

Article

Physiological Performance and Biomass Growth of Different Black Locust Origins Growing on a Post-Mining Reclamation Site in Eastern Germany

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Abstract: Black Locust/*Robinia* can play an important role in land reclamation due to its pronounced nitrogen fixation capability, fast initial growth and relative high drought tolerance. Hence, we set up a trial to test 12 Black Locust clones and three provenances growing on sandy overburden material within the open cast lignite mine *Welzow-Süd* (South Brandenburg) in March 2014. Since then, biomass growth of the Black Locust trees was examined and physiological performance was studied on several occasions using chlorophyll a fluorescence and Dualex[®] measuring technique. Plant physiological measurements revealed differences in photosynthetic vitality (PI_{ABS}), although the PI_{ABS} values followed a similar pattern and sequences across the plot. While the genotypes *Fra3* and *Roy* show the highest photosynthetic vitality, the clones *Rog* and *Rob* display the lowest PI_{ABS} mean values. Chlorophyll and phenol content as well as the nutrition supply of the test trees vary depending on their origin and site conditions. The annual biomass growth rate corresponds to photosynthetic vitality and both depend on weather conditions during the growing season. After six years, the growing biomass amounts to 14.7 Mg d.m. ha⁻¹ for clone *Rob* and 44.8 Mg d.m. ha⁻¹ for clone *Fra3*, i.e., 2.5 to 7.5 Mg d.m. ha⁻¹ year⁻¹. Our data demonstrate a good correlation between biophysical parameters and biomass growth. We, thus, infer that physiological measuring methods can be combined to strengthen predictions regarding the physiological performance of Black Locust origins.

Keywords: *Robinia pseudoacacia* L.; photosynthetic vitality; chlorophyll and phenol content; nutrition supply; dry matter yield; land reclamation



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1. Introduction

Black Locust (*Robinia pseudoacacia* L.)—once introduced to central Europe on the strength of its remarkable flowering—has established itself as a common tree species. Since the time of its introduction, the prolific *Robinia* has demonstrated its remarkable ability to spread [1,2], though it often remains neglected by silviculture. Current estimates suggest that Black Locust covers 34,000 hectares of German forest cultivation area, with over two thirds of this area situated in the north-east German lowlands [3]. Black Locust is considered a fast-growing tree species and stands out due to its superior wood characteristics which make it suitable for wide-ranging and high-quality usage. Furthermore, in times of climate change, *Robinia* is gaining in importance by virtue of its outstanding tolerance to drought and heat [4] as well as its good adaptability to climate change. Hence, Black Locust is predestined to contribute to the sustainable productivity of forests, even during critical weather situations such as drought and frost. In addition, *Robinia* plays an important role in the rehabilitation/reclamation of nutrient-poor lignite mining and

abandoned land on account of its ability to fix atmospheric nitrogen. Black Locust stands currently cover 4.9% (i.e., 102 hectares) of the total reclamation area managed by the lignite mining company LEAG.

The declared aim of breeding Black Locust genotypes is to provide vital plus trees prior to material utilisation. However, to date, Robinia breeding initiatives have predominantly focused on external characteristics such as the stem form and improving the biomass growth performance. Nonetheless, criteria such as plants' physiological performance and tolerance to abiotic stressors are becoming increasingly decisive for the successful establishment of forest stands as well as for short rotation coppices in agriculture. Despite this, surprisingly little is known about the physiological performance of the Black Locust clones and the Black Locust provenances available on the market, and the research on cultivating Robinia genotypes which meet both criteria—high biomass growth as well as promising stress tolerance—is still in its infancy [5]. There is evidence showing significant genetic variation with regard to drought tolerance [6–9].

Within the framework of the FastWOOD subprojects 6 and 7, we investigated biomass growth as well as individual physiological performance of different Black Locust origins (i.e., clones and provenances). Our goal was to determine specific reaction patterns of promising origins to abiotic stressors. We used so-called biomarkers as major indicators to identify and classify Robinia clones and provenances with regard to their climate adaptability [8,10]. We relied on an experimental blend consisting of plot experiments [10] as well as field trials [8] to test selected Black Locust origins. In this context, we applied physiological marker techniques to reveal the individual adaptive capacity of the Robinia clones and provenances to drought, nutrient deficiencies and late frost.

Given that Robinia clones and provenances differ in their physiological performance and stress tolerance, insights into a genotype's suitability for cultivation in short coppice rotations and on forest land are valuable. The earlier a measurement procedure can deliver reliable results predicting the physiological performance, the faster research into relevant tree breeding can progress. Hence, our hypothesis is that there is a relationship between biophysical parameters and biomass growth which can be used to deliver physiological performance predictions of different Robinia origins at an early stage.

2. Materials and Methods

2.1. Experimental Site

Our test plot ($51^{\circ}36'49''$ N, $14^{\circ}14'05''$ E) is situated in the northern part of *Welzow-Süd* open-cast lignite mining site, in the south of the Federal State of Brandenburg (Germany), Figure 1. Our experimental plot covers 0.4 hectares in total. We also run a weather station approximately 5 km east of our experimental plot which records standard meteorological data (i.e., precipitation, temperature, etc.).

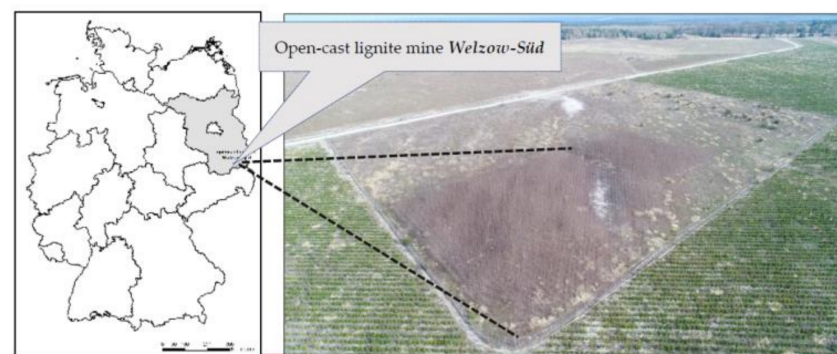


Figure 1. Map and aerial image showing our test plot in the north of lignite surface mine *Welzow-Süd*, photo taken on 12 April 2018.

2.2. Soil Conditions

The test area, situated in the northern part of the lignite mining pit *Welzow-Süd*, has been undergoing rehabilitation since 2012. Investigations on local dump soil substrate by Hanschke [11] revealed the prevalence of two different dumped materials:

- (1) Loamy Sand with few gravels;
- (2) Loamy Sand with few gravels and finely divided lignite material.

Note that the evaluation of the post-mining soil substrate by Hanschke [11] was carried out prior to lime application on our test site. Both dump substrates units are characterised by low pH value, low nutrition level and low water holding capacity [11], Table 1.

Table 1. Soil chemical composition of two identified mining substrates [11] related to the Robinia clonal test plot.

Substrate No.	pH (CaCl ₂)	10% HCl Extraction								
		C _{tot} [%]	N _{tot} [%]	C/N	S _{tot} [%]	CaCO ₃ [%]	CaO [mg kg ⁻¹]	MgO [mg kg ⁻¹]	K ₂ O [mg kg ⁻¹]	P ₂ O ₅ [mg kg ⁻¹]
1	3.8	0.62	0.016	38.8	0.07	<0.1	865	443	430	94
2	4.1	2.07	0.027	76.7	0.04	<0.1	1617	753	634	144

C_{tot}: total carbon content; N_{tot}: total nitrogen content; S_{tot}: total sulphur content; C/N: C/N ratio, total carbon content divided by total nitrogen content.

2.3. Test Design

The trial was designed to investigate 12 selected clones of Black Locust along with 3 provenances (Table 2). The original plus trees of the clones (ortets) were selected on the basis of their outstanding stem form and growth performance in 1990 [12]. The trial was established with one-year-old plants from tissue culture (clones) and provenances in spring 2014. All plants were cut after planting 5–7 cm above ground. Potential competitive shoots were removed during the first growing season. A complete randomised subplot design was used with 12 replications and 9 trees per plot. The spacing was 1.5 m by 1.5 m.

Table 2. Experimental set up for testing different Black Locust clones and provenances. The background colour is highlighting the 10 test units actually investigated in the study (10 of 15 in total).

No.	Name	Test Unit	Origin	Remarks
1	Bendida	Ben	Bulgaria	selection by company <i>Lignum</i>
2	Tangra	Tan	Bulgaria	selection by company <i>Lignum</i>
3	Fraport 1	Fra1	Hesse (GER)	identical to “ <i>Nyrségi</i> ”
4	Fraport 2	Fra2	Hesse (GER)	identical to “ <i>Jázkiséri</i> ”
5	Fraport 3	Fra3	Hesse (GER)	
6	Langen	Lan	Forest district <i>Langen</i> (GER)	
7	Habichtborn	Hab	Arboretum Habichtborn (GER)	clone “ <i>Appalachia 4183</i> ”
8	Robert	Rob	Brandenburg (GER)	Forest <i>Hasenholz</i>
9	Roger	Rog	Brandenburg (GER)	Forest <i>Hasenholz</i>
10	Romy	Rom	Brandenburg (GER)	Forest <i>Buckow</i>
11	Rowena	Row	Brandenburg (GER)	Forest <i>Bollersdorf</i>
12	Roy	Roy	Brandenburg (GER)	Forest <i>Waldsieversdorf</i>
15	Kiskunsagi	Kis	Hungary	origin: seed stocks
22	Schöneiche	Schö	Brandenburg (GER)	origin: seed stocks
23	Cuci	Cuc	Romania	origin: seed stocks

However, in order to reduce costs and delay we only examine 7 clones and the 3 provenances in this study (grey shaded in Table 2). Five of the investigated clones have their origins in the Federal State of Brandenburg (*Rob*, *Rog*, *Romy*, *Row* and *Roy*, respectively) and two in the Federal State of Hesse (*Fra3*, *Lan*).

According to the principle described in [8], we picked five out of nine test plants per patch for biophysical measurement (Figure 2).

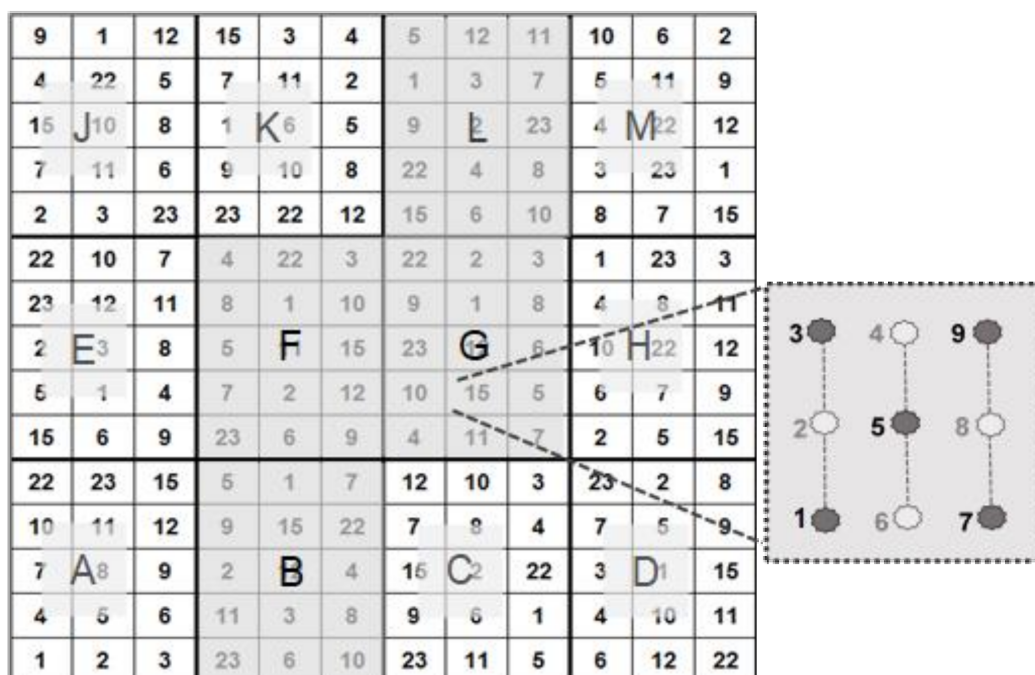


Figure 2. Test design, comprising 4 (B, F, G, L) out of a total of 12 test subplots. Per test unit, 5 out of 9 Robinia test plants were used in our investigations (see [8]).

2.4. Chlorophyll a Fluorescence Measurements and JIP-Test

A portable Plant Efficiency Analyser (Pocket PEA, Hansatech, King's Lynn, UK) was used to non-invasively measure chlorophyll fluorescence on the leaves' surface of Black Locust test plants in order to assess their physiological status (i.e., vitality). Lange et al. [13] successfully employed this methodology to determine plant physiological effects of different soil ameliorants applied to young sessile oaks (*Quercus petraea* (Matt.) Liebl.) growing on a uranium tailings dump in *Schlema* (Ore Mountains, Germany). Before taking the actual measurement, leaves of the test plants were dark-adapted for at least one hour before the chlorophyll a fluorescence measurements were performed. The fast phase fluorescence transients were quantified by means of the JIP-test [14,15] and using the Biolyzer software [15]. The JIP-test, developed and tested under both laboratory and practical conditions, is well accepted amongst experts to detect, describe and quantify the dynamic capacities of the photosynthetic sample. It has been widely and successfully used for the investigation of photosystem II behaviour in various photosynthetic organisms under different stress conditions and enables the study of synergistic and antagonistic effects of different co-stressors [10,13,16–18].

In order to quantify and compare the individual physiological performance of the Robinia test trees both under normal and stress conditions we chose to use the Performance Index (PI_{ABS}) as the JIP-test parameter. PI_{ABS} is a multiparametric expression which incorporates the independent parameters contributing to photosynthesis, namely absorption (RC/ABS), the quantum efficiency of trapping ($\phi Po/(1 - \phi Po)$) and efficiency of conversion of trapped excitation energy to electron transport ($\psi o/(1 - \psi o)$), see Strasser et al. [19]. The Performance index (PI_{ABS}) is presented below on absorption basis, Equation (1) [19]:

$$PI_{ABS} = \frac{\gamma RC}{1 - \gamma RC} \cdot \frac{\phi Po}{1 - \phi Po} \cdot \frac{\psi o}{1 - \psi o} = \frac{RC}{ABS} \cdot \frac{\phi Po}{1 - \phi Po} \cdot \frac{\psi o}{1 - \psi o} \quad (1)$$

where γ_{RC} is the fraction of reaction center chlorophylls relative to the total chlorophyll: $\gamma_{RC} = \text{Chl}_{RC} / \text{Chl}_{\text{total}}$. Since $\text{Chl}_{\text{tot}} = \text{Chl}_{\text{antenna}} + \text{Chl}_{RC}$, we get: $\gamma_{RC} / (1 - \gamma_{RC}) = \text{Chl}_{RC} / \text{Chl}_{\text{antenna}} = \text{RC} / \text{ABS}$.

2.5. Dualex[®] Scientific+TM for Determining Chlorophyll and Flavonols in Leaves

According to the manufacturer's specification, the Dualex[®] Scientific+TM system was developed on the basis of research conducted by the CNRS (Centre national de la recherche scientifique, Paris, France) and Force-A (University of Paris-Sud, Orsay, France). Using a photometric measurement principle, the Dualex[®] is able to perform a non-destructive and rapid measurement of the chlorophyll content in leaves as well as flavonol and anthocyanin contents in the epidermis with sufficient accuracy and in real time [20]. There is some evidence that polyphenols, especially antioxidative flavonols such as anthocyanin, are reliable indicators for plants' vitality. Under abiotic stress and/or as a result of nutrition deficiency, biosynthesis of chlorophyll decreases whereas the production of secondary plant substances such as flavonols increases [21]. The Nitrogen Balance Index (NBI), calculated from the ratio of chlorophyll content and flavonol concentration, indicates the nitrogen supply status of the tested plant [22,23]. Ultimately, an efficient and field-suited optical sensor is available to screen large datasets of leaf samples in a relatively short period of time.

2.6. Biophysical Measurements

Throughout the growing seasons in 2015 and 2016, we carried out *in vivo* chlorophyll a fluorescence and Dualex[®] measurements (usually five measurements per tree) on five out of nine test plants from seven different Robinia clones and three provenances located in the subplots B, F, G and L (Figure 2). Altogether, physiological test results were collected for 200 Black Locust trees over seven measuring dates.

2.7. Plants' Leaf Analysis

After sampling at the beginning of August 2015 and 2016, the leaf tissue was analysed to determine the (N, P, K, Ca, Mg and S) content. Leaves were dried at 80 °C for 48 h and then finely ground using a vibrating sample mill. The total nitrogen content was derived by combustion according to the Dumas principle using an element analyser. The other major elements (K, Ca, Mg, P, S) were measured with an inductively coupled plasma atomic emission spectrometer (ICP-AES). Prior to ICP-AES measurement, dried leaf powder was digested in a microwave pressure digestion system with HNO₃. Determination methods are listed in Table 3.

Table 3. Determination methods used for leaf sample analysis.

Parameter	Test Method	Detection Limit	Dimension
Sample preparation	VDLUFA Bd.III 2.1.1 (1983)		
Sample preparation	VDLUFA Bd. III 2.2.1–2.2.4 (1976)		
N _{tot}	DIN ISO 13878 (1998-11)	0.005	%
C _{tot}	DIN ISO 10694 (1996-08)	0.02	%
Nitric acid pressure digestion (microwave)	EPA Method 3052 (1996-12)		
Ca	DIN EN ISO 11885 (E22, 2009-09)	0.01	g kg ⁻¹
Mg	DIN EN ISO 11885 (E22, 2009-09)	0.002	g kg ⁻¹
K	DIN EN ISO 11885 (E22, 2009-09)	0.01	g kg ⁻¹
P	DIN EN ISO 11885 (E22, 2009-09)	0.005	g kg ⁻¹
Water/moisture content	VDLUFA Bd. III 3.1 (1976)	0.1	%

2.8. Biomass Growth Measurements

Towards the end of the particular growing season, we recorded plant height and diameter at breast height (i.e., 1.3 m above ground level) for 200 test specimens. Subsequently,

we calculated the mean annual height and diameter growth of the clones and provenances. In addition, we estimated the individual biomass growth rate (aboveground annual woody biomass) using an allometric equation [24–26]. The biometric investigations focusing on 265 Black Locust test trees in the joint project FastWOOD resulted in the allometric equation (Equation (2)) which is tailored toward young Robinia forests on reclamation sites [27,28].

$$BM_{\text{dry matter}} [\text{kg}] = 0.00059909 \cdot d_{1.3}^{2.356} \quad (2)$$

2.9. Quality Assessment

Parallel to growth measurements, the stem form and crown formation were also evaluated. The number of trees with multiple stems, forks and/or ramiforms was assessed as well as trees with stem and bark injuries, and branch and crown fractures.

3. Results and Discussions

3.1. Weather Conditions from 2014–2019

Weather recordings from the time period 2014–2019 presented in Table 4 help to characterise the growth conditions for our Robinia test plants.

Table 4. Monthly sum of rainfall [mm] and average air temperature [°C] throughout the years 2014–2019. Data recorded at FIB weather station *Welzow*, Brandenburg, Germany. The background colour is a stylistic instruments.

	Precipitation [mm]						Air Temperature [°C]					
	2014	2015	2016	2017	2018	2019	2014	2015	2016	2017	2018	2019
January	18.4	60.6	40.4	23.6	40.9	56.3	0.8	2.8	−0.3	−2.4	3.2	0.6
February	13.5	5.2	46.3	32.4	4.7	30.6	4.6	1.3	3.6	2.0	−2.6	3.5
March	24.9	38.0	30.6	37.8	27.1	47.2	7.4	5.7	4.3	6.9	1.4	7.0
April	20.1	28.9	35.6	24.2	31.3	12.3	11.3	8.9	8.5	7.9	13.5	10.4
May	102.3	23.9	29.3	29.0	31.6	61.0	13.3	13.5	14.8	14.7	16.7	12.3
June	41.5	57.2	97.9	106.4	11.5	7.6	17.0	16.3	18.6	18.5	19.0	22.5
July	100.7	62.2	84.9	112.3	33.0	36.9	20.7	20.1	19.2	19.0	20.7	20.0
August	124.9	21.6	39.3	74.4	26.8	24.9	17.1	22.1	17.9	19.0	21.4	20.5
September	79.6	68.7	8.2	27.0	35.0	31.3	15.6	14.0	16.8	13.3	16.0	14.8
October	55.8	42.2	71.0	64.2	20.5	34.3	12.0	8.0	8.6	11.7	11.1	11.2
November	6.5	68.2	35.4	47.3	13.6	20.6	6.7	7.6	3.6	5.5	5.4	6.2
December	36.6	16.8	39.6	41.7	69.1	26.9	2.6	6.6	2.1	2.9	4.3	4.2
Year (Jan–Dec)	624.8	493.5	558.5	620.3	345.1	389.9	10.8	10.6	9.8	9.9	10.9	11.1
Veg. (Apr–Oct)	524.9	304.7	366.2	437.5	189.7	208.3	15.3	14.7	14.9	14.9	16.9	15.9

As shown in Table 4, the total precipitation was considerably lower in 2018 and 2019 than in the years from 2014 to 2017, especially during the vegetation period (Apr–Oct). Indeed, the average precipitation during the vegetation period in 2018 and 2019 was, respectively, 219 mm (−53.5%) and 200 mm (−49.0%) lower than the 2014–2017 average. Compared to the average air temperature in the years 2014–2017, the mean air temperature also increased by 2° K (growth year 2018) and by 1° K (2019). Therefore, in 2018, the young Black Locust trees were faced with the most extreme conditions since their planting in March 2014.

3.2. Chlorophyll a Fluorescence and Photosynthetic Vitality

Results determined from the measurements taken during growth season 2015 and 2016 are presented in the following section. PI_{ABS} , a significant JIP-test parameter which enables the quantification of plants' vitality status at a specific time point, will be addressed in greater detail. PI_{ABS} average values vary across the Black Locust test units, but follow similar sequences and patterns over time (Figures 3 and 4). After starting on a relative low

level in June of both of the test years, average PI_{ABS} values of all the test trees were found to have increased on subsequent occasions.

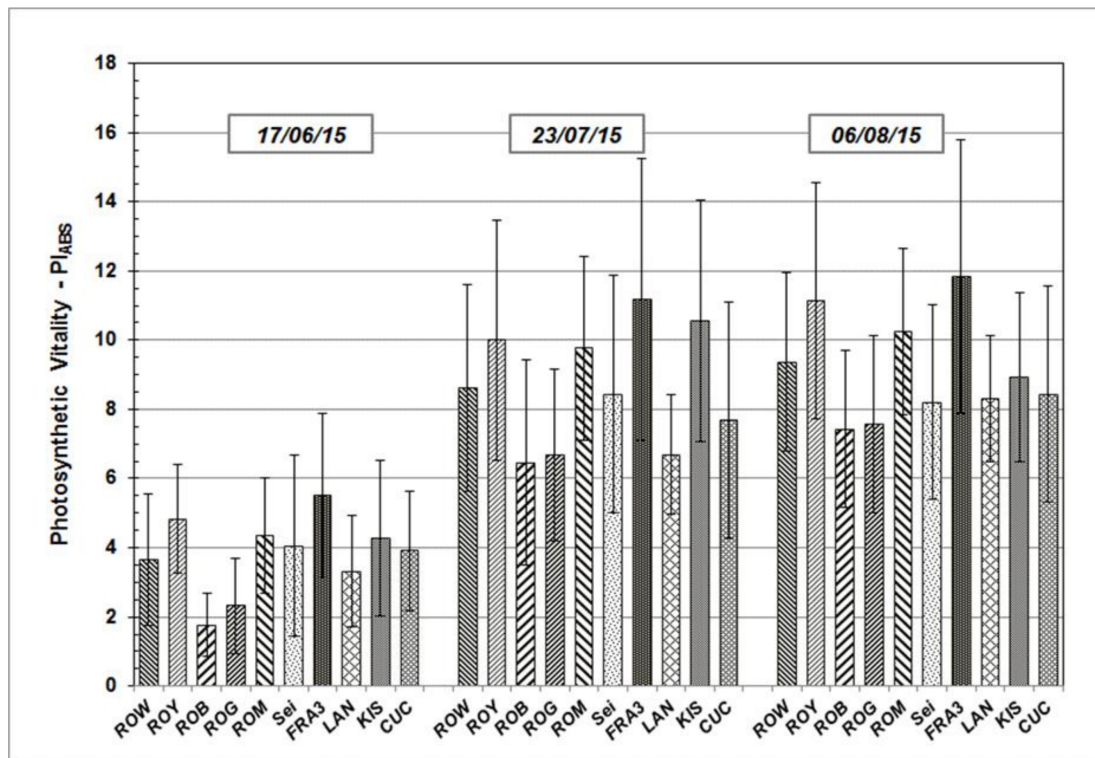


Figure 3. PI_{ABS} mean values and standard deviation of Black Locust clones and provenances during the growth season 2015 at clonal test plot *Welzow*.

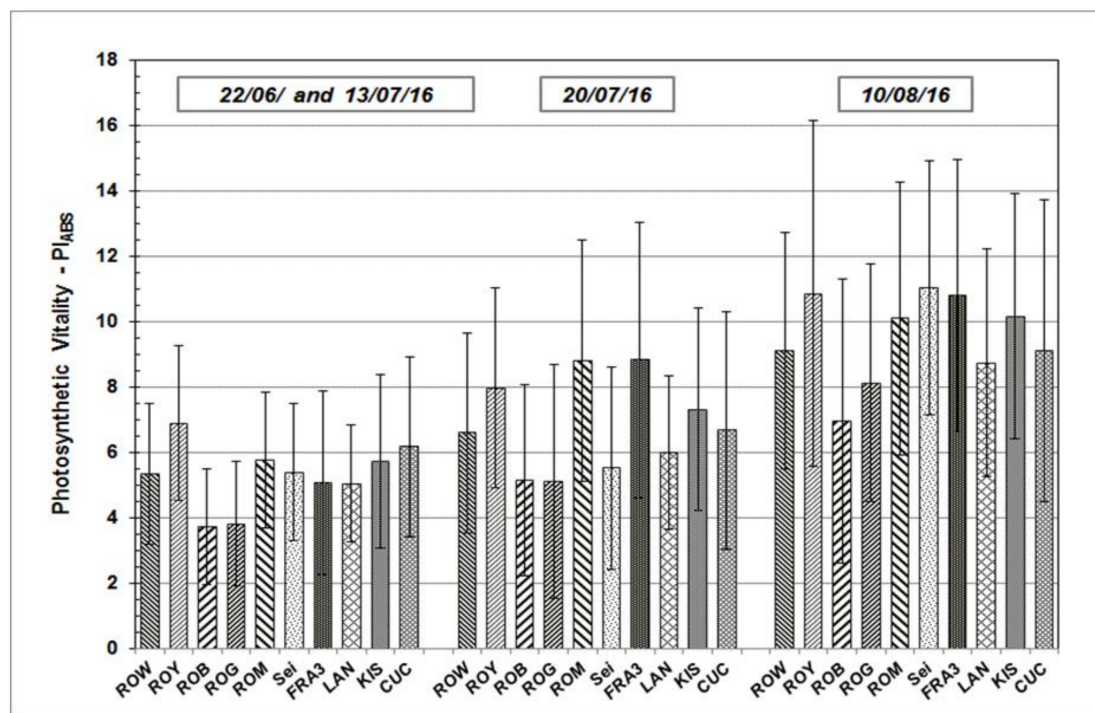


Figure 4. PI_{ABS} mean values and standard deviation of Black Locust clones and provenances during the growth season 2016 at clonal test plot *Welzow*.

As shown, individual PI_{ABS} mean values of each particular Robinia clone and provenance remain more or less the same in their relationship to each other, independent of measuring date and time. The highest average PI_{ABS} values were observed in the test clones *Fra3* and *Roy*. Test trees of *Rog* and *Rob* clones show the lowest PI_{ABS} mean values, indicating a weak photosynthetic vitality and low physiological performance.

3.3. Chlorophyll and Phenol Content

In addition to the chlorophyll a fluorescence described above, we also performed Dualex[®] measurements on exactly the same Robinia leaves. The results of these measurements enable us to check for correlations between parameters originating from different measuring methods. Table 5 contains average values of chlorophyll, flavonol and anthocyanin as well as the above-mentioned NBI collected over 7 measuring dates in 2015 and 2016.

Table 5. Mean values of Dualex[®] parameter determined on Robinia leaves of different clones and provenances at clonal test plot *Welzow* in 2015 and 2016.

		Chlorophyll [$\mu\text{g cm}^{-2}$]		Flavonol [$\mu\text{g cm}^{-2}$]		Anthocyanin [$\mu\text{g cm}^{-2}$]		NBI	
		2015	2016	2015	2016	2015	2016	2015	2016
Row	Avg	37.6	34.3	1.58	1.22	0.025	0.004	24.1	28.6
	SD	2.8	3.6	0.14	0.20	0.017	0.004	2.9	3.7
Roy	Avg	37.5	35.3	1.56	1.22	0.031	0.008	24.3	29.5
	SD	2.9	3.8	0.10	0.20	0.016	0.009	2.6	4.3
Rob	Avg	37.0	37.2	1.68	1.49	0.049	0.023	22.2	25.7
	SD	3.5	3.1	0.13	0.25	0.017	0.023	2.5	4.4
Rog	Avg	34.8	33.8	1.70	1.35	0.050	0.016	20.6	25.9
	SD	2.5	3.8	0.12	0.27	0.023	0.017	2.2	4.2
Romy	Avg	39.5	38.3	1.71	1.48	0.025	0.007	23.1	26.5
	SD	2.2	4.0	0.08	0.26	0.015	0.011	1.6	3.5
Schö	Avg	37.8	37.3	1.58	1.26	0.032	0.004	24.2	30.3
	SD	3.3	3.7	0.12	0.20	0.018	0.008	2.8	4.0
Fra3	Avg	35.5	32.5	1.56	1.33	0.038	0.017	23.0	25.3
	SD	2.1	2.9	0.13	0.25	0.017	0.019	2.8	3.5
Lan	Avg	40.5	38.0	1.40	1.09	0.017	0.005	29.3	36.0
	SD	3.5	4.5	0.11	0.20	0.012	0.008	4.1	6.4
Kis	Avg	38.0	35.3	1.53	1.11	0.026	0.003	25.1	32.6
	SD	5.0	5.1	0.12	0.18	0.027	0.006	4.3	4.7
Cuc	Avg	38.2	36.8	1.63	1.31	0.033	0.010	23.7	29.3
	SD	3.6	4.3	0.13	0.25	0.018	0.013	3.2	6.5
All	Avg	37.6	35.9	1.59	1.29	0.033	0.010	24.0	29.0
	SD	1.7	1.9	0.09	0.13	0.011	0.007	2.3	3.4

When comparing the data collected in 2015 and in 2016, it becomes apparent that both the mean chlorophyll content as well as average flavonol and anthocyanin concentrations were higher in 2015, although the average NBI was found to be lower in 2015. The increase in the plant's own secondary substances in the leaves during growing season 2015 and the decrease in NBI can likely be attributed to the weather conditions (relatively dry and warm August 2015, Table 4).

3.4. Plant Nutrition

As mentioned above, the NBI indicates the nitrogen supply status of the test plant. The nutrient supply of test trees belonging to Robinia clones and provenances *Row*, *Roy*, *Fra3*, *Lan*, *Kis* and *Cuc* is therefore discussed in the next section (Table 6).

Table 6. Results of the determination of plant nutrients in the leaf tissue of Robinia test trees taken on 10 August 2015 and 10 August 2016 at clonal test plot *Welzow*. The font colour and italics are stylistic instruments.

	N_{tot} [w.-%]		P [w.-%]		K [w.-%]		Ca [w.-%]		Mg [w.-%]		S_{tot} [w.-%]	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Row	2.87	3.76	0.12	0.15	1.34	1.35	2.38	1.84	0.24	0.16	0.18	0.19
Roy	3.29	3.70	0.15	0.17	1.25	1.18	1.95	1.55	0.19	0.13	0.20	0.21
Fra3	2.67	3.14	0.11	0.14	0.98	1.22	3.33	2.17	0.30	0.17	0.18	0.21
Lan	3.02	3.97	0.12	0.18	1.33	1.35	2.08	1.40	0.27	0.15	0.19	0.23
Kis	3.21	3.93	0.14	0.17	1.35	1.35	2.02	1.74	0.21	0.15	0.21	0.21
Cuci	3.16	3.55	0.13	0.16	1.26	1.17	2.13	1.76	0.27	0.15	0.25	0.23
Mean	3.04	3.67	0.13	0.16	1.25	1.27	2.32	1.74	0.25	0.15	0.20	0.21
SD	0.23	0.30	0.01	0.02	0.14	0.09	0.52	0.27	0.04	0.01	0.03	0.02

The data presented in Table 6 indicate that with a few exceptions, the uptake of nitrogen, phosphorus, potassium and sulphur in Robinia leaves was slightly higher during the growing season in 2016 than in 2015. In contrast, calcium and magnesium contents were found to have decreased from 2015 to 2016, regardless of plant origin. Test trees of *Kis* and *Roy* displayed the highest N and P concentrations (valid for 2015), whereas *Fra3* and *Row* exhibited the highest Ca and Mg contents in general.

Bearing in mind that the growing season in 2015 was warmer and drier than in 2016 (Table 4), it is highly likely that drought, monitored in late summer 2015, limited nitrogen fixation and resulted in lower N and P content in the Robinia leaf tissue.

Our measured leaf nutrient concentrations correspond closely with previously published data, e.g., [29,30]. Heinsdorf [30] investigated nutrient uptake and nutrient supply of a seven-year-old Black Locust stand growing on a former open cast lignite mining site in East Germany and reported the following leaf analysis data (mean values): N_{tot} 3.64%, $N_{soluble}$ 0.92%, P 0.18%, K 1.19% and Mg 0.16%. Our findings also showed a K-to-Ca-antagonism, as reported by Heinsdorf [30], where high K concentrations in Robinia leaves correspond to low Ca concentrations (Figure 5).

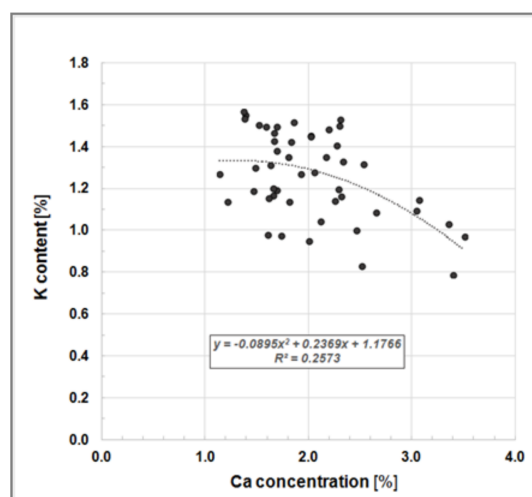


Figure 5. Bivariate scatter plot illustrating the K-to-Ca-antagonism, i.e., a negative relationship between K and Ca concentration in leaves of Black Locust trees growing on clonal test plot *Welzow*.

Furthermore, we examined our data for a systematic relationship between Dualex[®]-derived NBI values and N_{tot} content as well as NBI and the P concentration, respectively (Figure 6).

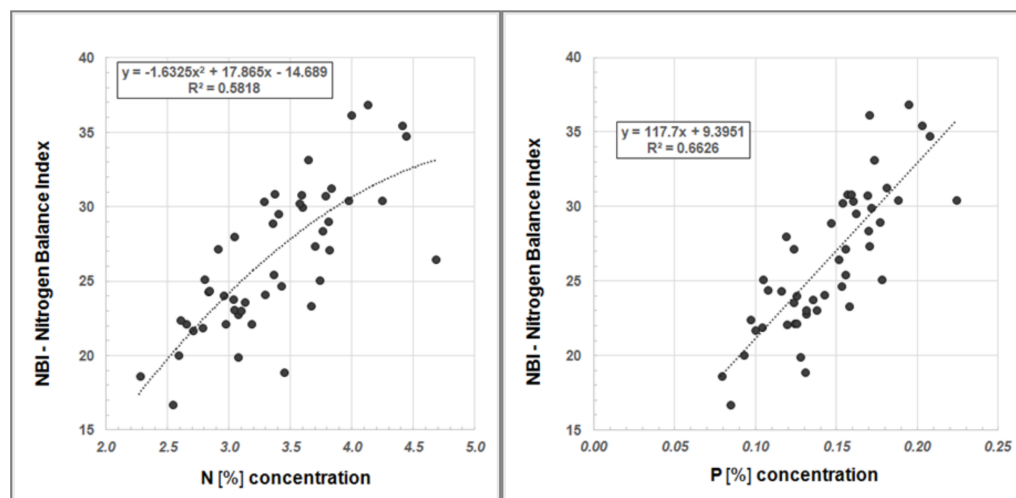


Figure 6. Regression analysis between NBI and N content (**left**) and between NBI and P concentration (**right**) in Robinia leaves.

The regression analyses show a good correlation between the NBI, determined using the Dualex[®], and N_{tot} content in Robinia leaves as well as NBI and P concentration, respectively (Figure 6). Hence, Dualex[®] measurements offer a more cost-effective and time-efficient method for determining the nutrient status of plants.

3.5. Biomass Growth

As already mentioned in the previous section, PI_{ABS} mean values vary depending on the origin of the plant sampled. Generally, it can be stated that higher PI_{ABS} values, indicating greater efficiency in primary photosynthetic processes, are likely to result in greater plant growth. Clear and significant differences can be observed between the clones and provenances with regard to the mean annual height growth (Figure 7).

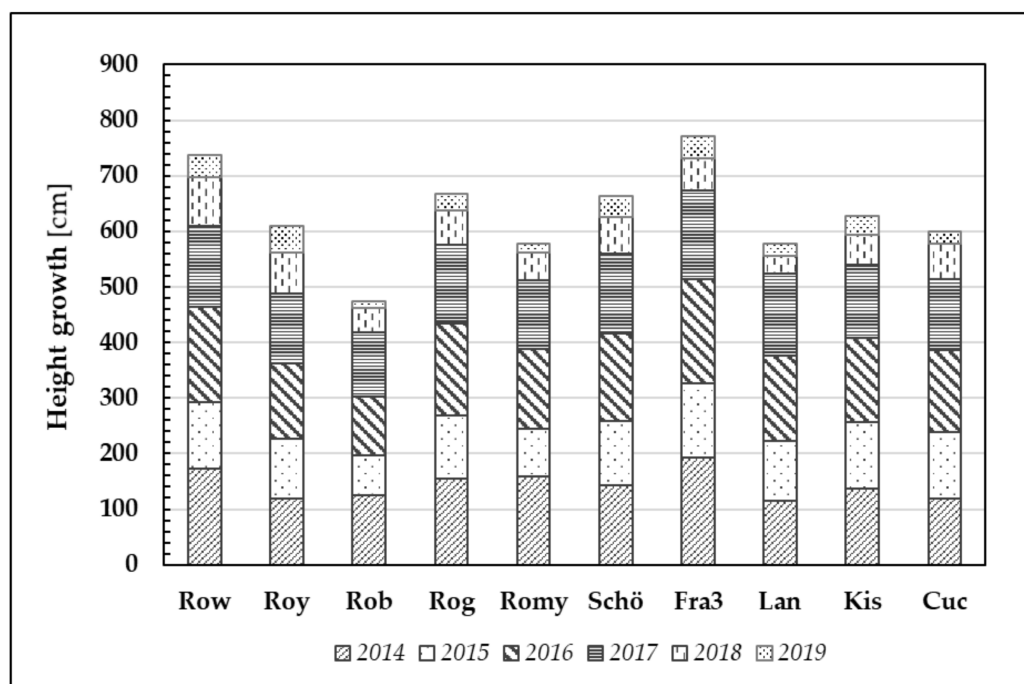


Figure 7. Plant height growth through the years 2014–2019 of ten Robinia test units. Note that one bar usually comprised 20 Robinia test trees per origin and per year.

Clones *Fra3* and *Row* demonstrated the best height growth (mean values) after six years of growth (\varnothing 772 cm and \varnothing 738 cm, respectively). In contrast, the clone *Rob* has the lowest performance (\varnothing 474 cm).

After the first growing season, Robinia test trees gained a height increment of 144 cm (averaged over all trees). Despite the relatively dry year that followed (2015), the trees nonetheless produced 110 cm growth on average. To date, even the 2018 and 2019 growing seasons, namely the driest and hottest vegetation periods, resulted in a minimum average height increment of 59 cm (2018) and 30 cm (2019).

Furthermore, we examined biomass formation during the growth periods of 2014 to 2019 (Figure 8). Annual biomass growth rates vary depending on the weather conditions during the growing season and corresponding to origin. After six years, the mean biomass yield averaged over all tested Robinia clones and provenances was 28.20 Mg d.m. ha⁻¹, whereas the mean annual increment was 4.70 Mg d.m. ha⁻¹ year⁻¹.

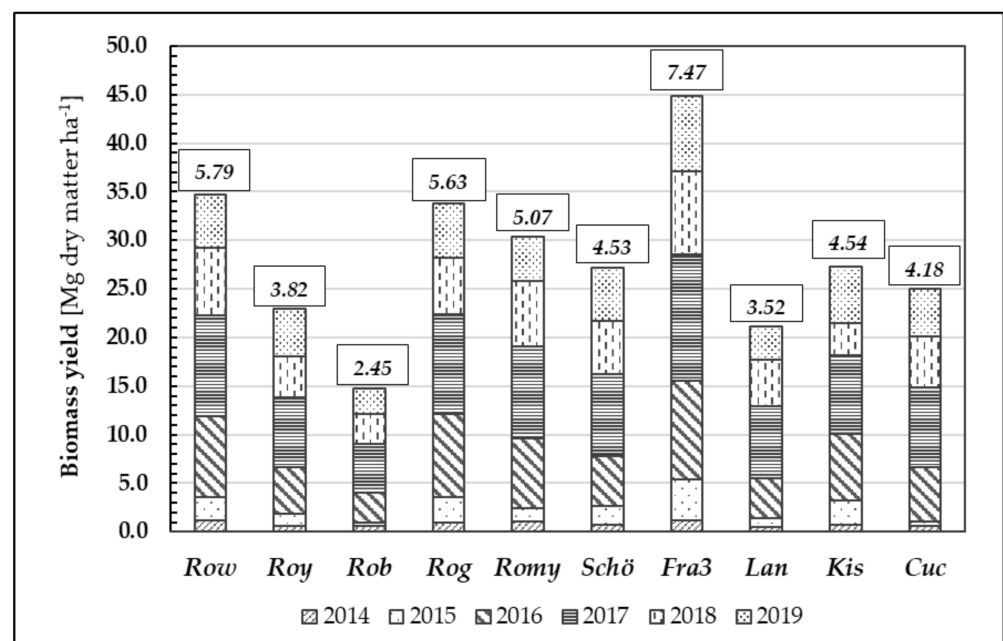


Figure 8. Biomass growth [Mg dry matter ha⁻¹] through the years 2014–2019 of different Robinia test units; data given in small boxes represent individual average increment of biomass [Mg dry matter ha⁻¹] in 6 years of time.

Similar to their performance in height growth, the clones *Fra3* and *Row* achieved the highest mean biomass growth values (7.47 Mg d.m. ha⁻¹ year⁻¹ and 5.79 Mg d.m. ha⁻¹ year⁻¹, respectively) whilst the low-performing clone *Rob* yielded only 2.45 Mg d.m. ha⁻¹ year⁻¹. The differences in biomass yield between the test units were statistically significant.

3.6. Quality Assessment

A quality assessment of all the test trees revealed a frequent occurrence of crooked stem forms and unfavourable crown formation. Altogether, 66.1% of all trees tested showed multiple stem formation with varying severity between individuals. Only 30% of the test trees belonging to clone *Romy* were found to have forks, whereas more than two thirds (83.3%) of the plants of the provenance *Kis* exhibited forks. These findings raise the question of whether it is possible to generate a sufficient number of straight-boled trunks for material utilisation.

4. Discussion

Our results show that the drought, monitored in late summer 2015, is very likely to have caused limitations of nitrogen fixation and resulted in lower N and P content in the Robinia leaves' tissue (Table 6). Such findings have been confirmed by other authors. Mantovani et al. [31], for example, studied carbon allocation, nodulation, and biological nitrogen fixation of two-year-old Black Locust (*Robinia pseudoacacia* L.) saplings under soil water limitation. The authors used stable isotopic composition of C ($\delta^{13}\text{C}$) and N ($\delta^{15}\text{N}$) of the leaves to investigate adverse effects of drought as well as to identify the portion N accrued from the atmosphere by biological nitrogen fixation. They also found that drought stress significantly reduces total aboveground biomass production of the test plants as well as increases the nodule biomass of Black Locust in order to maintain biological nitrogen fixation and counteract the lower soil nitrogen availability.

Regarding adaptation to climate change, Mantovani et al. [32] showed that Black Locust plants can adapt to prolonged drought conditions by lessening water loss through both reduced transpiration and leaf size. However, under well-watered conditions, Robinia does not regulate its transpiration. It, therefore, cannot be considered a water-saving tree species. Veste and Kriebitzsch [7] carried out pot experiments in order to evaluate the growth and ecophysiological performance of Black Locust under drought stress. They demonstrated that when Black Locust is exposed to drought, it drastically reduces leaf area in order to minimise transpiration. Moreover, their test plants showed different adaptations and a high plasticity of the ecophysiological processes to cope with long-term drought stress and high temperatures, which also enables them to grow in drier regions [7]. Bhusal et al. [33] have shown that drought resistance is indicated by leaf mass per area, photosynthetic rate, leaf water potential and further factors. While drought resistance was not concretely explored in this study, it opens avenues to combine our results with investigations into drought response of Black Locust origins in the future.

Furthermore, Seserman et al. [34] pointed out that tree yields in Black Locust short rotation coppices were positively impacted by air temperature increase and negatively by decreasing precipitation.

The reduction in biomass growth of Black Locust during the drought years (Figure 8) is in accordance with previous studies [34,35]. Hence, Mantovani et al. [35] investigated spatial and temporal variation of drought impact on Black Locust's water status and growth. They conducted their study at two different sites: one site with fertile agricultural soil (site 1) and a reclaimed post-mining site with heterogeneous unstructured soil (site 2). They found that stem growth was drastically reduced during a period of summer drought, particularly in the post-mining area, as a result of the adverse edaphic conditions (below the critical pre-dawn water potential value of -0.5 MPa). However, the trees could cope with the extreme soil and weather conditions in the post-mining site without perishing.

Our biomass growth rates are in range with average annual values gained from other sites in the Lusatian post-mining area reported by Knoche et al. [28]. The latter reported biomass yields after six growth years for study site *Drebkau 1* = 37.7 Mg d.m. ha^{-1} (Ø 6.3 Mg d.m. ha^{-1} year^{-1}) and for site *Drebkau 2* = 40.7 Mg d.m. ha^{-1} (Ø 6.8 Mg d.m. ha^{-1} year^{-1}) as well as 19.3 Mg d.m. ha^{-1} (Ø 3.2 Mg d.m. ha^{-1} year^{-1}) for site *Senftenberg*.

To give a comprehensive evaluation of all clones and provenances studied, plant physiological performance, biomass growth and quality data were individually evaluated and ranked (Table 7). Note that parameters given in the table are not weighted and deep balanced but may nonetheless assist in identifying the best Robinia plant material for land users in post-mining areas.

Table 7. Ranking of major plant-physiological, biomass growth and quality parameters of ten Black Locust clones and provenances. The background colour is a stylistic instrument.

	<i>Row</i>	<i>Roy</i>	<i>Rob</i>	<i>Rog</i>	<i>Romy</i>	<i>Schö</i>	<i>Fra3</i>	<i>Lan</i>	<i>Kis</i>	<i>Cuc</i>
PI_{ABS} — <i>photosynthetic vitality</i>	5	2	10	9	3	6	1	8	4	7
Chlorophyll content	8	7	5	9	2	3	10	1	6	4
Phenol content	3	4	10	9	6	5	6	1	2	6
Nutrition supply (only N, P, K)	4	2	-	-	-	-	6	3	1	5
NBI— <i>Nitrogen Balance Index</i>	6	4	9	10	7	3	8	1	2	5
Height growth (2014–2019)	2	6	10	3	9	4	1	8	5	7
Height increment (2018–2019)	1	2	9	5	8	3	4	10	6	7
Total biomass formation (2014–2019)	2	8	10	3	4	6	1	9	5	7
Biomass increment (2018–2019)	2	7	10	3	4	5	1	9	8	6
Multiple stem formation 2019	3	6	5	2	1	6	6	9	10	4
Evaluation stem quality 2019	7	3	9	5	2	4	1	8	10	5
<i>Final Rank</i>	1	5	10	6	4	2	2	9	7	8

5. Conclusions

Our study reveals pronounced differences in the physiological performance, biomass growth and stem quality of the Black Locust clones and provenances studied. Under the challenging climatic and edaphic conditions of our test site—considering photosynthetic vitality, chlorophyll and phenol content, nutrition state, biomass growth and stem quality—Robinia genotypes *Rowena*, *Fra3* and *Romy* as well as the Brandenburg provenance *Schöneiche* show the most promise with regard to growth performance, especially for cultivation in short coppice rotation and on forest land.

Biophysical measurements using the Pocket PEA and Dualex[®], especially when combined and used in parallel, are reliable indicators for detecting abiotic stress already in an early stage. We were able to show that while mean PI_{ABS} values vary across Black Locust test units, they still follow similar sequences/patterns. We found correlations between biophysical parameters resulting from chlorophyll a fluorescence and Dualex[®] measurements. Correlation analysis revealed good accordance of Dualex[®]-derived NBI (Nitrogen Balance Index) and N_{tot} content as well as P content, detected in Robinia leaves. Hence, using Dualex[®] measurements can provide insights into the nutrient status (especially nitrogen) of plants in a cost and time efficient manner.

We conclude that biophysical measurements have the potential to shorten otherwise long-lasting research plans in tree breeding. In addition, biophysical measurements enable the early assessment of the physiological performance and stress tolerance of different Robinia clones and provenances.

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