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Biomass Losses Caused by *Teratosphaeria* Leaf Disease in *Eucalyptus globulus* Short Rotation Forestry

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Abstract: This article presents the results of a study that examines the loss of biomass and energy, per hectare, caused by *Teratosphaeria* leaf disease (TLD) in *Eucalyptus globulus* short rotation forestry. The 95 *Eucalyptus globulus* taxa analyzed are from seeds of open pollinated families of both Spanish and Australian origin. Tree height and diameter were measured and the crown damage index (CDI) assessed at 27 months of age. Taxa that have a certain tolerance to the disease have been identified. The taxon identified as code 283 is the one that shows lower CDI (42%) and with an average volume that exceeded 0.017 m^3 at 27 months of age. Biomass losses were determined for each fraction of dry biomass of the tree (leaves, branches, twigs and bark) based on CDI. These losses were translated into terms of energy lost per hectare, depending on the CDI. A comparison was then carried out between the productivity of *Eucalyptus globulus* exhibiting various levels of TLD severity and poplar and willow clones used for bioenergy in Europe. In our region, the results show that despite the losses of biomass associated with TLD, *Eucalyptus globulus* remains competitive as long as CDI values are lower than 56%.

Keywords: biomass losses; short rotation forestry; *Eucalyptus globulus*; *Mycosphaerella* leaf disease; energy density

1. Introduction

The replacement of fossil fuels by bio-based energy sources contributes to a more sustainable world [1]. Spain is a country with huge foreign energy dependence, however, it has a great deal of potential energy that could be derived from renewable resources. For the particular case of Cantabria in northern Spain, forest based biomass has a promising future since adequate soil and climatic characteristics are present. The region has an extensive non-exploited forest area which may be used for new plantings with energy purposes.

Biomass is the third largest source of energy in the world [2]. In addition to abundance, biomass offers great versatility when being used as primary energy for the generation of electricity, heat or fuel for transportation [3]. One way to generate biomass is through short rotation forestry (SRF). These are characterized by fast growing species used in planting densities ranging from 1000 to 20,000 stools ha^{-1} in poor soils and reduced short tree shifts. Some examples are species belonging to the genera *Populus*, *Eucalyptus*, *Pinus*, *Acacia* and *Salix* [4–13]. Most research today involves the genera *Populus* and *Salix*, commonly known as poplar and willow. In fact, clones of these genera have been specifically selected for biomass generation in short rotations [14–19]. Research has focussed on these genera due to interest of countries of northern Europe and America where the amount of water that these species require during the vegetative stage occurs naturally.

The genus *Eucalyptus* performs exceedingly well as an energy crop in temperate forests, such as those of northern Spain, where water availability is a limiting factor for the growth of poplar and willow during the spring and summer [10,11,20]. The appropriateness of this genus is justified from the production and the energy point of view, since it combines high density biomass [21] and good calorific values [22,23]. At present, the superiority of the genus *Eucalypus* to generate biomass in SRF is limited by the appearance of a biotic agent that produces the disease known as *Theratosphaeria Leaf Disease* (TLD), especially in the *Eucalyptus globulus*. The genus *Eucalyptus* can suffer from a large number of fungal leaf diseases, however TLD is seen as the most serious [24–27]. A single *Theratosphaeria* species, *Theratosphaeria nubilosa,* is responsible for the bulk of the damage to *Eucalyptus* trees in Spain. Infection of leaves occurs when acospores germinating on the leaf surface produce germtubes which enter the leaf via stomata [28]. The most intense attack occurs during the months of late summer and early autumn, while a recovery of the tree normally occurs in springtime. There are many studies in the scientific literature that examine the impact and control of diseases on forest trees that generate energy in short cycles [29–33]. These studies, mainly based on the genera poplar and willow, examine ways to manage diseases by the use of chemicals or by selection of genotypes tolerant to pests and diseases.

Harvest age ranges from two to four years. *Eucalyptus* species are characterized by two types of foliage over their lifetime: juvenile and adult foliage. TLD affects juvenile foliage causing extensive defoliations and a marked growth reduction, which, in combination with frost, can kill the tree [28,34–39]. It is worth highlighting that the juvenile stage of the species is of greatest interest for energy crops, because of short tree shifts. This article is the result of a research project that began in 2006 with the establishment of a trial with genetic material from *Eucalyptus globulus* stands of Australian and northern Spanish origin. Each family is identified by a code. The goal was to compare the losses of biomass and energy, per hectare, versus the degree of importance of the disease. This will allow the productivity of this genus to be evaluated respect to other species used in SRF. The experimental results enabled us to identify those codes showing some tolerance to TLD. At 27 months of age, for each code, the heights and diameters of the trees have been measured, obtaining the corresponding volume. At this time, the crown damage index was defined and evaluated and also assessed. The biomass loss as a function of CDI was then determined for each fraction that forms the tree (leaves, branches, twigs and bark), and the total loss of biomass per hectare. Productivity (t ha^{-1}) and energy losses (Megajoules ha^{-1}) have been calculated based on the CDI. This allows the calculation of CDI levels below which the cultivation of *Eucalyptus globulus* can be viable and/or comparable with clones of poplar and willow used in short rotation coppice. This can be a first step in obtaining tolerant genetic material that can be used to generate biomass in areas with prevalence of TLD.

2. Materials and Methods

In March and April 2006, a *E. globulus* short rotation stand was established in Cantabria (northern Spain), latitude 43°28′ N, longitude 3°48′ W at 120 meters above sea level. This period is very suitable for the development of this species and *TLD* appears to be the single most limiting biotic agent. The site is characterised by a climate with moderate temperature variation and regular rainfall. Long-term values for mean air temperature and annual rainfall are 13.8 °C and 598 mm respectively. The stand consists of 2375 trees belonging to taxa from two sources: Australian (50 taxa), supplied by CSIRO Forestry, and Spanish (45 taxa), obtained from seeds of trees from forests in northern Spain. Both sources are from open-pollinated families. The trial contained 25 replicates with 95 individuals per replicate. Each replicate contained one individual (code) arranged at random. The stand frame used was 2.5 × 2.5 m which corresponds to 1600 plants per ha.

At the time of planting, the soil was fertilized with 20–30 g per plant of a controlled release fertilizer 11-22-9 (NPK) + 6 MgO. At the age of one year, the soil was again fertilized with 300 g per plant of complex fertilizer 15-15-15 (NPK).

At 27 months of age, for each tree, one branch was taken randomly at breast height. Once each branch was cut, they were then transported to the laboratory in a sealed polyethylene bag. Simultaneously, the degree of defoliation (D, %) due to the disease was evaluated in the field using the diagrams given by [39,40] for this purpose. Once in the laboratory, the samples were evaluated for severity (S, %), defined as the percentage of leaf area affected [39,40]. Taking into account the severity and the defoliation, the overall rate of damage Crown Damage Index, (CDI, %) is defined by the expression (1) encompassing both variables [41]. Obviously, the severity affects only the leaves that have not yet fallen.

$$\mathrm{CDI} = D + \frac{S(100 - D)}{100} \tag{1}$$

Height (H, m) and diameter (D, m) was measured using a laser hypsometer Vertex and a mechanical calliper respectively. In order to calculate the volume (V, m^3) with bark, the formula given by [42] based on the total height and diameter at breast height over bark (DBHOB), was followed. From the volumes, the amount of biomass lost as a function of CDI due to TLD was determined. For this, the weight of dry biomass of each fraction "i" (W_i, kg) was first calculated for each code, using the expression (2) [43] and the parameters in Table 1. The amount of biomass lost was obtained by means of the difference between the CDI zero (obtained by regression) and the CDI evaluated for each code.

$$W_i = \exp^{(\alpha + \beta \ln D + \gamma \ln H)} \tag{2}$$

Table 1. Regression coefficients for *Eucalyptus globulus* stands [43].

Fraction	α	β	γ
Total biomass	−2.8982	0.1984	1.7425
Leaves	0.7897	0.2921	0.8769
Wood + bark	−6.8579	0.2474	2.2294
Rest	−2.5669	0.3346	1.3349

From the amount of biomass calculated, the loss has been estimated for each CDI.

In order to determine the amount of energy loss, the gross and the net calorific values (*GCV* and *NCV*) of *Eucalyptus globulus* in the juvenile stage have been obtained using the method proposed by [44] in a calorimeter. The characteristics of the apparatus and the methodology used are described in [23].

The average *NCV* of the fractions (dry biomass) that make up the biomass of *Eucalyptus globulus* at juvenile age, and that were studied in the laboratory [23]. For the calculation of the weighted average value of *NCV*, the weight percentage of each fraction of the tree, given by [45], has been taken into account. These percentages are related to Cantabria since they were determined in juvenile stands of north western Spain. Studies carried out elsewhere [46] show that the weight percentages of tree fractions vary with age and diameter, however, this effect is neglectable in SRF.

Data analyses were performed using the Statistical package SPSS (PASW) 18.0 (SPSS Inc., Chicago, IL, USA), comparing CDI means and tree volume for the different codes analyzed.

The experimental results enabled us to identify those codes showing some tolerance to MLD. At 27 months of age, for each code, the heights and diameters of the trees have been measured, obtaining the corresponding volume. At this time, the Crown Damage Index (CDI) was defined, evaluated and also assessed. The biomass loss as a function of CDI was then determined for each fraction that forms the tree (leaves, branches, twigs and bark), and the total loss of biomass per hectare. Productivity (t ha^{-1}) and energy losses (MJ ha^{-1}) have been calculated based on the CDI. This allows the calculation of CDI levels below which the cultivation of *Eucalyptus globulus* can be viable and/or comparable with clones of poplar and willow used in short rotation coppice. This can be a first step in obtaining tolerant genetic material that can be used to generate biomass in areas with prevalence of MLD.

3. Results and Discussion

Table 2 shows the results of the measurements in the stands. Heights and diameters are related to the CDI at the age of 27 months. The first column gives the origin identification code. ANOVA revealed significative differences in the CDI mean values for the different codes (p-value = 0.05). The average value of CDI is 60.64%, however, the codes 283, 105, 255, 102 and 341 present severities below the average, with values of 42.67, 49.03, 51.09, 52.09, 52.40% respectively. This study shows that there are some codes less sensitive to TLD than others. In the scientific literature, this variation in the resistance to the disease is associated to genetic factors and not environmental ones [27,38,47,48]; this way, tolerant plants show this property everywhere.

There are significative differences between the average volumes achieved at 27 months by each code (p-value = 0.001). The codes previously mentioned achieved an average volume of 17.13×10^{-3}, 14.99×10^{-3}, 10.96×10^{-3}, 10.82×10^{-3} and 11.04×10^{-3} m^3, respectively, which can be compared with the average value of 9.20×10^{-3} m^3. There is also a significative negative correlation ($r = -0.638$; $p < 0.0001$) between the individual average volume at 27 months and the CDI. This means that those codes showing lower sensitivity to TLD are the most suitable for the biomass generation. In experimental stands with no damage from TLD established in previous years, average volumes of only 9.7×10^{-3} m^3 per tree were obtained at 49 months of age without fertilisation. The high volumes we obtained in this study are due to the fertilization provided in conjunction with the soil quality in the stands on which this study is based. These two factors enable a swift recovery from the attack of TLD and permit the subsequent development of the tree during the second spring, allowing the change from youth to adult leaf at a younger age. As a result, these data cannot be compared with those of other stands with different bioclimatic features and fertilizations. However, one can compare the effects of TLD among the various families included in this study and provide an estimate of the biomass loss in terms of TLD damage.

Table 2. Mean, standard deviation (Std Dev) and standard error mean (Std Err Mean) of heights (H), diameters (D), volumes ($V \times 10^{-3}$) and CDI for all codes of *Eucalyptus globulus* at 27 months of age.

Code	H (m)	Std Dev	Std Err Mean	$D \times 10^2$ (m)	Std Dev	Std Err Mean	$V \times 10^3$ (m^3)	Std Dev	Std Err Mean	CDI (%)	Std Dev	Std Err Mean
32	4.22	1.06	0.249	3.34	1.07	0.252	6.32	2.69	0.635	67.05	7.42	1.749
65	5.19	1.14	0.238	4.33	1.31	0.272	9.65	4.44	0.926	56.26	15.46	3.225
68	4.93	1.31	0.272	3.97	1.36	0.284	8.60	3.96	0.826	64.88	8.66	1.806
86	4.64	1.34	0.292	3.76	1.48	0.323	7.97	4.50	0.981	59.72	14.30	3.121
89	4.61	1.04	0.216	3.74	1.06	0.220	7.47	2.92	0.609	62.91	6.65	1.387
90	5.07	1.25	0.272	4.10	1.26	0.274	8.94	3.81	0.831	66.30	8.18	1.785
92	4.88	1.08	0.221	3.88	1.02	0.207	8.03	2.95	0.602	63.77	8.30	1.694
96	4.72	0.95	0.203	3.82	0.96	0.205	7.68	2.99	0.637	62.29	8.64	1.842
101	4.80	0.92	0.196	3.87	1.15	0.246	8.00	3.52	0.751	63.90	5.90	1.257
102	5.03	1.58	0.372	4.65	1.64	0.397	10.82	5.93	1.439	52.09	19.65	4.632
104	5.61	0.97	0.206	4.89	1.18	0.251	11.44	4.02	0.856	57.33	12.87	2.744
105	6.00	1.27	0.259	5.65	1.80	0.367	14.99	7.68	1.568	49.03	17.98	3.670
152	5.05	1.96	0.408	4.76	1.63	0.355	11.47	5.71	1.245	60.75	9.97	2.125
213	4.02	1.24	0.358	3.04	1.06	0.305	5.70	2.83	0.816	68.40	8.28	2.391
216	4.76	0.99	0.197	3.89	0.93	0.187	7.84	2.81	0.561	62.60	7.31	1.462
223	5.38	1.32	0.269	4.44	1.47	0.301	10.40	5.73	1.170	63.33	5.42	1.107
225	5.65	0.89	0.182	4.68	0.93	0.189	10.78	3.45	0.704	61.83	8.71	1.779
232	4.89	1.19	0.239	4.06	1.16	0.232	8.55	3.69	0.738	63.50	7.49	1.497
235	5.23	0.87	0.186	4.36	0.86	0.183	9.39	3.12	0.665	64.01	7.00	1.493
238	5.31	1.36	0.278	4.49	1.30	0.265	10.24	4.73	0.965	60.58	10.91	2.227
239	5.71	1.39	0.279	5.25	1.32	0.269	13.13	6.13	1.251	60.72	8.54	1.744
241	5.40	1.15	0.235	4.63	1.13	0.231	10.48	3.93	0.803	54.35	13.29	2.712
246	5.32	1.00	0.199	4.49	1.05	0.210	9.97	3.94	0.788	62.22	6.81	1.362
248	4.76	1.27	0.253	3.79	1.23	0.246	7.92	3.55	0.710	58.46	12.70	2.540
255	5.29	1.30	0.265	4.68	1.60	0.326	10.96	6.28	1.281	51.09	13.83	2.823
256	4.73	0.95	0.198	3.82	0.95	0.197	7.66	2.58	0.537	63.14	7.15	1.491
257	4.76	1.00	0.204	3.72	1.08	0.220	7.65	3.45	0.705	62.74	6.96	1.420
259	4.91	1.19	0.254	4.15	1.16	0.247	8.77	4.00	0.852	61.26	8.78	1.873
261	5.43	0.89	0.186	4.60	1.00	0.209	10.31	3.72	0.775	57.62	11.73	2.447

Table 2. *Cont.*

Code	*H* (m)	Std Dev	Std Err Mean	$D \times 10^2$ (m)	Std Dev	Std Err Mean	$V \times 10^3$ (m^3)	Std Dev	Std Err Mean	CDI (%)	Std Dev	Std Err Mean
265	5.48	1.01	0.207	4.68	1.25	0.255	10.76	3.93	0.802	62.47	8.70	1.775
267	5.82	1.18	0.245	4.89	1.23	0.257	11.91	4.99	1.041	56.33	9.26	1.932
270	5.30	1.06	0.226	4.40	0.98	0.209	9.69	3.86	0.822	56.88	18.17	3.874
271	4.98	1.18	0.240	4.10	1.17	0.245	8.93	4.16	0.866	61.05	11.97	2.444
275	5.02	0.97	0.199	4.35	1.12	0.228	9.16	3.24	0.661	59.63	8.16	1.665
279	5.15	0.93	0.198	4.05	0.84	0.179	8.60	2.82	0.600	64.83	6.15	1.311
282	4.96	1.28	0.280	4.14	1.28	0.280	8.91	3.88	0.847	63.33	9.76	2.130
283	6.24	1.19	0.248	6.18	1.63	0.340	17.13	8.22	1.714	42.67	16.59	3.460
286	5.06	1.24	0.259	4.33	1.26	0.262	9.46	4.61	0.962	55.45	12.91	2.691
287	4.93	0.95	0.202	4.20	1.20	0.256	8.86	3.86	0.822	58.50	9.80	2.090
338	5.11	1.01	0.212	4.36	1.26	0.263	9.55	4.88	1.017	57.60	10.10	2.107
339	5.26	1.12	0.228	4.30	1.24	0.253	9.62	4.30	0.878	59.28	14.57	2.973
340	4.87	0.70	0.146	4.02	0.77	0.160	8.10	2.42	0.505	64.96	8.38	1.747
341	5.36	1.16	0.237	4.82	1.31	0.267	11.04	4.46	0.911	52.40	10.80	2.204
342	4.44	1.62	0.324	3.76	1.79	0.358	8.19	6.24	1.247	59.50	14.21	2.843
343	4.41	1.32	0.270	3.71	1.12	0.233	7.44	3.38	0.706	58.88	12.36	2.577
345	4.93	1.29	0.268	3.93	1.42	0.297	8.64	4.83	1.006	65.38	7.97	1.662
346	4.78	1.12	0.233	4.20	1.30	0.270	8.78	4.32	0.902	58.30	10.57	2.204
347	4.64	1.58	0.322	3.48	1.57	0.321	7.66	4.97	1.014	62.27	8.35	1.704
348	5.32	1.25	0.272	4.31	1.19	0.259	9.71	4.28	0.933	66.70	5.90	1.287
349	4.88	1.17	0.245	4.34	1.15	0.239	9.09	3.85	0.803	59.27	10.44	2.226
350	5.13	0.97	0.199	4.09	0.94	0.191	8.74	3.18	0.650	65.03	6.02	1.228
351	4.62	1.00	0.224	3.96	1.29	0.288	8.03	3.56	0.797	55.39	13.16	2.944
352	5.41	1.59	0.331	4.71	1.78	0.372	11.42	5.56	1.159	58.52	11.18	2.331
353	5.46	1.46	0.291	4.53	1.26	0.253	10.56	5.60	1.119	59.96	9.56	1.912
354	4.77	1.09	0.227	3.91	1.00	0.209	7.95 [a]	3.08	0.643	60.73	9.30	1.939
355	5.13	1.03	0.215	4.25	1.11	0.232	9.20	3.79	0.790	59.97	10.46	2.182
356	5.06	1.13	0.230	4.34	1.32	0.270	9.47	4.09	0.834	62.50	6.26	1.277
357	5.23	1.17	0.239	4.45	1.28	0.262	10.03	5.40	1.103	59.46	10.21	2.084
358	5.02	1.67	0.340	4.32	1.42	0.296	9.87	5.65	1.178	61.46	9.22	1.883
359	4.79	1.05	0.215	3.85	1.09	0.222	7.92	3.23	0.659	64.98	7.18	1.466
360	5.02	0.77	0.157	4.35	0.92	0.188	9.07	2.90	0.593	59.60	7.56	1.544
361	5.18	1.45	0.295	4.20	1.50	0.307	9.67	5.85	1.194	65.01	7.51	1.534
362	4.72	0.96	0.192	3.79	1.02	0.205	7.66	3.00	0.600	63.99	10.66	2.131
363	4.61	1.42	0.295	3.87	1.45	0.302	8.10	4.24	0.883	62.21	7.37	1.538
364	5.71	0.77	0.157	5.17	1.19	0.244	12.31	4.63	0.945	55.40	14.07	2.872
365	4.56	1.11	0.226	3.43	1.03	0.210	6.86 [a]	2.67	0.545	66.15	10.11	2.063
366	4.65	1.47	0.300	4.06	1.48	0.303	8.60	4.66	0.951	57.82	8.73	1.782
367	4.85	1.23	0.251	4.12	1.34	0.274	8.77	4.30	0.877	59.33	11.42	2.382
368	5.54	0.87	0.177	4.45	0.82	0.167	9.98	3.13	0.638	65.95	5.10	1.040
370	4.74	1.13	0.303	4.04	1.17	0.311	8.30	3.66	0.978	61.01	4.22	1.127
372	4.80	0.85	0.185	4.24	1.31	0.287	8.80	4.00	0.872	55.71	11.00	2.401
377	4.53	0.94	0.200	3.57	0.97	0.206	7.03	3.00	0.639	60.88	7.29	1.555
379	5.28	0.97	0.194	4.36	1.05	0.210	9.60	3.95	0.790	61.44	8.64	1.728
380	5.36	1.48	0.309	4.50	1.04	0.221	10.37	3.78	0.806	63.72	9.33	1.946
381	5.30	0.89	0.186	4.81	1.07	0.223	10.64	3.64	0.759	57.32	7.76	1.619
384	5.06	1.22	0.244	4.48	1.52	0.305	9.97	4.92	0.984	60.10	11.18	2.237
388	5.13	1.35	0.276	4.13	1.31	0.267	9.13	3.69	0.753	64.43	9.02	1.881
389	5.50	1.11	0.222	4.38	1.10	0.220	10.05	4.58	0.916	62.28	7.69	1.538
390	4.94	1.15	0.239	4.16	1.01	0.210	8.70	3.39	0.707	62.99	7.89	1.645
391	5.47	0.99	0.203	4.59	1.10	0.225	10.46	4.13	0.842	64.30	4.30	0.878
393	4.24	1.45	0.303	3.64	1.54	0.327	7.46	4.34	0.925	60.87	10.10	2.106
395	5.31	0.89	0.239	4.51	0.94	0.252	9.89	3.79	1.014	60.34	8.06	2.153
402	4.70	1.15	0.235	3.63	1.11	0.227	7.44	3.27	0.667	66.09	7.17	1.464
403	5.09	1.13	0.247	4.19	1.28	0.279	9.14	3.98	0.868	62.31	12.54	2.736
404	4.67	1.11	0.232	3.82	1.21	0.253	7.84	3.64	0.759	61.36	9.14	1.906
405	5.05	1.16	0.231	4.14	1.13	0.225	8.91	3.90	0.779	57.36	14.28	2.856
406	4.62	1.36	0.284	3.86	1.49	0.312	8.18	5.12	1.067	61.64	8.99	1.875
407	5.04	1.47	0.307	4.12	1.25	0.261	9.03	4.50	0.938	60.14	12.51	2.609
408	5.06	1.09	0.227	4.24	1.34	0.280	9.22	3.93	0.819	63.20	7.07	1.474
410	4.96	1.25	0.267	4.00	1.36	0.291	8.75	5.00	1.065	64.13	11.95	2.547
411	5.52	1.35	0.270	4.83	1.62	0.325	11.80	6.83	1.366	53.29	16.84	3.368
412	5.44	1.09	0.233	4.58	1.35	0.289	10.68	5.78	1.232	59.35	11.57	2.467
423	5.18	1.35	0.296	4.54	1.50	0.327	10.35	5.22	1.139	58.69	11.58	2.526
424	3.94	1.46	0.344	3.02	1.27	0.298	5.81	3.70	0.872	63.77	13.40	3.159
425	3.28	0.44	0.139	2.28	0.43	0.137	3.74	0.74	0.235	63.25	7.46	2.359

Figure 1 shows the weight percentage of leaf loss over a null CDI. It is worth noting that as the CDI increases the leaf loss is more pronounced, reaching values close to 48% for CDI above 60%. In more specific terms, at 27 months, the average loss of leaves per tree is approximately 1.5 kg. This loss not only results in a reduction of biomass and of tree growth, but also can favour the development of other pathogens due to the weakness of the tree after the first attack of TLD, and could possibly lead to the tree's death [49].

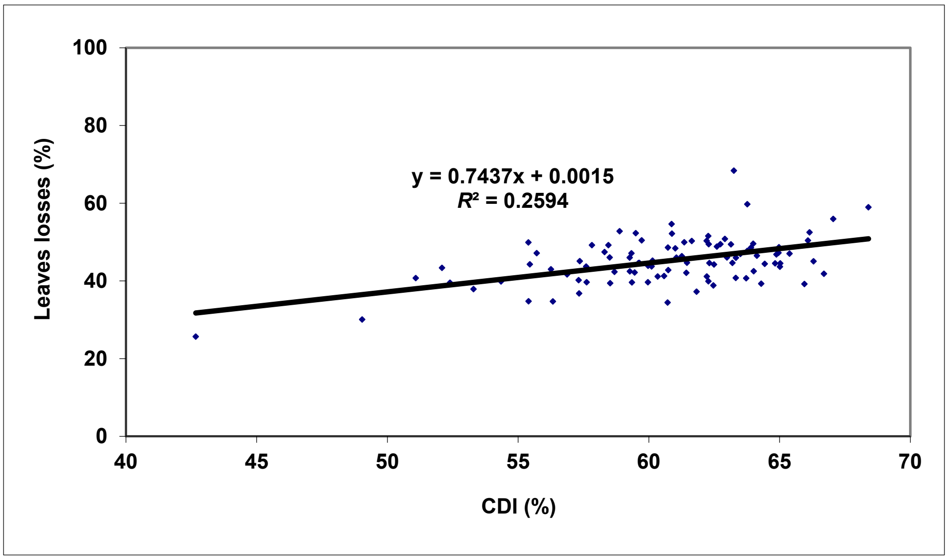

Figure 1. Leaves losses at 27 months of age according to the CDI.

Similarly, Figure 2 shows the loss percentage of wood with bark. It can be observed that the loss of this fraction at a CDI of 60% is between 50% and 60% in weight. Thus, for juvenile *Eucalyptus globulus* with 60% CDI, the average losses of dry wood and bark per tree are about 3 kg.

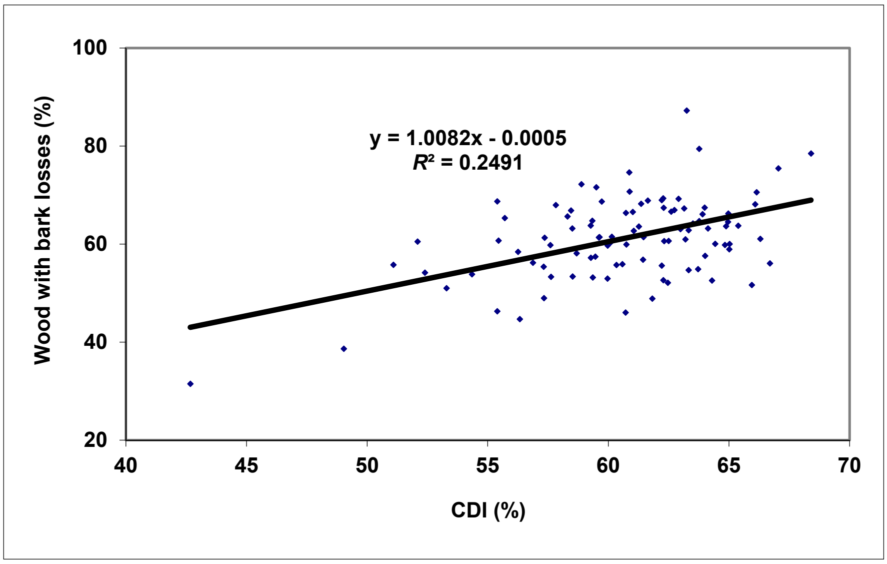

Figure 2. Wood with bark loss at 27 months of age according to the CDI.

Regarding the branches and twigs fraction, Figure 3 shows the results of the losses depending on the CDI value. For a 60% CDI, the average loss percentage reaches 50%, compared to a CDI of zero. This implies a loss per tree of around 1.25 kg of branches and twigs for this species, at 27 months of age.

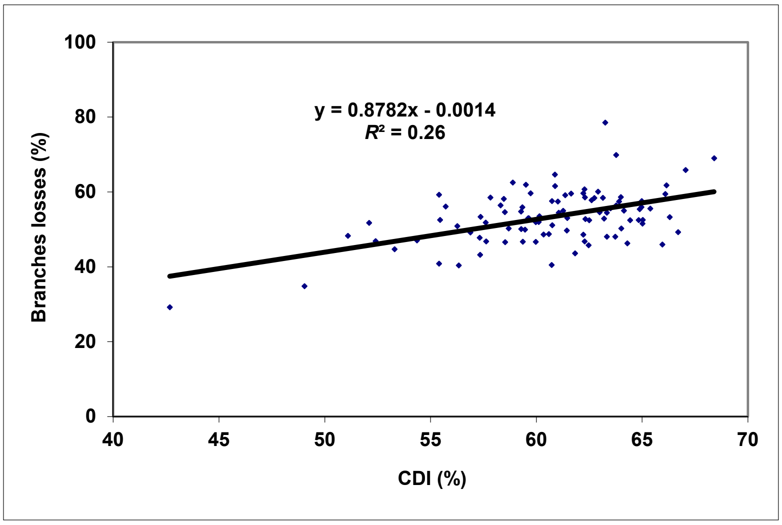

Figure 3. Branch and twig loss percentage at 27 months of age, according to the CDI.

Comparing Figures 1–3, it can be seen that losses increase with increasing CDI, and that wide differences appear between codes. For example, code 241 incurs lower losses than other codes with a similar CDI, and it exhibits comparable losses to other codes with lower CDI levels. For a given CDI, the disease causes greater loss percentages in wood and bark than in the other fractions that comprise the tree. Figure 4 brings together all the biomass (leaves + branches + twigs + bark) representing the weight in kg of dry biomass per tree, depending on the CDI. For a CDI of around 42%, the dry biomass production at the age of 27 months is more than 8 kg per tree, whereas for CDI higher than 66%, the dry biomass production is lower than 3.8 kg per tree. These results show a 200% difference in production when the CDI varies by only 20%. This fact suggests a possible method of selection for the future, based on the observation that distinct families or individuals present a certain tolerance to TLD. They could be the genetic basis of viable energy stands of *Eucalyptus globulus* in the future. In our case, the codes 105 and 283 are those that generate more biomass in areas with high prevalence of TLD, manifesting a certain tolerance to the disease.

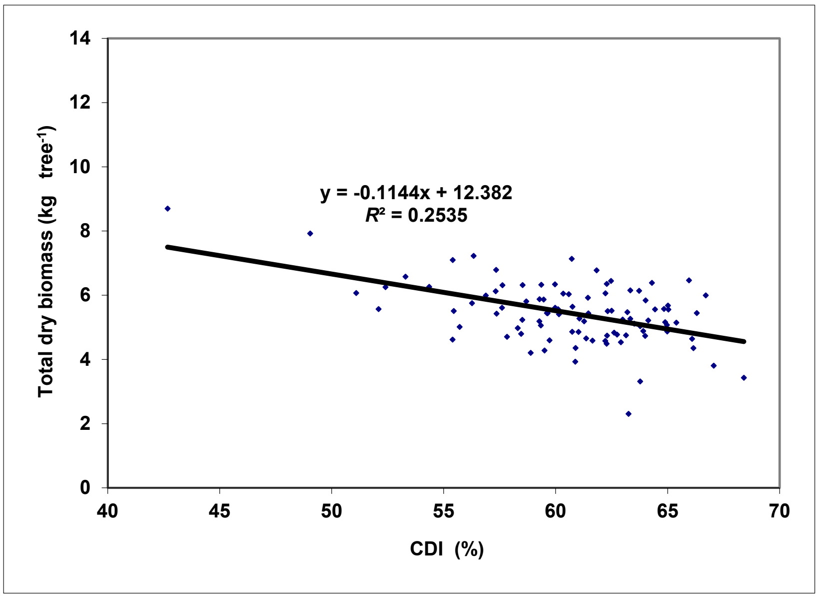

Figure 4. Total weight of dry biomass per tree depending on the CDI (age 27 months).

Considering the data in [23] and the biomass losses, the amount of energy lost (MJ ha^{-1}) in a juvenile stage *Eucalyptus globulus* stand, based on the CDI, has been estimated (see Figure 5). A planting density of 1600 stems ha^{-1} and a rate (number of trees that mortalyted 12 months of age) of 10%, were taken into account. This death rate can be considered as an extensively managed plantation representative of an *E. globulus* stand that has not been damaged by external agents. The results shown in Figure 5 relate biomass losses with energy losses. In our case, it is observed that between CDI$_{maximum}$ and CDI$_{minimum}$ the losses range between 83,000 and 184,000 MJ ha^{-1} respectively.

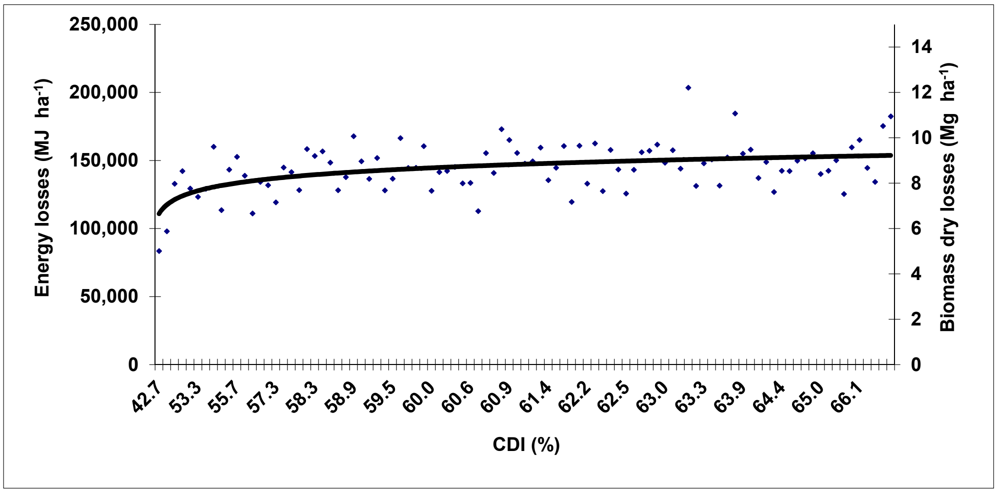

Figure 5. Dry biomass and energy losses per ha, at 27 months of age, according to the CDI.

Figure 6 compares the *E. globulus* dry biomass yields, in Megagrams ha^{-1}, from our study, with yields from other genera used as SRF (poplar and willow clones) in northern Europe.

According to [8,50], poplar and willow clone production, in experimental stands with densities of 16,600 stems ha^{-1}, varies between 10 and 40 Mg ha^{-1} and 15 and 38 Mg ha^{-1}, respectively, at five years of age and depending on fertilization treatments and soil characteristics. This implies an annual average yield of 6 and 4 Mg ha^{-1} for poplar and willow, respectively. It is worth highlighting that two codes of *Eucalyptus globulus*, even with much lower planting densities (see Figure 6), can achieve annual productions exceeding those of poplar and willow clones. It can be concluded that these two codes, namely 283 and 105, are most suitable for bioenergy stands in areas with high TLD prevalence.

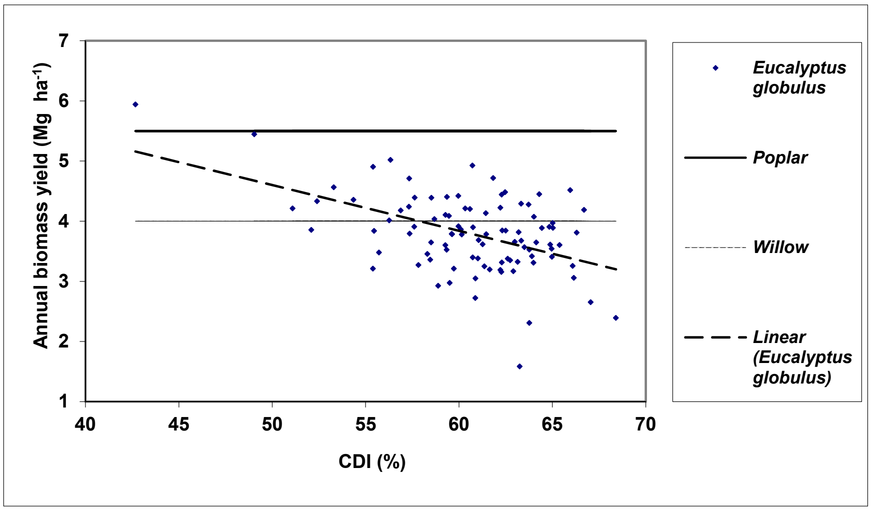

Figure 6. *Eucalyptus globulus* annual productivity (Mg ha^{-1}) versus *Poplar* and *Willow* clones.

It should be noted that average productions of poplar and willow clones are calculated for five year rotations and planting densities 10 times higher than those in this study. Moreover, in this study, the analysis was performed on trees at the age of 27 months. This fact has relevance since the damage caused by TLD in *Eucalyptus globulus* occurs mainly from the second year on, specifically between 22 and 30 months of age [50]. Thus, if the rotation period is less than two years, the damage caused by TLD would be lower, and productivity per hectare would increase. This option would entail much higher planting densities, difficulty with regrowth, soil depletion due to overexploitation; in a word, significantly greater environmental impact. In practice, this strategy, from a purely business standpoint, would involve costs not feasible today. However, it would be interesting to test different rotation periods and planting densities in order to optimize production of this tree species in areas with TLD prevalence.

In the stands of poplar and willow clones, planting densities are around 16,600 stems ha^{-1}, [8] which represents a significant increase in the planting and fertilization costs compared to those of *Eucalyptus globulus*. From an economic standpoint, this fact favours *Eucalyptus globulus*, since, with planting densities several times lower than poplar and willow, this species can attain similar biomass productivities, even when TLD is present. Despite the impact of the TLD on the plantations of *Eucalyptus globulus*, this previously selected species can be used as SRF in temperate places where the fungus is a limiting factor during the spring and summer months. Knowledge about the control of the disease [51] together with the selection of individuals will allow the establishment of viable *E. globulus* plantations.

4. Conclusions

The attack on *Eucalyptus globulus* short rotation stands by the foliar disease TLD significantly reduces its productivity, since it is precisely the juvenile stage that is affected. There are taxa (identified by codes in this study) of *E. globulus* in which the disease severity is significantly lower than the average, suggesting that these families are endowed with a certain tolerance to the disease, as compared to their counterparts.

The loss of dry biomass varies according to the CDI. For trees at 27 months of age, with a CDI of around 60%, the loss ranges between 8 and 10 Mg ha^{-1}. This total loss corresponds to the sum of the partial losses that make up the biomass. The greatest losses are seen in the fraction representing wood and bark, which, at 60% CDI, experiences a reduction of approximately 70% in weight.

The weighted average NCV of juvenile *E. globulus*, (dry biomass), is 16,774 kJ kg^{-1}. In this study, when combined with the loss of biomass per ha, the energy loss per area unit range between 83,000 and 184,000 MJ ha^{-1}. This loss could be translated into economic terms by considering the current high prices for electricity obtained from forest energy crops.

Despite the incidence of TLD in stands of *E. globulus*, their productivity is similar (when CDI values are low enough), to other species used for energy purposes (clones of poplar and willow), and this happens even when the planting densities of the *E. globulus* stands are much lower. In general, *E. globulus* biomass yields can be considered similar to those of poplar and willow when the CDI is lower than 56%. This finding suggests a future research focus which could involve the selection of families tolerant to the disease that would serve as the genetic basis for future stands. In our case, the families represented by codes 283 and 105 are best suited for this purpose.

The presence of the foliar disease TLD in stands of *E. globulus* influences the forestry and management of such plantations. An effective response to the threat posed involves the selection of individuals and families tolerant to disease. One barrier to overcome is the known difficulty of *E. globulus* to be cloned by cuttings due to low rooting capacity. This limitation, as well as the tolerance to TLD appears to be strongly linked to the genetics of each individual and/or family.

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Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "S. Pérez conceived and designed the experiments; C.J. Renedo, performed the experiments; A. Ortiz and F. Ortiz analyzed the data; A. Santisteban contributed reagents/materials/analysis tools; S. Pérez, wrote the paper." Authorship must be limited to those who have contributed substantially to the work reported.

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

References

1. Pacala, S.; Socolow, R. Stabilization wedges: Solving the climate problem for the next 50 years with current technologies. *Science* **2004**, *305*, 968–972. [CrossRef] [PubMed]
2. Werther, J.; Saenger, M.; Harthge, U.; Ogada, T.; Siagi, Z. Combustion of agricultural residues. *Prog. Energy Combust. Sci.* **2000**, *26*, 1–27. [CrossRef]
3. Strelher, A. Technologies of wood combustion. *Ecol. Eng.* **2000**, *16*, 25–40. [CrossRef]
4. Walle, I.V.; Camp, N.V.; Van de Casteele, L.; Verheyen, K.; Lemeur, R. Short-rotation forestry of birch, maple, poplar and willow in Flanders (Belgium) II. Energy production and CO_2 emission reduction potential. *Biomass Bioenergy* **2007**, *31*, 276–283. [CrossRef]
5. Sims, R.E.; Senelwa, K.; Maiava, T.; Bullock, B.T. Eucalyptus species for biomass energy in New Zealand—Part II: Coppice performance. *Biomass Bioenergy* **1999**, *17*, 333–343. [CrossRef]
6. Zewdie, M.; Olsson, M.; Verwijst, T. Above-ground biomass production and allometric relations of *Eucalyptus globulus* Labill coppice plantations along a chronosequence in the central highlands of Ethiopia. *Biomass Bioenergy* **2009**, *33*, 421–428. [CrossRef]
7. Sixto, H.; Hernández, M.; Barrio, M.; Carrasco, J.; Cañellas, I. Plantaciones del género *Populus* para producción de biomasa con fines energéticos: Revisión. *Investig. Agrar. Sist. Recur. For.* **2007**, *16*, 277–294. [CrossRef]
8. Hofmann-Schielle, C.; Jug, A.; Makeschin, F.; Rehfuess, K. Short-rotation plantations of balsam poplars, aspen and willows on former arable land in the Federal Republic of Germany. I. Site-growth relationships. *For. Ecol. Manag.* **1999**, *121*, 67–83. [CrossRef]
9. Misra, R.K.; Turnbull, C.R.A.; Cromer, R.N.; Gibbons, A.K.; LaSala, A.V. Bellow-and above ground growth of *Eucalyptus nitens* in a young plantation I. Biomass. *For. Ecol. Manag.* **1998**, *106*, 283–293. [CrossRef]
10. Van den Broek, R.; Vleeshouwers, L.; Hoogwijk, M. The energy crop growth model SILVA: Description and application to *Eucalyptus* plantations in Nicaragua. *Biomass Bioenergy* **2001**, *21*, 335–349. [CrossRef]
11. Sochacki, S.J.; Harper, R.J.; Smettem, K.R.J. Estimation of woody biomass production from a short-rotation bio-energy system in semi-arid Australia. *Biomass Bioenergy* **2007**, *31*, 608–616. [CrossRef]
12. Rockwood, D.L.; Rudie, A.W.; Ralph, S.A. Energy product options for *Eucalyptus* species grown as short rotation woody crops. *Int. J. Mol. Sci.* **2008**, *9*, 1361–1378. [CrossRef] [PubMed]
13. Sims, R.E.H.; Maiava, T.G.; Bullock, B.T. Short rotation coppice tree species selection for woody biomass production in New Zealand. *Biomass Bioenergy* **2001**, *20*, 329–335. [CrossRef]
14. Gasol, C.M.; Gabarrilla, X.; Antón, A.; Rigolad, M.; Carrascoe, J.; Ciriae, P.; Rieradevalla, J. LCA of poplar bioenergy system compared with Brassica carinata energy crop and natural gas in regional scenario. *Biomass Bioenergy* **2009**, *33*, 119–129. [CrossRef]
15. Zalesny, R.S.; Wiese, A.H.; Bauer, E.O.; Riemenschneider, D.E. Ex situ growthand biomass of *Populus* bioenergy crops irrigatedand fertilized with landfill leachate. *Biomass Bioenergy* **2009**, *33*, 62–69. [CrossRef]
16. Coylea, D.R.; Colemana, M.D.; Durante, J.A.; Newman, L.A. Survival and growth of 31 *Populus* clones in South Carolina. *Biomass Bioenergy* **2006**, *30*, 750–758. [CrossRef]
17. Ceulemans, R.; Mcdonaldt, A.J.S.; Pereira, J.S.A. A comparison among eucalypt, poplar and willow characteristics with particular reference to a coppice, growth-modelling approach. *Biomass Bioenergy* **1996**, *11*, 215–231. [CrossRef]
18. Arevalo, C.B.M.; Volk, T.A.; Bevilacqua, E.; Abrahamson, L. Development and validation of aboveground biomass estimations for four *Salix* clones in central New York. *Biomass Bioenergy* **2007**, *31*, 1–12. [CrossRef]
19. Aravanopoulos, F.A.; Kimb, K.H.; Zsuffa, L. Genetic diversity of superior *Salix* clones selected for intensive forestry plantations. *Biomass Bioenergy* **1999**, *16*, 249–255. [CrossRef]
20. Kidanu, S.; Mamo, T.; Stroosnijder, L. Biomass production of *Eucalyptus* boundary plantations and their effect on crop productivity on Ethiopian highland vertisols. *Agrofor. Syst.* **2005**, *63*, 281–290. [CrossRef]

21. Tejedor, C. Basic density selection for *Eucalyptus globulus* in Northern Spain. Within-tree and between-tree variation. In Proceedings of the IUFRO Conference Eucalyptus in a Changing World, Aveiro, Portugal, 11–15 October 2004.
22. Faúndez, P. Potential costs of four short-rotation silvicultural regimes used for the production of energy. *Biomass Bioenergy* **2003**, *24*, 373–380. [CrossRef]
23. Pérez, S.; Renedo, C.J.; Ortiz, A.; Mañana, M.; Silió, D. Energy evaluation of the *Eucalyptus globulus* and the *Eucalyptus nitens* in the North of Spain (Cantabria). *Thermochim. Acta* **2006**, *451*, 57–64. [CrossRef]
24. Hunter, G.C.; Crous, P.W.; Carnegie, A.J.; Burgess, T.I.; Wingfield, M.J. *Mycosphaerella* and *Teratosphaeria* diseases of Eucalyptus: Easily confused and with serious consequences. *Fungal Divers.* **2011**, *50*, 145–166. [CrossRef]
25. Park, R.F.; Keane, P.J.; Wingfield, M.J.; Crous, P.W. Fungal disease of eucalypt foliage. In *Diseases and Pathogens of Eucalypts*; Keane, P.J., Kile, G.A., Podger, F.D., Brown, B.N., Eds.; CSIRO Publishing: Collingwood, Australia, 2000; pp. 153–239.
26. Crous, P.W.; Hong, L.; Wingfield, B.D.; Wingfield, M.J. ITS rDNA phylogeny of selected Mycosphaerella species and their anamorphs ocurring on Myrtaceae. *Mycol. Res.* **2001**, *105*, 425–431. [CrossRef]
27. Milgate, A.W.; Potts, B.M.; Joyce, K.; Mohammed, C.; Vaillancourt, R.E. Genetic variation in *Eucalyptus globulus* for susceptibility to *Mycosphaerella nubilosa* and its association with tree growth. *Australas. Plant Pathol.* **2005**, *34*, 11–18. [CrossRef]
28. Park, R.F.; Keane, P.J. Three *Mycosphaerella* species from leaf diseases of *Eucalyptus*. *Trans. Br. Mycol. Soc.* **1982**, *79*, 95–100. [CrossRef]
29. Royle, D.J.; Hubbes, M. Diseases and pests in energy crop plantations. *Biomass Bioenergy* **1992**, *2*, 45–54. [CrossRef]
30. Royle, D.J.; Ostry, M.E. Disease and pest control in the bioenergy crops Poplar and Willow. *Biomass Bioenergy* **1995**, *9*, 69–79. [CrossRef]
31. Sage, R.B. Short rotation coppice for energy: Towards guidelines. *Biomass Bioenergy* **1998**, *15*, 39–47. [CrossRef]
32. Pinkard, E.A.; Mohammed, C.L. Photosynthesis of *Eucalyptus globulus* with *Mycosphaerella* leaf disease. *New Phytol.* **2006**, *170*, 119–127. [CrossRef] [PubMed]
33. Eyles, A.; Barry, K.; Quentin, A.; Pinkard, E. Impact of defoliation in temperate Eucalypt plantations: Physiological perspectives and management. *For. Ecol. Manag.* **2013**, *304*, 49–64. [CrossRef]
34. Tuskan, G.A. Short-Rotation woody crop supply systems in the United States: What do we know and what do we need to know? *Biomass Bioenergy* **1998**, *14*, 307–315. [CrossRef]
35. Dickmann, D.I. Silviculture and biology of short-rotation woody crops in temperate regions: Then and now. *Biomass Bioenergy* **2006**, *30*, 696–705. [CrossRef]
36. Park, R.F. Epidemiology of *Mycosphaerella nubilosa* and *M. cryptica* on *Eucalyptus* spp. in South-Eastern Australia. *Trans. Br. Mycol. Soc.* **1988**, *91*, 261–266. [CrossRef]
37. Carnegie, A.J.; Addes, P.K. The proportion of leaf spots caused by *Mycosphaerella cryptica* and *M. nubilosa* on *Eucalyptus globulus*, *E. nitens* and their F1 hybrids in a family trial in Tasmania, Australia. *Australas. Mycol.* **2002**, *21*, 53–63.
38. Maxwell, A. The Taxonomy Philogeny and Impact of Mycosphaerella Species on Eucalypts in South-Western Australia. Ph.D. Thesis, School of Biothecnology and Biological Science, Murdoch University, Murdoch, Australia, 2004.
39. Carnegie, A.J.; Keane, P.J.; Ades, P.K.; Smith, I.W. Variation in susceptibility of *Eucalyptus globulus* provenances to *Mycosphaerella* leaf disease. *Can. J. For. Res.* **1994**, *24*, 1751–1757. [CrossRef]
40. Lundquist, J.E.; Purnell, R.C. Effects of *Mycosphaerella* Leaf Spot on growth of *Eucalyptus nitens*. *Plant Dis.* **1987**, *71*, 1025–1029. [CrossRef]
41. Stone, C.; Matsuki, M.; Carnegie, A.J. *Pest and Disease Assessment in Young Eucalypt Plantations: Field Manual for Using the Crown Damage Index*; Parson, M., Ed.; National Forest Inventory, Bureau of Rural Sciences: Canberra, Australia, 2003.
42. Pita, P.A. *Tablas de CubicacióN Por DiáMetros Normales y Alturas Totales*; Instituto Forestal de Investigaciones y Experiencias (I.F.I.E. Ministerio de Agricultura): Madrid, Spain, 1967.
43. Reed, D.; Tomé, M. Total aboveground biomass and net dry matter accumulation by plant component in young *Eucalyptus globulus* in response to irrigation. *For. Ecol. Manag.* **1998**, *103*, 21–32. [CrossRef]

44. Hubbard, W.N.; Scott, D.W.; Waddinton, G. *Experimental Thermochemistry*; Rossini, F.D., Ed.; Interscience: New York, NY, USA, 1956.
45. Brañas, J.; González-Río, E.; Merino, A. Content and distribution of nutrients in *Eucalyptus globulus* plantations in Northwestern Spain. *Investig. Agrar.* **2000**, *2*, 317–355.
46. Singh, R.P.; Sharma, V.K. Biomass estimation in five different aged plantations of *Eucalyptus tereticornis* Smith in Western Uttar Pradesh. In *Oslo Biomass Studies*; College of Life Sciences and Agriculture, University of Maine: Orono, ME, USA, 1976; pp. 143–161.
47. Soria, S. Especies de *Mycosphaerella*, y *Teratosphaeria* en Plantaciones Jóvenes de *Eucalyptus globulus*: Evaluación de Daño y Control. Ph.D. Thesis, Facultad de Ciencias, Universidad de Uruguay, Montevideo, Uruguay, 2016.
48. Dungey, H.S.; Potts, B.M.; Carnegie, A.J.; Ades, P.K. *Mycosphaerella* leaf disease: Genetic variation in damage to *Eucalyptus nitens*, *Eucalyptus globulus*, and their F1 hybrid. *Can. J. For. Res.* **1997**, *27*, 750–759. [CrossRef]
49. Balmelli, G.; Altier, N.; Marroni, V. Daños provocados por enfermedades foliares y por heladas en E. globulus I. Efecto fenotípico sobre el comportamiento productivo posterior. *Bol. CIDEU* **2007**, *3*, 67–75.
50. Vande Walle, I.; Van Camp, N.; Van de Casteele, L.; Verheyen, K.; Lemeur, R. Short-rotation forestry of birch, maple, poplar and willow in Flanders (Belgium) I—Biomass production after 4 years of tree growth. *Biomass Bioenergy* **2007**, *31*, 267–275. [CrossRef]
51. Pérez, S.; Renedo, C.J.; Ortiz, A.; Ortiz, F.; Tejedor, C. Strategies to combat *Mycosphaerella* leaf disease in *Eucalyptus globulus* plantations in northern Spain. *Forests* **2016**, *7*, 190. [CrossRef]