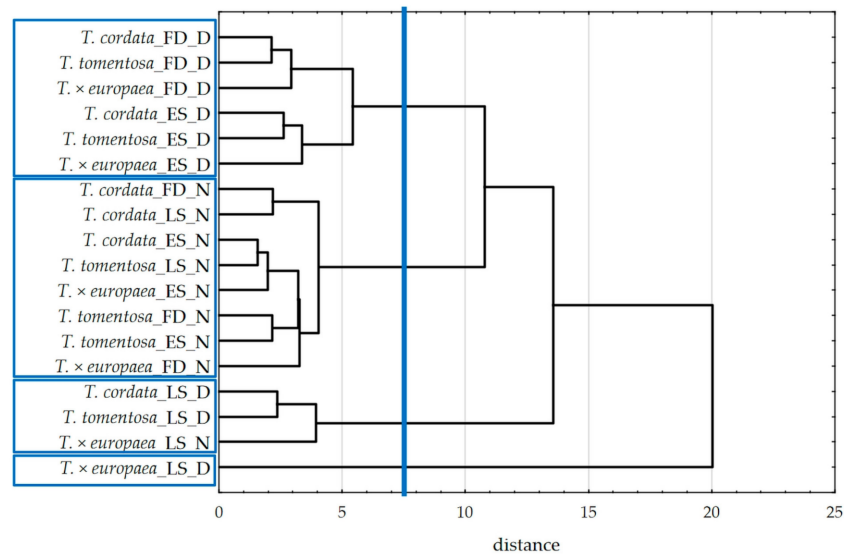
Figure 6 was prepared based on the Euclidean distance of a given development phase from full development for each species and precipitation deficit category, separately. It can be noticed that *T. × europaea* analysed during late senescence was classified separately than other species late senescence leaves from the specific year (D or N, respectively). Late senescence leaves during the year without precipitation deficit of *T. tomentosa* and *T. cordata* were included in cluster 2 and the case of *T. × europaea* in cluster 3. In addition, during the year with precipitation deficit, the late senescence leaves of *T. tomentosa* and *T. cordata* were separated in a different cluster than *T. × europaea* (clusters 3 and 4, respectively).

In addition, in Figure 6, the distance from the full development phase to early senescence and late senescence during N year is small, except for *T. × europaea*\_LS\_N. The distance between the full development phase and ES of plants from the year with a rainfall deficit is slightly higher. Much larger is the distance from full development to phases/treatments included in cluster 3, which emphasizes their difference compared to the full development (changes during senescence). However, the largest changes from FD to LS are observed in the case of *T. × europaea* during year with a precipitation deficit, where the greatest distance was recorded.

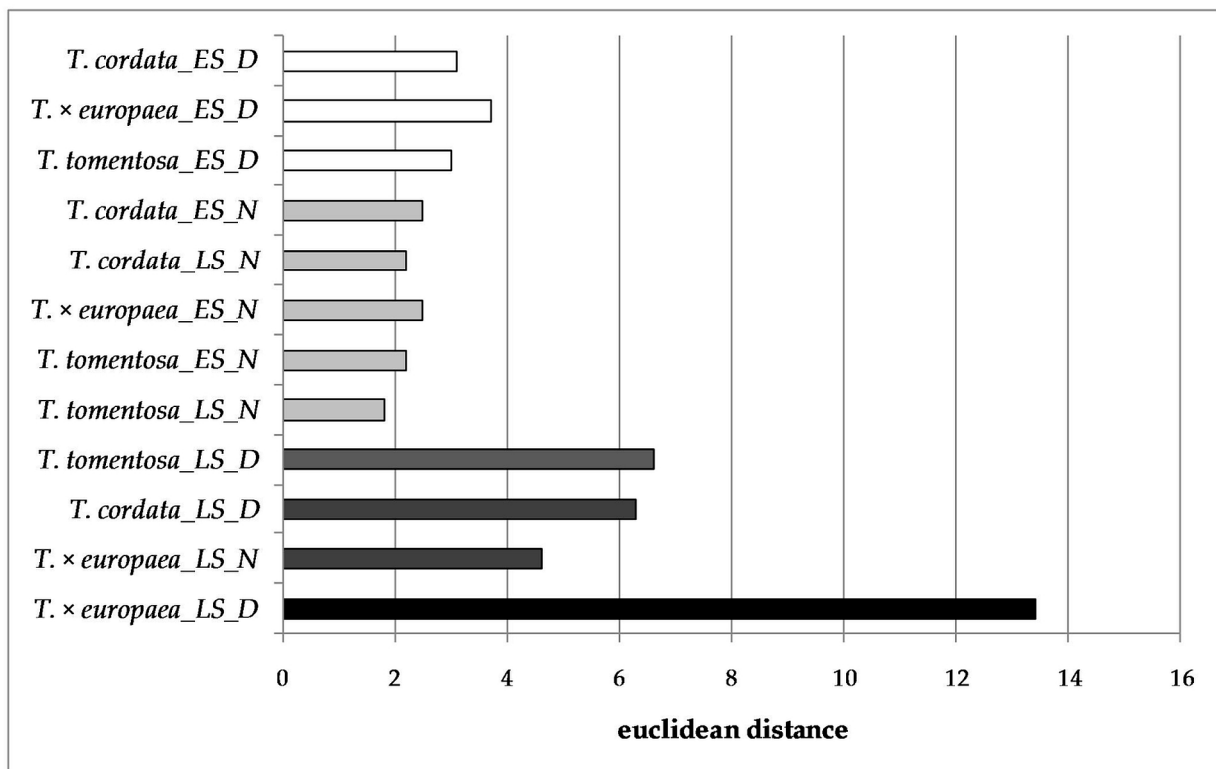


**Figure 4.** JIP-test and photosynthetic pigment parameters, calculated for investigated *Tilia* species presented as a spider plot. Fully developed leaves were set as 1, and early and late senescence presented in relation to fully developed leaves. Statistical analysis was performed separately to distinguish statistical differences between early senescence and full development (first mark), and late senescence and full development (second mark; – means there were no statistical differences, \* means there were statistical differences according to the Kruskal–Wallis method and Dunn test with  $p < 0.05$ ).





**Figure 5.** The results of the cluster analysis presented as a dendrogram. All treatments were classified based on all of the presented parameters (pigment contents and ratio as well as the ChlF parameters) using the Euclidean distance and Ward’s method; the blue line indicates the cut-off point; and blue rectangles show separate clusters.



**Figure 6.** Euclidean distance between full development and other developmental phases in each species and category (D or N), separately. White bars indicate objects classified into cluster 1, light grey bars—cluster 2, dark grey bars—cluster 3 and the black bar—cluster 4.

#### 4. Discussion

Drought is a complex phenomenon that can be measured and considered in many ways. For that cause, it is difficult to indicate the exact beginning and duration of such an

event [3]. In addition to in-depth techniques testing the soil moisture and evapotranspiration rates, there are also simplified methods that enable the determination of drought events under limited climatic data [33].

To estimate the drought event appearances during years of study, we used the Gaussem–Walter climatic diagram, which facilitates estimation of the precipitation deficit with specific chart plotting. According to this method, each group of investigated trees were examined during one year with a summer (months VII–VIII) precipitation deficit, and one with the summer precipitation classified as normal or moist (according to the presented climatic diagram and analysis of precipitation norm—Figures 1 and 2).

Stressful conditions, such as drought, severely affect photosynthesis in all its phases and at many levels. Drought can cause the degradation of chlorophyll and thylakoid membranes, hence, disturbing photosystem functioning and mainly the light-absorbing efficiency [42]. Chlorophyll *a* fluorescence is one of the effective measurements that provides information about PSII functioning. It has been proven suitable in measurements of tree reactions to specific stresses, such as drought [43,44].

In most of the available research, the examination of stress effects was conducted close to the presence of the stressor. We decided to apply the innovative way of prolonged investigation, after the relief from stress conditions. We did not observe an evident reaction of investigated trees PSII during stress occurrence. Therefore, we decided to evaluate if drought events can influence the autumnal senescence course.

Autumnal senescence is a programmed process that enables nutrient relocation before the dormancy period. Aging patterns are strongly connected to the plant species, and environmental conditions can alter the natural course of those reactions [45]. It is known that, during the senescence of deciduous tree leaves, the content of photosynthetic pigments, such as chlorophylls and carotenoids, decreases; however, the degradation processes take place at different rates [46]. The chlorophyll degradation traits were different among investigated species.

It has been proven that deciduous tree species (maple, oak and beech) vary in the chlorophyll degradation trend during senescence [47]. The mentioned authors also assumed that species differed in the onset of senescence processes. As presented for four different tree species (*S. aucuparia*, *A. platanooides*, *B. pendula* and *P. padus*), individual leaves maintained relatively high chlorophyll content for the whole autumnal senescence period, estimated at 2 months [48]. We underline that the fast decrease of chlorophyll was observed only about a week before leaf abscission. A similar reaction was observed in the investigated linden trees (Figure 4).

The chlorophyll sum decreased significantly in the year with a summer precipitation deficit during late senescence for all of the investigated species in comparison to full development and in early senescence for *T. × europaea*. However, it did not decrease in early or late senescence for any species after the summer with ‘normal’ precipitation. Such a result indicates disturbances in the natural chlorophyll degradation in the year with a summer precipitation deficit. This observation indicates a delayed reaction of physiological traits to summer natural drought.

It was also presented, for *Tilia cordata* growing in Poland, that the content of Chl *a* was lower in autumn in comparison to spring and summer, while the Chl *b* and carotenoid contents did not change significantly [49]. This is connected with the faster senescence-related degradation of chlorophyll *a* over chl *b* and carotenoids, and this is consistent with our results during the year with normal precipitation (Figure 4). The chlorophyll to carotenoid ratio, which was decreased during senescence (D), was unaffected or even higher during senescence (N) in comparison to full development. This indicates a more drastic reaction during senescence in the year with precipitation deficit in comparison to the ‘normal’ year.

The mentioned changes in the rate of chlorophyll pigment degradation were not so evident in the presented chl *a/b* ratio. This is caused by the decrease in the chl *b* content in early and late senescence (D). For that reason, the difference between chl *a/b* during

senescence (D) and full development (D) was insignificant in most cases. The general degradation of both chlorophylls was observed during year with a precipitation deficit. The reduction of total chlorophyll in LS compared to FD in the year with a precipitation deficit was about 58% in *T. cordata*, 73% in *T. tomentosa* and 95% in *T. × europaea*, which emphasizes the differences in reactions to drought between these species.

Under natural leaf senescence, the OJIP transient became flatter, and the values of the fluorescence in the J, I, and P decreased [50]. The responses of the chlorophyll *a* fluorescence transient (OJIP curve) were changed after drought in summer (D), which was especially observed during late senescence (Figure 3). The reduction in the I-P phase was observed under the drought stress of *Fraxinus ornus*. It correlates with active plastocyanin (PC) and P700, and reflects the ability for reducing final acceptors beyond PSI.

In addition, the reduction in the J-phase is connected with the down-regulation of PSII photochemistry as a photoprotective mechanism observed in *Fraxinus ornus* under drought [43]. In the presented results for all investigated *Tilia* species, both photosystems were disturbed in the late senescence (D) while, in the 'normal' year, this occurred only in *T. × europaea* (decrease in the J-phase and I-P phases). These findings are contrary to drought-stressed *T. cordata* growing in controlled conditions in pots [51]. In such conditions, the authors observed the increase of K and J bands of OJIP test after 28 days without plant watering.

As presented for two young (15–20 years old) deciduous tree species *Acer saccharum* and *Quercus bicolor*, a high photochemical efficiency was maintained during autumnal senescence [52]. Selig and Bohne [53] observed no differences between the control and drought-treated *Tilia cordata* Fv/Fm parameters. In some cases, this parameter is not sensitive enough, as was suggested previously [44,54,55]. Similar results were presented in our study for investigated linden species during the year with 'normal' precipitation in the summer (Figure 4). There was no significant decrease in the maximum photochemical efficiency of PSII (measured with Fv/Fm) during early senescence for any species. However, such a decrease was observed in the late senescence for *T. × europaea*, which again had a distinct reaction.

In the year with a summer precipitation deficit, there was a significant decrease of the Fv/Fm parameter during late senescence in all of the investigated species. However, the reduction of this parameter was not uniform in all the species studied, in the case of *T. cordata*, it was about 4%, in *T. tomentosa* 30% and in *T. × europaea*, 62%. The decrease of the Fv/Fm parameter during drought was also observed in *Tilia cordata* by Kalaji et al. [51]. Such a reaction was also observed in *Acer saccharum* leaves growing in the city park area, during autumnal leaf coloration [56]. The change from green to yellow and orange leaves resulted in the decrease of the Fv/Fm parameter from 0.75 to 0.4 and 0.25, respectively. Such a decrease of the maximum quantum yield of PSII is connected with a loss of photosynthetic capacity.

Changes in the specific fluxes were observed during natural leaf senescence. As presented for *Acer platanoides* leaves, during yellowing, ABS/RC, TR<sub>0</sub>/RC and DI<sub>0</sub>/RC increase and ET<sub>0</sub>/RC decrease was observed [50]. However, the changes in investigated *Tilia* leaves were more evident during senescence after natural summer drought than after a 'normal' summer. Similarly to our results, after 28 days of drought, Kalaji et al. [51] observed an increase of F<sub>0</sub>, ABS/RC, TR<sub>0</sub>/RC and DI<sub>0</sub>/RC as well as a decrease of Fm, Fv/F<sub>0</sub>, Fv/Fm and PI<sub>total</sub> in the investigated *Tilia cordata* leaves. An increase of DI<sub>0</sub>/RC is an indicator of the dissipation of absorbed photosystem energy when the photochemistry is disrupted [57,58].

This indicates that the drought stress observed in summer inhibited electron transport and stimulated the dissipation of energy in late senescence in *Tilia* leaves. Although Kalaji et al. [41] did not observe changes in ET<sub>0</sub>/RC, in our experiment, a decrease of electron transport was significant in the LS\_D in all *Tilia* species. Absorbed radiation energy was redirected not to electron transport and photochemical reactions but to the processes of its

dissipation. Furthermore, the redirection of absorbed energy was also confirmed by the decrease of  $F_v/F_0$  parameter.

This observation could be an indicator of a lower photochemical process efficiency in PSII [59]. In addition, the decrease of  $F_m$  or  $F_v/F_m$  could be the information about the reduced potential of primary acceptor QA of PSII for receiving electron due to the dissociation of the light-harvesting complex (LHC) from the PSII [51]. During the dehydration of plant tissue, the  $TR_0/RC$  increased as a result of the increase of inactive RCs and decrease of active RCs until 30% of RWC (relative water content in tissue) [60]. The decrease of active RCs was also possible in our experiment in late senescence *Tilia* leaves after a summer drought.

During the year with normal precipitation, *T. cordata* showed no changes in the  $ABS/RC$ ,  $TR_0/RC$  and  $DI_0/RC$  parameters during senescence, while, in the case of *T. × europaea*, significant changes were observed. In addition, the *T. cordata* PI total parameter during aging (comparison of FD with LS) was reduced by 65%; in *T. × europaea*, this was reduced by 74%; and, in *T. tomentosa*, a decrease of 31% was observed. During the year with a deficit of precipitation, changes in the  $ABS/RC$ ,  $TR_0/RC$ ,  $ET_0/RC$  and  $DI_0/RC$  as well as in the PI total parameters were observed in all the species studied during senescence.

However, the levels of change were not similar. The PI total decreased by 73% and 75% in the case of *T. cordata* and *T. tomentosa*, while in *T. × europaea* decreased by 99%. The increase in energy dissipation expressed by the  $DI_0/RC$  parameter was 2.3 fold in the case of *T. cordata*, 9 fold in *T. tomentosa* and as much as 25 fold in *T. × europaea*. This clearly shows the differences in the response to summer drought stress between the studied species.

Cluster analysis is a useful tool for treatment classification. The usefulness of cluster analysis in the classification of similarity of *Tilia* species based on morphological features was proven [61]. However, in the presented study, the most important comparison was the reaction of different species to stress conditions (summer with precipitation deficit). Therefore, the result of cluster analysis shows treatments with similar and distant responses. Four distinct groups were separated, where treatments exposed to a precipitation deficit in summer were clearly separated from those collected during years with better rainfall distribution.

Moreover, some growth phases of plants exposed to summer precipitation deficits were separated: full development and early senescence from late senescence. This indicates significant changes in the tested parameters and deterioration of the functioning state of the photosynthetic apparatus. There was no phase separation during the better distribution of rainfall (N), except for the late senescence of *T. × europaea*. It is known that plants differ in their 'drought resistance' adaptations, and reactions are species-dependent [62].

The results of the cluster analysis suggest that the response to senescence and summer drought stress is different for *T. cordata* and *T. × europaea*, despite the species relationship. Swoczyna et al. [63] classified *T. cordata* and *T. × europaea* into one group based on fluorescence parameters and found that, as forest species, they are sensitive to drought stress. However, those classification also included other, not-related urban tree species.

Three species of linden were examined in the presented study, while, in the studies of Swoczyna et al. [63], various tree species were compared (*Tilia*, *Acer*, *Quercus*, *Gleditsia*, *Pyrus*, *Platanus* and *Ginkgo*), which likely contributed to the classification of *T. cordata* and *T. × europaea* into one group. In our research, in the late senescence phase, both species are classified into different groups, which indicates that the pattern of senescence and response to drought stress in both species is different. The greatest change from full development to late senescence was seen in *T. × europaea* during the year with precipitation deficit.

## 5. Conclusions

1. Investigated species of linden trees, i.e., *Tilia tomentosa*, *T. × europaea* and *T. cordata* revealed delayed reactions to precipitation deficits. The photosynthetic performance of the investigated species was disturbed in the late senescence phase after summer

- with precipitation deficits. Those changes were less evident during senescence after summer without precipitation deficits.
2. Changes in the OJIP test included mostly parameters related to the number of active reaction centres. After the summer precipitation deficits, increases of the ABS/RC, DI<sub>0</sub>/RC and TR<sub>0</sub>/RC and a decrease of ET<sub>0</sub>/RC were related to the decrease of active RCs.
  3. Different severities of the senescence-related reactions were observed in *T. × europaea*. This heterotic species demonstrated a more intense decrease of certain parameters during late senescence (N) compared with other species (i.e., Fm, Fv/Fm and Fv/F<sub>0</sub>). In the years with precipitation deficits, those differences were more evident. For example, compared to full development, the decrease in the maximum fluorescence (Fm) during late senescence (D) was about 30% for *T. cordata* and *T. tomentosa* and about 75% for *T. × europaea*. Despite the close relation of *T. cordata* and *T. × europaea*, the species showed different physiological traits during senescence after precipitation deficits in summer.
  4. The presented results underline the need for monitoring tree conditions not only during stress but also in the wider perspective, concerning a prolonged time of measurement for at least few weeks after the stress appearance. Practitioners and urban planners need to take into account that the impact of a stress factor on tree physiology can occur many weeks beyond its occurrence.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/f12060761/s1>, Table S1: Locations of investigated trees with number of individuals. Figure S1: Locations of investigated trees presented on Krakow's map, red is for *T. × europaea*, blue is for *T. tomentosa* and green is for *T. cordata*; Table S2: Table with the means of parameters derived from OJIP-test and chlorophyll pigment content, for investigated *Tilia* species. In the column 'term' FD is for full development, ES is for early senescence and LS is for late senescence. In the column 'year' N is for normal year and D is for dry year (with precipitation deficit in summer).

**Author Contributions:** Conceptualization, M.C., A.K. and P.M.; methodology, A.K.; formal analysis, M.C. and A.K.; investigation, M.C. and A.K.; resources, M.C. and A.K.; data curation, M.C. and A.K.; writing—original draft preparation, M.C., A.K. and P.M.; writing—review and editing, M.C., A.K. and P.M.; visualization, M.C. and A.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** The presented study was supported by The Ministry of Education and Science, Subsidy SUB2020-050014-D011 and Subsidy SUB/2020-050012-D011.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors are very grateful to Grażyna Sierant for her great technical assistance and support.

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

## References

1. Van Loon, A.F.; Stahl, K.; Di Baldassarre, G.; Clark, J.; Rangelcroft, S.; Wanders, N.; Gleeson, T.; Van Dijk, A.I.; Tallaksen, L.M.; Hannaford, J.; et al. Drought in a human-modified world: Reframing drought definitions, understanding, and analysis approaches. *Hydrol. Earth Syst. Sci.* **2016**, *20*, 3631–3650. [[CrossRef](#)]
2. Long, A.L. Drought. In *Disturbance and Sustainability in Forests of the Western United States*; Barrett, T.M., Robertson, G.C., Eds.; Gen. Tech. Rep. PNW-GTR-992; US Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, OR, USA, 2021; Volume 231, p. 992.
3. Spinoni, J.; Vogt, J.V.; Naumann, G.; Barbosa, P.; Dosio, A. Will drought events become more frequent and severe in Europe? *Int. J. Climatol.* **2018**, *38*, 1718–1736. [[CrossRef](#)]
4. World Meteorological Organization (WMO); Global Water Partnership (GWP). *Integrated Drought Management Programme (IDMP)*; WMO: Geneva, Switzerland, 2017.
5. Cook, B.I.; Mankin, J.S.; Anchukaitis, K.J. Climate change and drought: From past to future. *Curr. Clim. Chang. Rep.* **2018**, *4*, 164–179. [[CrossRef](#)]



6. Ghadami, M.; Razi, T.; Amini, M.; Modarres, R. Regionalization of drought severity–duration index across Iran. *Nat. Hazards* **2020**, *103*, 2813–2827. [[CrossRef](#)]
7. United Nations, Department of Economic, Social Affairs, Population Division. *World Urbanization Prospects: The 2014 Revision, Highlights (ST/ESA/SER.A/352)*; United Nations: New York, NY, USA, 2015.
8. Czaja, M.; Kołton, A.; Muras, P. The Complex Issue of Urban Trees—Stress Factor Accumulation and Ecological Service Possibilities. *Forests* **2020**, *11*, 932. [[CrossRef](#)]
9. Yang, J.-L.; Zhang, G.L. Formation, characteristics and eco-environmental implications of urban soils—A review. *Soil Sci. Plant Nutr.* **2015**, *61* (Suppl. 1), 30–46. [[CrossRef](#)]
10. Day, S.D.; Wiseman, P.E.; Dickinson, S.B.; Harris, J.R. The Root Ecology in the Urban Environment and Implications for a Sustainable Rhizosphere. *Arboric. Urban* **2010**, *36*, 193–204.
11. Zhang, P.; Ariaratnam, S.T. Meta-analysis of storm water impacts in urbanized cities including runoff control and mitigation strategies. *J. Sustain. Dev.* **2018**, *11*, 27–40. [[CrossRef](#)]
12. Moser, A.; Rötzer, T.; Pauleit, S.; Pretzsch, H. The Urban Environment Can Modify Drought Stress of Small-Leaved Lime (*Tilia cordata* Mill.) and Black Locust (*Robinia pseudoacacia* L.). *Forests* **2016**, *7*, 71. [[CrossRef](#)]
13. Gupta, A.; Rico-Medina, A.; Caño-Delgado, A.I. The physiology of plant responses to drought. *Science* **2020**, *368*, 266–269. [[CrossRef](#)]
14. Zhang, C.; Stratopoulos, L.M.F.; Pretzsch, H.; Rötzer, T. How do *Tilia cordata* Greenspire trees cope with drought stress regarding their biomass allocation and ecosystem services? *Forests* **2019**, *10*, 676. [[CrossRef](#)]
15. Cornic, G. Drought stress inhibits photosynthesis by decreasing stomatal aperture—Not by affecting ATP synthesis. *Trends Plant Sci.* **2000**, *5*, 187–188. [[CrossRef](#)]
16. Kalaji, H.M.; Jajoo, A.; Oukarroum, A.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Łukasik, I.; Goltsev, V.; Ladle, R.J.; et al. Chapter 15—The Use of Chlorophyll Fluorescence Kinetics Analysis to Study the Performance of Photosynthetic Machinery in Plants. In *Emerging Technologies and Management of Crop Stress Tolerance*; Ahmad, P., Rasool, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; Volume 2, pp. 347–384. [[CrossRef](#)]
17. Gururani, M.A.; Venkatesh, J.; Tran, L.S.P. Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Mol. Plant* **2015**, *8*, 1304–1320. [[CrossRef](#)]
18. Banks, J.M. Continuous excitation chlorophyll fluorescence parameters: A review for practitioners. *Tree Physiol.* **2017**, *37*, 1128–1136. [[CrossRef](#)] [[PubMed](#)]
19. Stirbet, A.; Lazár, D.; Guo, Y.; Govindjee, G. Photosynthesis: Basics, history and modelling. *Ann. Bot.* **2020**, *126*, 511–537. [[CrossRef](#)] [[PubMed](#)]
20. Wong, C.Y.; D’Odorico, P.; Bhatena, Y.; Arain, M.A.; Ensminger, I. Carotenoid based vegetation indices for accurate monitoring of the phenology of photosynthesis at the leaf-scale in deciduous and evergreen trees. *Remote Sens. Environ.* **2019**, *233*, 111407. [[CrossRef](#)]
21. Yang, H.; Yang, X.; Heskell, M.; Sun, S.; Tang, J. Seasonal variations of leaf and canopy properties tracked by ground-based NDVI imagery in a temperate forest. *Sci. Rep.* **2017**, *7*, 1–10. [[CrossRef](#)]
22. Koller, S.; Holland, V.; Brueggemann, W. Seasonal monitoring of PSII functionality and relative chlorophyll content on a field site in two consecutive years: A case study of different oak species. *Photosynthetica* **2020**, *58*, 379–390. [[CrossRef](#)]
23. Pflug, E.E.; Buchmann, N.; Siegwolf, R.T.; Schaub, M.; Rigling, A.; Arend, M. Resilient leaf physiological response of European beech (*Fagus sylvatica* L.) to summer drought and drought release. *Front. Plant Sci.* **2018**, *9*, 187. [[CrossRef](#)]
24. Moser, A.; Rahman, M.A.; Pretzsch, H.; Pauleit, S.; Rötzer, T. Inter- and intra-annual growth patterns of urban small-leaved lime (*Tilia cordata* mill.) at two public squares with contrasting microclimatic conditions. *Int. J. Biometeorol.* **2017**, *61*, 1095–1107. [[CrossRef](#)]
25. Gillner, S.; Korn, S.; Hofmann, M.; Roloff, A. Contrasting strategies for tree species to cope with heat and dry conditions at urban sites. *Urban Ecosyst.* **2017**, *20*, 853–865. [[CrossRef](#)]
26. Jarosińska, E.; Bodziony, M. Temporal and spatial rainfall variability in the urbanized area of Cracow. *Acta Sci. Pol. Formatio. Circumiectionis* **2019**, *18*, 43–55. [[CrossRef](#)]
27. Pigott, D. *Lime-Trees and Basswoods. A Biological Monograph of the Genus Tilia*; Cambridge University Press: New York, NY, USA, 2012.
28. Pauleit, S.; Jones, N.; Nyhuus, S.; Pirnat, J.; Salbitano, F. Urban Forest Resources in European Cities. In *Urban Forests and Trees*; Konijnendijk, C., Nilsson, K., Randrup, T., Schipperijn, J., Eds.; Springer: Berlin, Germany, 2005.
29. Zhang, C.; Stratopoulos, L.M.F.; Xu, C.; Pretzsch, H.; Rötzer, T. Development of Fine Root Biomass of Two Contrasting Urban Tree Cultivars in Response to Drought Stress. *Forests* **2020**, *11*, 108. [[CrossRef](#)]
30. Brodzińska, B.; Czekerda, D.; Czernercki, B.; Dołęga, E.; Kowalik, A.; Krupa-Marchlewska, J.; Marcinkowski, M.; Mizera, M.; Moskwiński, T.; Nowak, B.; et al. *Bulletin of the National Hydrological and Meteorological Service*; Institute of Meteorology and Water Management National Research Institute: Warsaw, Poland, 2013; Volume 13, p. 15. (In Polish)
31. Brodzińska, B.; Czekerda, D.; Dołęga, E.; Gabryelewicz, M.; Kowalik, A.; Mizera, M.; Pawelec, W.; Pietrzykowska, A.; Sawicka, M.; Strzelczak, A.; et al. *Bulletin of the National Hydrological and Meteorological Service*; Institute of Meteorology and Water Management National Research Institute: Warsaw, Poland, 2014; Volume 13, p. 15. (In Polish)

32. Brodzińska, B.; Cebulak, E.; Czekierda, D.; Gabryelewicz, M.; Kowalewski, M.; Kowalik, A.; Nowak, D.; Pawelec, W.; Pietrzykowska, A.; Sawicka, M.; et al. *Bulletin of the National Hydrological and Meteorological Service; Institute of Meteorology and Water Management National Research Institute: Warsaw, Poland, 2015; Volume 13, p. 14.* (In Polish)
33. Treder, W.; Klamkowski, K.; Wójcik, K. A new approach to the method of drawing the Gausse–Walter climate diagram. *Meteorol. Hydrol. Water Manag.* **2018**, *6*, 3–9. [[CrossRef](#)]
34. Łukasiewicz, S. A suggested modification of the method of drawing the wet ‘humid’ period in the Gausse–Walter climate diagram. *Bad. Fizjogr. Nad Pol. Zach. Ser. A Geogr. Fiz.* **2006**, *57*, 95–99. (In Polish)
35. Lichtenthaler, H.K.; Babani, F.; Navrátil, M.; Buschmann, C. Chlorophyll fluorescence kinetics, photosynthetic activity, and pigment composition of blue-shade and half-shade leaves as compared to sun and shade leaves of different trees. *Photosynth. Res.* **2013**, *117*, 355–366. [[CrossRef](#)] [[PubMed](#)]
36. Lichtenthaler, H.K.; Babani, F. Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In *Advances in Photosynthesis and Respiration. Chlorophyll a fluorescence. A Signature of Photosynthesis*; Papageorgiou, G.C., Govindjee, Eds.; Springer: Dordrecht, The Netherlands, 2004; Volume 19, pp. 713–736. [[CrossRef](#)]
37. Govindjee, S.A. On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: Basics and applications of the OJIP fluorescence transient. *J. Photochem. Photobiol. B Biol.* **2011**, *104*, 236–257. [[CrossRef](#)]
38. Střibet, A.; Lazár, D.; Kromdijk, J.; Govindjee. Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica* **2018**, *56*, 86–104. [[CrossRef](#)]
39. Wellburn, A.R. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [[CrossRef](#)]
40. D’Amato, R.; De Feudis, M.; Hasuoka, P.E.; Regni, L.; Pacheco, P.H.; Onofri, A.; Businelli, D.; Proietti, P. The selenium supplementation influences olive tree production and oil stability against oxidation and can alleviate the water deficiency effects. *Front. Plant Sci.* **2018**, *9*, 1191. [[CrossRef](#)]
41. Akula, R.; Ravishankar, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* **2011**, *6*, 1720–1731. [[CrossRef](#)] [[PubMed](#)]
42. Ashraf, M.H.P.J.C.; Harris, P.J. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [[CrossRef](#)]
43. Salvatori, E.; Fusaro, L.; Manes, F. Chlorophyll fluorescence for phenotyping drought-stressed trees in a mixed deciduous forest. *Ann. Bot.* **2016**, *6*, 39–49. [[CrossRef](#)]
44. Banks, J.M. Chlorophyll fluorescence as a tool to identify drought stress in *Acer* genotypes. *Environ. Exp. Bot.* **2018**, *155*, 118–127. [[CrossRef](#)]
45. Munné-Bosch, S. Senescence: Is it universal or not? *Trends Plant Sci.* **2015**, *20*, 713–720. [[CrossRef](#)]
46. Croft, H.; Chen, J.M. Leaf pigment content. *Compr. Remote Sens. Ref. Modul. Earth Syst. Environ. Sci.* **2017**, *3*, 117–142. [[CrossRef](#)]
47. Primka, E.J.; Smith, W.K. Synchrony in fall leaf drop: Chlorophyll degradation, color change, and abscission layer formation in three temperate deciduous tree species. *Am. J. Bot.* **2019**, *106*, 377–388. [[CrossRef](#)]
48. Mattila, H.; Valev, D.; Havurinne, V.; Khorobrykh, S.; Virtanen, O.; Antinluoma, M.; Mishra, K.B.; Tyystjärvi, E. Degradation of chlorophyll and synthesis of flavonols during autumn senescence—The story told by individual leaves. *Aob. Plants* **2018**, *10*, ply028. [[CrossRef](#)]
49. Ciupak, A.; Dziwulska-Hunek, A.; Gładyszewska, B.; Kwaśniewska, A. The relationship between physiological and mechanical properties of *Acer platanoides* L. and *Tilia cordata* Mill. leaves and their seasonal senescence. *Sci. Rep.* **2019**, *9*, 1–10. [[CrossRef](#)]
50. Lepeduš, H.; Jurkovič, V.; Štolfa, I.; Čurković-Perica, M.; Fulgosi, H.; Cesar, V. Changes in photosystem II photochemistry in senescing maple leaves. *Croatia Chem. Acta* **2010**, *83*, 379–386.
51. Kalaji, H.M.; Račková, L.; Paganová, V.; Swoczyna, T.; Rusinowski, S.; Sitko, K. Can chlorophyll-*a* fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? *Environ. Exp. Bot.* **2018**, *152*, 149–157. [[CrossRef](#)]
52. Moy, A.; Le, S.; Verhoeven, A. Different strategies for photoprotection during autumn senescence in maple and oak. *Physiol. Plant.* **2015**, *155*, 205–216. [[CrossRef](#)]
53. Selig, M.; Bohne, H. Drought stress reactions of different populations of *Quercus robur* L. and *Tilia cordata* Mill. *J. Environ. Hortic.* **2017**, *35*, 6–12. [[CrossRef](#)]
54. Tsimilli-Michael, M. Revisiting JIP-test: An educative review on concepts, assumptions, approximations, definitions and terminology. *Photosynthetica* **2019**, *58*, 275–292. [[CrossRef](#)]
55. Kalaji, H.M.; Rastogi, A.; Živčák, M.; Brestic, M.; Daszkowska-Golec, A.; Sitko, K.; Alsharafa, K.Y.; Lotfi, R.; Stypiński, P.; Samborska, I.A.; et al. Prompt chlorophyll fluorescence as a tool for crop phenotyping: An example of barley landraces exposed to various abiotic stress factors. *Photosynthetica* **2018**, *56*, 953–961. [[CrossRef](#)]
56. Junker, L.V.; Ensminger, I. Relationship between leaf optical properties, chlorophyll fluorescence and pigment changes in senescing *Acer saccharum* leaves. *Tree Physiol.* **2016**, *36*, 694–711. [[CrossRef](#)] [[PubMed](#)]
57. Lee, T.Y.; Woo, S.Y.; Kwak, M.J.; Inkyin, K.; Lee, K.E.; Jang, J.H.; Kim, I.R. Photosynthesis and chlorophyll fluorescence responses of *Populus sibirica* to water deficit in a desertification area in Mongolia. *Photosynthetica* **2016**, *54*, 317–320. [[CrossRef](#)]

58. Meng, L.L.; Song, J.F.; Wen, J.; Zhang, J.; Wei, J.H. Effects of drought stress on fluorescence characteristics of photosystem II in leaves of *Plectranthus scutellarioides*. *Photosynthetica* **2016**, *54*, 414–421. [[CrossRef](#)]
59. Roháček, K. Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* **2002**, *40*, 13–29. [[CrossRef](#)]
60. Strasser, R.J.; Tsimilli-Michael, M.; Qiang, S.; Goltsev, V. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochim. Biophys. Acta* **2010**, *1797*, 1313–1326. [[CrossRef](#)]
61. Clark, J.Y. Neural networks and cluster analysis for unsupervised classification of cultivated species of *Tilia* (Malvaceae). *Bot. J. Linn. Soc.* **2009**, *159*, 300–314. [[CrossRef](#)]
62. Basu, S.; Ramegowda, V.; Kumar, A.; Pereira, A. Plant adaptation to drought stress. *F1000Research* **2016**, *5*, Faculty Rev-1554. [[CrossRef](#)] [[PubMed](#)]
63. Swoczyna, T.; Kalaji, H.M.; Pietkiewicz, S.; Borowski, J. Ability of various tree species to acclimation in urban environments probed with the JIP-test. *Urban For. Urban Green.* **2015**, *14*, 544–553. [[CrossRef](#)]