

## Article

# Effect of Poultry Manure-Derived Compost on the Growth of *eucalypts* spp. Hybrid Clones

Pedro F. Rizzo <sup>1,\*</sup>, María C. Salinas <sup>1,2</sup>, Virginia Della Torre <sup>3</sup>, Juan P. Diez <sup>4</sup>, Leonardo F. Salleses <sup>5</sup>, Nicolás I. Riera <sup>3</sup>, Pablo S. Pathauer <sup>6</sup>, Dimitrios Komilis <sup>7</sup> and Antoni Sánchez <sup>7,\*</sup>

- <sup>1</sup> Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Mendoza, Agrotecnología Sostenible, Luján de Cuyo 5507, Argentina
  - <sup>2</sup> National Council of Scientific and Technical Research (CONICET), Mendoza 5500, Argentina
  - <sup>3</sup> Laboratorio de Transformación de Residuos, Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Microbiología y Zoología Agrícola (IMyZA), Hurlingham 1686, Argentina
  - <sup>4</sup> Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Bariloche, Rio Negro, San Carlos de Bariloche 8400, Argentina
  - <sup>5</sup> Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Balcarce, Balcarce 7620, Argentina
  - <sup>6</sup> Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Recursos Biológicos (IRB), Buenos Aires 1033, Argentina
  - <sup>7</sup> Composting Research Group, Department of Chemical Engineering, Universitat Autònoma de Barcelona, Bellaterra, 08193 Cerdanyola del Vallès, Spain
- \* Correspondence: rizzo.pedro@inta.gob.ar (P.F.R.); antoni.sanchez@uab.cat (A.S.); Tel.: +54-(261)-4963020 (P.F.R.); +34-935811019 (A.S.)



**Citation:** Rizzo, P.F.; Salinas, M.C.; Della Torre, V.; Diez, J.P.; Salleses, L.F.; Riera, N.I.; Pathauer, P.S.; Komilis, D.; Sánchez, A. Effect of Poultry Manure-Derived Compost on the Growth of *eucalypts* spp. Hybrid Clones. *Processes* **2022**, *10*, 2182. <https://doi.org/10.3390/pr10112182>

Academic Editor: Davide Dionisi

Received: 4 October 2022

Accepted: 21 October 2022

Published: 25 October 2022

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**Abstract:** Interspecific hybrids of *E. grandis* × *E. camaldulensis* were generated to widen the plantation area. The aim of this study was to assess root capability and development for six different clones of eucalyptus grown in substrates made with three different composts derived from poultry manure. A factorial design was used to assess the effect of different composts on six growth variables. The analysis detected a greater effect from the genotype than the substrate. *E. grandis* × *E. camaldulensis* hybrid vegetative propagation was successful in alternative substrates formulated from composted poultry manure. GC8 was the genotype that showed the greatest differences for four the different variables among the substrates, being both the most sensitive and the one with the highest values for all parameters measured. The hybrids' vegetative propagation was determined in alternative substrates formulated from poultry manure compost. The physicochemical characteristics of substrates composed of pine bark and sawdust provided adequate conditions for the growth of eucalyptus. GC8 was the genotype most sensitive to the use of different substrates, showing significant differences in the ratio of roots/callus, radicular dry weight, and cutting dry weight. These clones might be a good option for evaluating compost-based substrates for forestry applications.

**Keywords:** substrates; vegetative propagation; forest valorization

## 1. Introduction

Eucalyptus is one of the most important economic forest species worldwide [1,2]. *E. grandis* has fast growth and light wood; however, it is sensitive to frost, and it is mainly destined for solid use. *E. camaldulensis* is a slow growth species with a dark and dense wood. It has wide genetic variation and plasticity, which allows it to adapt to different climatic and soil conditions, including those with high salinity and low moisture [3]. Hybrids are able to produce adaptive features and production combinations which are not genetically possible within species.

A hybrid can only be used if it can be cloned, and hybrid vigor is achieved using clonal silviculture [4]. In this sense, *E. grandis* and *E. camaldulensis*, have the advantage of easy rooting if variables that intervene in their vegetative propagation are adjusted, such

as humidity, temperature, rooting promoter concentration, and substrate, among others. The substrate is the material that allows the radicular system anchorage [5]. It can be made up of one or more materials, and must have high porosity and hydric retention capacity as well as good drainage and aeration [6]. Different materials such as composted pine bark, peat, perlite, and burnt rice bran are often used, either pure or mixed and in different proportion, as substrates in eucalyptus vegetative propagation.

Developing peat alternative substrates is necessary for three different reasons: (i) the sources of peat are limited worldwide; (ii) the pressure for using waste coming from human or industrial activities is increasing rapidly; and (iii) the economic necessity of using locally produced waste products is increasing [7]. The most commonly used material in substrate mixture for forest plant production in nurseries is peat moss, thanks to its physicochemical characteristics [8]. This material comes from mosses, such as *Sphagnum*; however, its use is being debated because of its high cost [9] and its questionable future availability due to environmental limitations [10]. The substrate mixture should favor well-developed fibrous root systems to produce quality plants and improve their survival and growth in the field [11]. To achieve the optimal conditions of the substrate, the mixtures should have adequate physical characteristics to retain water and facilitate drainage and aeration [12,13].

The use of organic waste derived from agricultural and forestry activities is becoming important for plant production [4,14]. Pine sawdust and bark are wood waste products of the forestry industry, and can be obtained at a relatively low cost [15]. However, there is a lack of information on the possibility of commercial use of these growing mediums in forest nurseries [16]. In substrate mixtures, different percentages are used depending on the compost properties and the species under cultivation. Generally, these mixtures have alkaline pH and high salt content. For this reason, it is convenient to combine them with materials with lower pH and salt levels [17].

A consequence of the amplification of animal production systems worldwide is the concentration of animals in small areas, resulting in large amounts of manures and excreta. This leads to environmental problems, including water and soil pollutions and bad odors around animal breeding sites [18–20]. Common treatment alternatives for poultry manure are anaerobic digestion and composting or a combination of both [21–23].

Argentinian eucalyptus and poultry production are mainly concentrated in the same region of the Littoral, namely, Entre Ríos, one of the most important provinces [24]. This makes it an ideal area to study strategies for using poultry manure as a potential substrate component for substrates in eucalyptus plantations. The use of a problematic waste as an alternative to peat for substrate formulation has multiple benefits, for example, recovery of poultry manure, improving the biogeochemical cycling of nutrients, and minimizing contamination [25]. For this reason, the aim of this study was to evaluate the effect of different substrates which included poultry manure derived compost in the development of six different eucalyptus hybrid clones.

## 2. Materials and Methods

### 2.1. Production of Compost and Substrates

The compost was made in the Laboratorio de Transformación de Residuos (LTR) IMyZA, CICVyA—INTA using manure from hens in automatized production facilities. The three composts (C1, C2, C3) used to formulate the substrates (S1, S2, S3) had different initial compositions. Table 1 shows the percentage composition of the three composts [25]. Sawdust and bark have different physical characteristics; sawdust has a larger percentage of fine particles and can cause problems with excess moisture, while wood shavings have larger particles and a low water holding capacity [26]. Crushed bare corn cobs combine fine particles and large ones.

**Table 1.** Initial waste percentages (%) <sup>1</sup> used in each compost to formulate the substrates (S1, S2, and S3).

Type of Compost	Poultry Manure	Intact Corn Bare Cobs	Crushed Corn Bare Cobs	Sawdust	Wood Savings
C1 <sup>2</sup>	40	0	20	20	20
C2 <sup>3</sup>	60	0	20	20	0
C3 <sup>4</sup>	60	30	10	0	0

<sup>1</sup> All percentages are on a wet weight basis. <sup>2</sup> Compost 1. <sup>3</sup> Compost 2. <sup>4</sup> Compost 3.

Poultry manure is rich in nitrogen waste and can be mixed with materials rich in available carbon, such as sawdust. Sawdust can retain nitrogen, thereby avoiding ammonia volatilization, because organisms that decompose organic matter use nitrogen as well. Poultry manure contains a low C:N ratio and high porosity. Therefore, degradation processes can be enhanced by adding carbon-rich materials (co-substrates). The co-composting of poultry manure with other agricultural wastes improves its physicochemical characteristics and reduces phytotoxicity, in addition to promoting and managing better use of other local residues, thereby generating added value. In this sense, we were able to produce compost with 40–60% poultry manure and other agricultural wastes as co-substrates [25].

Three mixtures were prepared using a capacity of 0.5 m<sup>3</sup> mix at the beginning of the assay. Piles were built in trapezoidal shapes (1.5 m high, 2 m wide and 2 m length). Each treatment was carried out using three repetitions of 2 m<sup>3</sup> piles each. The composting piles were manually turned every three days during the first active decomposition phase of the process and every five days when the cell temperature was similar to the ambient temperature. Moisture content was maintained through irrigation and taking into account local precipitation. The composting process lasted 83 days. All the composts reached the stage of stability and maturity as measured from the static respirometric index (SRI < 0.5 mg O<sub>2</sub> g<sup>-1</sup> OM h<sup>-1</sup>) and the NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratio (<0.3), respectively (Table 2). Table 2 shows the main parameters of the three composts.

The substrates used in this study (S1, S2, and S3) were made of 40% of compost 1 (C1), compost 2 (C2), or compost 3 (C3) and 60% composted pine leaves (Table 3). These formulations were defined taking into account previous pH and electrical conductivity measurements of the substrates that had been prepared with different proportions of composts and composted pine leaves. In addition, we took into account the fact that the optimum pH for substrates used in plant production should be between 5.2 and 6.3, while a value below 4.0 can cause root disease [6]. While livestock manure compost has good physical properties, the soluble salt content is too high and the pH is alkaline [27]. For this reason, it is necessary to mix it with an acidic material, such as peat, or with compost with high cationic exchange capacity [7]. The control substrate (C) was made with a 50:50 (v/v) mixture of composted pine bark and burned rice husks, which is routinely used for vegetative propagation and is recommended by various authors [28,29].

**Table 2.** Physicochemical characterization of composts used in the substrate preparation.

Parameters <sup>1</sup>	Unit	C1	C2	C3	Target Value or Range/Upper Limit	Reference
pH		8.0	8.1	8.7	6–8/9	[30]
EC	mS cm <sup>-1</sup>	2.0	3.4	2.7	<0.6/1.5	[30]
D	Mg m <sup>-3</sup>	0.7	0.8	0.5	0.45–0.50/0.55	[30]
OM	%	35.0	37.0	87.0	≥20	[31]
SRI	mg O <sub>2</sub> g <sup>-1</sup> OM h <sup>-1</sup>	0.20	0.40	0.37	0.5–1.0	[32]
C:N	%	14.4	13.6	18.0	<20	[31]
Ca	mg L <sup>-1</sup>	8170	9810	2210	≥1%	[33]
Mg	mg L <sup>-1</sup>	251	348	470	≥0.05%	[33]
K	mg L <sup>-1</sup>	6563	8700	11,350	NPK ≥ 6%	[33]
NH <sub>4</sub> <sup>+</sup> /NO <sub>3</sub> <sup>-</sup>	%	0.0	0.0	0.004	<0.3	[31]

<sup>1</sup> EC: electrical conductivity, D: apparent density, OM: organic matter, SRI: static respirometric index, C:N: carbon nitrogen ratio, Ca: calcium, Mg: magnesium, K: potassium, NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup>: ammonium nitrate ratio.

**Table 3.** Initial substrate formulation, pH, and EC values (mean  $\pm$  standard deviation with  $n = 3$ ).

Substrates	Composition <sup>1</sup>	pH	EC *
1	40% Compost 1 + 60% composted pine leaves	6.55 $\pm$ 0.09	871 $\pm$ 7.1
2	40% Compost 2 + 60% composted pine leaves	6.64 $\pm$ 0.17	1387 $\pm$ 121.1
3	40% Compost 3 + 60% composted pine leaves	7.03 $\pm$ 0.06	1956 $\pm$ 48.8
Control	50% burned rice husks + 50% composted pine bark	7.05 $\pm$ 0.02	653 $\pm$ 1.1

<sup>1</sup> The percentages are on a volume basis. The control substrate is routinely used for vegetative propagation in the IRB; \* Electrical conductivity ( $\mu\text{S cm}^{-1}$ ).

## 2.2. Forest Assessment of Compost and Substrates

The study was carried out at the Instituto de Recursos Biológicos (IRB), CIRN–INTA in Buenos Aires, Argentina. Lopez (2017) [34] indicates that in Argentina the first experimental records of interspecific hybrids were reported by Alliani (1990) [35]. In the Mesopotamia region of Argentina, the first controlled crosses for the selection of hybrid clones of *E. grandis*  $\times$  *E. camaldulensis* and *E. grandis*  $\times$  *E. tereticornis* were generated by the INTA clonal program (Harrand and Schenone, 2002) [36]. More recently, Harrand et al. (2016) [37] report that the INTA clonal program has 150 hybrid clones in different stages of evaluation, several of which have been registered in the National Registry of Cultivars (INASE), and have since 2014 been transferred to nurseries in the region through transfer agreements. In this study, four clones of *E. grandis*  $\times$  *E. camaldulensis* (GC6, GC8, GC19 and GC24) and two clones of *E. grandis*  $\times$  *E. tereticornis* (5–105 and 5–128) were used, all originating from the program clone of INTA. The cuttings were obtained from 30 to 50 cm long young regrowth of the six genotypes from a cloning garden at the IRB. The cuttings were 10 cm long with an internode and preferably two opposite leaves, with its foliar area reduced by 50% to minimize evapotranspiration. Each cutting apical part was cut in a straight shape, while the basal extreme was cut in beveled edge to originate a greater contact zone with the growth promoter. Then, the cuttings were put under a Captan<sup>®</sup> ( $2 \text{ g L}^{-1}$ ) fungicide solution for one minute and treated at their bases with indolbutiric acid 3000 ppm dispersed in industrial talcum powder. Then, they were implanted in individual  $145 \text{ cm}^3$  substrate capacity tubes.

The trial was located within a  $100 \mu$  crystal polyethylene greenhouse with an automated irrigation and relative humidity control system. Environmental humidity values were near 80%, and environmental temperature ranged between  $22 \text{ }^\circ\text{C}$  and  $30 \text{ }^\circ\text{C}$ . The trial cuttings were sprinkled weekly with COMBO brand ( $2.5 \text{ cm}^3 \text{ L}^{-1}$ ) complete fertilizing solution with foliar absorption mixed with Captan<sup>®</sup> ( $2 \text{ g L}^{-1}$ ). The fungicide was altered weekly with Carbendazim<sup>®</sup> ( $2 \text{ cm}^3 \text{ L}^{-1}$ ) to avoid fungal resistance.

From the 55 days from the trial beginning, the humidification system was stopped to simulate environmental conditions until the plants were removed from the substrates and their roots washed. Then, the live cuttings were divided in three fractions (shoots, cuttings, and roots) and dried in a  $50 \text{ }^\circ\text{C}$  oven until constant weight was reached. Each fraction was weighed separately in a precision balance.

The tested variables to assess the growth of the eucalypts clones were survival (SUR), defined as the proportion of live cuttings from five of each plot, primary leave number (PLN), average number of shoots (ANS), average amount of leaves per shoot (ALS), total leaves (TL), average shoot length (ASL), longest shoot length (LSL), roots/callus (R/C), root length (RL), cutting diameter (CD), radicular dry weight (RDW), shoot dry weight (SDW), cutting dry weight (CDW), root type (RT), and shoot type (ST).

To evaluate the physicochemical substrate and compost characteristics, the following parameters were measured in the substrates according to USDA and USCC (2001) [32] and INTA (2021) [38] (Tables 2 and 3): pH, electrical conductivity (EC), humidity, dry matter, organic matter, ashes, total organic carbon (TOC), and total nitrogen (TN).

### 2.3. Experimental Design and Statistical Analysis

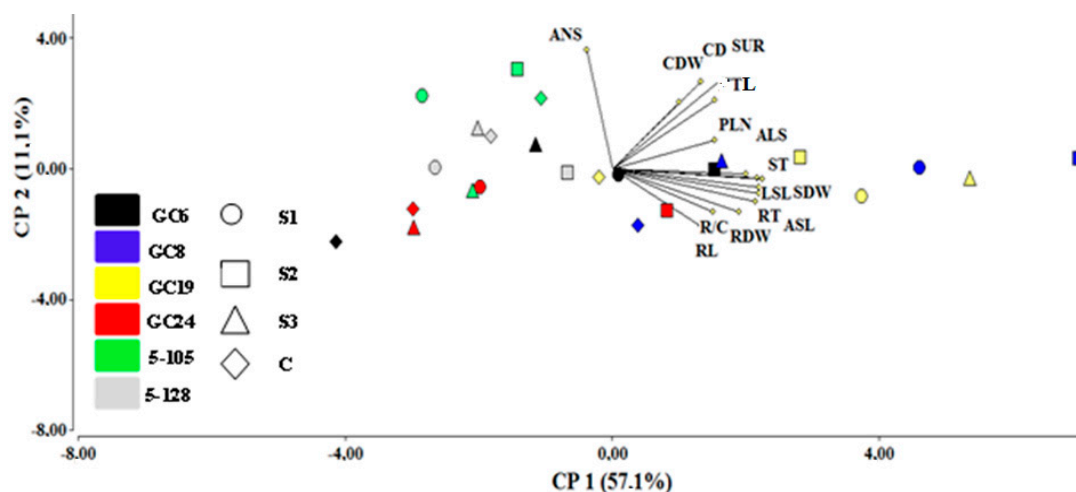
A factorial experimental design was used in which four substrates and six clones were tested. Eight repetitions for each combination of substrate and clone were analyzed, and five eucalyptus cuttings were used in each repetition.

A principal component analysis (PCA) was carried out to study the multivariate differences between the clones grown in different substrates. Subsequently, a Kruskal–Wallis non-parametric test was chosen to study the differences between the substrates for each clone and for each variable in particular ( $p \leq 0.05$ ). All the statistical analyses were run using InfoStat [39].

## 3. Results

### 3.1. Principal Component Analysis

PCA component 1 explained 57.1% of the variability, while component 2 explained 11.1%. Component 1 allowed us to group different eucalypts genotypes. The highest vegetable growth values were associated with GC8 and GC19, whereas the others clones were associated with lower vegetable growth values (Figure 1). The average number of shoots (ANS) is the variable that best explained Component 2, although there were no significant differences within the treatments in a later analysis within this particular variable.



**Figure 1.** Principal component analysis plot. References: ANS (Average number of shoots), CDW (cutting dry weight), CD (cutting diameter), SUR (survival), TL (total leaves), PLN (primary leave number), ALS (average amount of leaves per shoot), ST (shoot type), LSL (longest shoot length), SDW (shoot dry weight), RT (root type), ASL (average shoot length), R/C (roots/callus), RDW (radicular dry weight), RL (root length).

The analysis showed that most of the variables were actually correlated. The ones considered for the analysis were those that had correlations with ten or less variables, namely, survival, number of shoots per cutting, total leaves per cutting, roots number/callus ratio, root length, cutting diameters, dried root weight, and dried cutting weight.

### 3.2. Substrates Properties in Eucalypts Nurseries

Table 3 shows that the initial pH values are close to the optimum range cited in the bibliography [40]. Substrate 3 and control showed values significantly higher than the remaining substrate. On the other hand, the EC values were significantly higher in those substrates with larger amounts of poultry manure in their initial composition (substrates 2 and 3).

### 3.3. Growth Variables of the Clones Evaluated

#### 3.3.1. Survival Rates

Table 3 shows that the total average survival was 50%. S1 and S3 manifested a 56% survival rate and C showed 41% survival, while S2 showed 47%. GC19 and GC8 showed a survival rate greater than 60%. GC24 had the highest percentage of mortality. The highest survival rate (90%) was found in the combinations of GC8 with S1 and S3 and GC19 with S2 (Table 4).

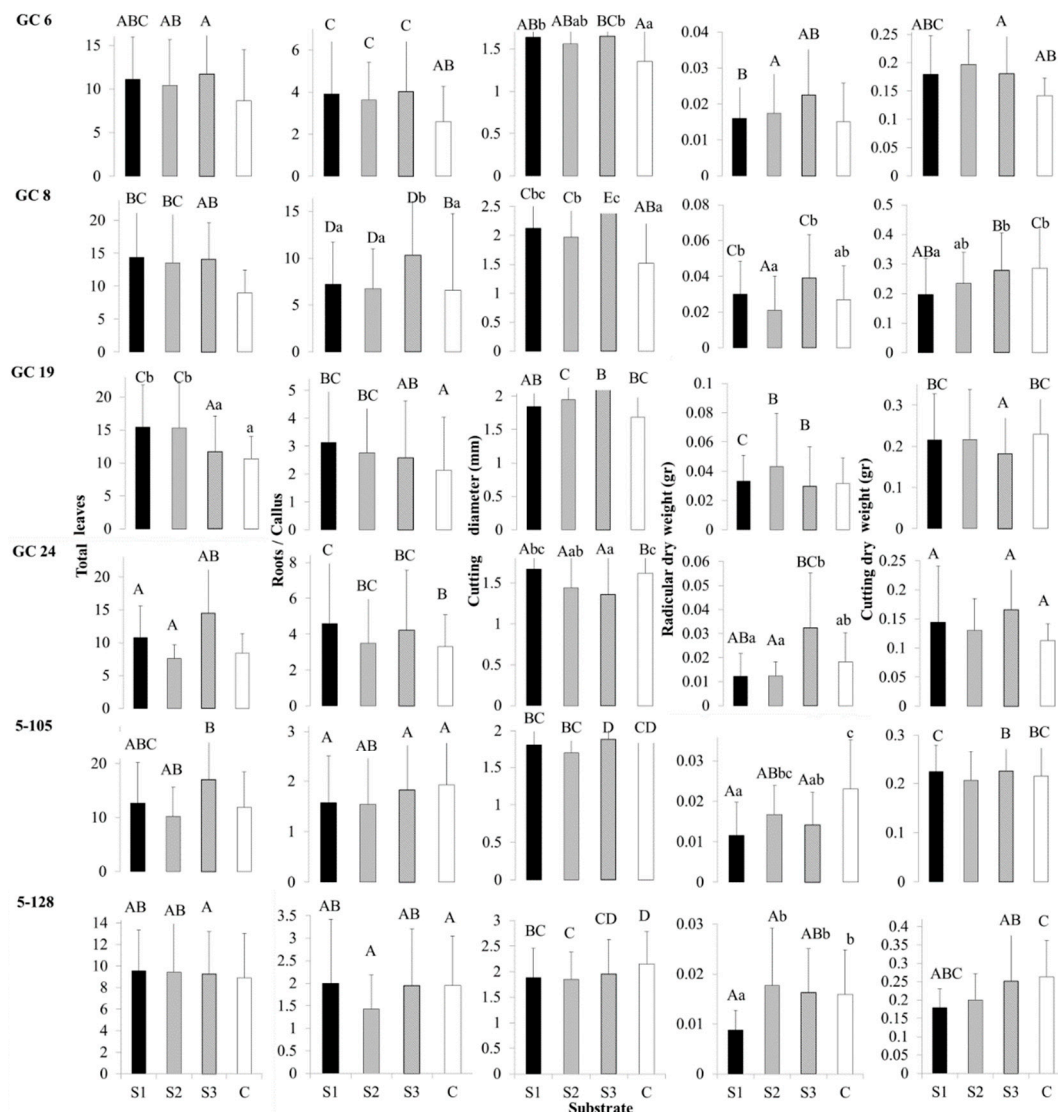
**Table 4.** Survival Percentages (%).

Treatments	S1	S2	S3	C	Clone
GC6	60	55	68	8	48
GC8	88	53	88	25	63
GC19	60	85	65	50	65
GC24	45	25	15	35	30
5–105	55	30	60	75	55
5–128	30	35	43	55	41
Substrates	56	47	56	41	50

#### 3.3.2. Substrate Effect Analysis Considering Each Genotype

When analyzing the substrate effect, neither the shoots number per cutting or cutting root length showed significant differences in any of the genotypes.

Only GC19 showed significant differences within total leaves. It was higher in S1 and S2 ( $16 \pm 6$ ) than in S3 and C ( $11 \pm 5$ ) (Figure 2). An opposite result was obtained when considering cutting dry weight. GC8 showed significant differences, having a higher CDW with S3 and C ( $0.3 \pm 0.1$  g) than with S1 ( $0.2 \pm 0.1$  g). Again, only GC8 showed significant differences, in this case considering the roots/callus variable. Figure 2 shows that this ratio was significantly higher in S3 than in the rest of the substrates (S3 =  $10 \pm 6$ ; S1, S2 and C =  $7 \pm 6$ ). The effect of the substrates was significant in more clones when analyzing the radicular dry weight. GC8 significantly differed between S2 (with the lowest value  $0.02 \pm 0.02$  g) and S1 and S3 (the highest value  $0.04 \pm 0.02$  g). GC24 significantly differed between S1 and S2 (with the lowest value,  $0.01 \pm 0.01$  g) and S3 (the highest value  $0.03 \pm 0.02$  g). In 5–105 clones, C and S1 had significant differences, with C having the highest RDW ( $0.02 \pm 0.01$  gr) and S1 the lowest ( $0.01 \pm 0.01$  g). In the case of 5–128, S1 ( $0.01 \pm 0.00$  g) differed significantly from the rest of the substrates, which had a greater RDW value ( $0.02 \pm 0.01$  g). Finally, when we evaluated the effect of the substrates on the cutting diameter of the clones, we obtained GC6 grown in S1 and S3 showed a significantly greater diameter than the control (S1 and S3  $1.7 \pm 0.5$  mm and C =  $1.4 \pm 0.4$  mm). The diameter in GC8 was significantly higher in S3 ( $2.5 \pm 0.8$  mm), medium in S2 ( $2.0 \pm 0.8$  mm), and lower in C ( $1.5 \pm 0.7$  mm). Those in S1 had an intermediate value between S2 and S3, with no significant differences between them. GC24 clones had significantly larger cutting diameter in C than in S3 ( $1.6 \pm 0.3$  mm and  $1.4 \pm 0.5$  mm).



**Figure 2.** Growth variables (Total Leaves, Roots/callus, Cutting Diameter, Radicular Dry Weight, Cutting Dry Weight) in four substrates (1, 2, 3, and control) and six clones (GC6, GC8, GC19, GC24, 5–105 and 5–128). The bars indicate mean values of Total Leaves, Roots/callus, Cutting Diameter, Radicular Dry Weight, and Cutting Dry Weight of eight replicates  $\pm$  standard deviation. Different lowercase letters indicate significant differences between substrates for each variable analyzed ( $p \leq 0.05$ ). Different capital letters indicate significant differences between genotypes for each variable analyzed ( $p \leq 0.05$ ). For no significant difference, no letters are shown.

### 3.3.3. Genotype Effect Analysis Considering Each Substrate

When we focused on genotype effect, the shoot number per cutting did not show significant differences in any of the substrates.

S1 presented significant differences in total leaves. GC19 obtained the highest total leaves per cutting ( $15 \pm 7$ ), while GC24 presented the lowest ( $9 \pm 6$ ). Only these two genotypes were different; the rest had intermediate values (Figure 2). The same trend was found using S2 ( $15 \pm 7$  for GC19 and  $8 \pm 2$  for GC24). Within S3, the total number of leaves per cutting was significantly higher in 5–105 ( $17 \pm 8$ ) than in 5–128, GC19 and 5–128 ( $11 \pm 5$ ), which had the lowest. There were no differences within the genotypes grown in the control substrate.

Using S1, GC8 showed the highest root number/callus ( $7 \pm 4$ ) and 5–105 the lowest ( $2 \pm 1$ ). The same results were found using S3 (GC8:  $10 \pm 6$  and 5–105:  $2 \pm 1$ ). In S2, GC8 had the highest ratio value by a significant margin ( $7 \pm 4$ ), while 5–128 had the lowest

(1 ± 1). The control presented significant differences as well. GC8 and GC24 had the highest ratio (4 ± 5) and GC19, 5–105 and 5–128 had the lowest (2 ± 1), while GC6 had a value between these groups.

Regarding root length, there were differences for genotypes grown in S1. GC19 had significantly longer roots than the rest of the genotypes (18.4 ± 6.3 cm), 5–105 had the shortest roots (11.7 ± 2.9 cm), and GC8 had an intermediate value (14.3 ± 4.8 cm). GC6, GC24, and 5–128 had intermediate length between 5–105 and GC8, with no significant differences. GC19 grown in S2 had significantly longer roots (17.8 ± 5.1 cm) than the rest of the genotypes (12.9 ± 4.5 cm). In S3, GC19 had significantly longest root than 5–105 (17.2 ± 6.3 cm and 11.4 ± 3.0 cm, respectively).

Another variable that showed significant differences was the cutting diameter. In S1, the GC8 diameter was significantly higher than that of GC24, which had the lowest value (2.1 ± 0.7 mm and 1.7 ± 0.7 mm, respectively). Using S2, GC8, GC19 and 5–128 had significantly higher diameters (1.9 ± 0.7 mm) than that of GC24 (1.4 ± 0.4 mm). Finally, in S3, GC8 had the highest diameter (2.5 ± 0.8 mm), the diameters of GC6, GC19, 5–105 and 5–128 were grouped in an intermediate level, and the diameter of GC24 was significantly the lowest one (1.4 ± 0.5 mm). GC8 grown in S3 had the highest diameter (2.5 ± 0.8 mm), while GC 24 had the lowest (1.4 ± 0.5 mm). In the control (C), only 5–128 and GC6 differed significantly, with 5–128 having the highest diameter and GC6 having the lowest (2.2 ± 0.6 mm and 1.4 ± 0.4 mm respectively).

Another variable considered was the radicular dry weight. Focusing on S1, GC8 and GC19 had significantly higher RDW than 5–105 and 5–128 (0.03 ± 0.02 g and 0.01 ± 0.01 g respectively). GC6 and GC24 had no significant differences in values between them. In S2, GC19 had a significantly higher RDW than the rest of the substrates (0.04 ± 0.04 g) except 5–105, which showed no significant differences with any of the genotypes. The genotypes with the lowest values were GC6, GC8, GC24, and 5–128 (0.02 ± 0.01 g). In S3, there were significant differences between GC8 and 5–105, the first with the highest RDW and the second with the lowest (0.04 ± 0.02 g and 0.01 ± 0.01 g, respectively); the remaining genotypes had values between these levels. Again, the genotypes showed no differences when using the control substrate.

The last variable considered that showed significant differences was cutting dry weight. For S1, 5–105 had the greatest value (0.2 ± 0.1 g) and GC24 had the lowest one (0.1 ± 0.1 g). In S3, 5–105 and GC8 (0.3 ± 0.1 g) had significantly higher values than GC6, GC19, and GC24 (0.2 ± 0.1 g). In C, GC8 and 5–128 (0.3 ± 0.1 g) showed heavier CDW than GC24 (0.1 ± 0.0 g).

#### 4. Discussion

The differences between the treatments were affected principally by the genotype (clone). Component 1 from the PCA explained 57.1% of the variability. This fact agrees with the findings of Woodward et al. (2006) [41], who highlighted the genetic differences that exist between clones within a species in their investigation. Wherever more than one clone was used in an experiment, there was a significant difference in the number of shoots and roots produced between the clones. The authors highlighted the use of PCA, which allowed them to verify that B availability was the attribute that explained most of eucalypt biomass variation. Additionally, these analyses showed the characteristics that are crucial to improving the organic waste compost-based substrates in comparison to commercial substrates [42]. Rinaldi et al. (2014) [43] used a multivariate approach to data analysis and summarized the information of a complex substrate–plant system based on a large set of tested treatments and several observed variables. Their results suggest that a combination of indicators is needed to evaluate the physiological response of ornamental rosemary to the different treatments explored and to classify treatments into homogeneous groups based on plant response. They concluded that multivariate analysis evaluates the influence of different composts on the substrate properties and the complexity of plant response



better than single-variable analysis; this analysis should be routinely used in the evaluation of substrate performance.

Genetic variation for productive and adaptive characteristics has been reported in various *Eucalyptus* spp. [44–46]. There is information about variability in rooting and tolerance to salinity of *E. grandis* × *E. camaldulensis* hybrid [1,47]. However, there is a lack of information about the development of this eucalyptus clone in different substrate media. Salleses et al. (2015) [13] evaluated different organic substrates in one eucalyptus clone. This work contributes to expanding the knowledge on the growth abilities of six Argentinean hybrid clones of eucalyptus in different substrates.

Rizzo et al. (2015) [24] have found that the compost formulated from initial mixtures with 40–60% poultry manure is alkaline. Although the authors did not find pH variations with the increase of poultry manure in the initial mixture, they found higher EC values in the treatment with 60% poultry manure. Generally, the compost derived from livestock manure had a lightly alkaline pH and a high salt content; therefore, it is necessary to amend these conditions before the use of such composts as substrate [30]. In this study, the initial pH values of the substrates were close to the optimum range.

The highest EC values of organic waste compost-based substrates were strongly influenced by the amount of poultry manure in the mixes, and ranged from 9.8 to 21.5 dS m<sup>-1</sup> when the proportions of poultry manure in the substrates increased from 0 to 41.7% [42].

Jayasinghe et al. (2010) [48] found that the components which most contribute to salinity are Na<sup>+</sup>, K<sup>+</sup>, ammonia, nitrate, and sulfate, which were found in greater amounts in compost-based substrates. The differences in the chemical and physicochemical properties of the organic wastes and in their proportions in the mixes markedly influenced the available contents of nutrients in the compost-based substrates [28].

In general, the organic waste compost-based substrates had EC values above the threshold limit level and high contents of available P, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>-N, and Na<sup>+</sup>, while the commercial substrates had adequate levels of B for plants and presented lower concentrations of NH<sub>4</sub><sup>+</sup>-N, P, K<sup>+</sup>, and micronutrients. The compost-based substrate with 33.4% of poultry manure and 25% of sewage sludge in its composition approaches the commercial substrate in terms of eucalyptus biomass production among the organic wastes compost-based substrates. Salt leaching during irrigation could have mitigated the possible negative effects of high EC, which is an important attribute to consider when young plants are cultivated. However, salts can be managed to adequate levels [25]. Even the propagation of seedlings obtained from vegetative parts of eucalyptus clones instead of seeds can have their growth impaired by high salt concentrations, even if the plants do not exhibit any deleterious effects of salinity.

According to Marrón (2015) [49] the presence of heavy metals in the soil following the spreading of sewage sludge, wastewater, compost, manure, or ash has been shown by numerous studies, mainly in the upper soil horizons. He found that all studies were unanimous on the fact that the levels found were under the limit of the regulatory pollution thresholds (thresholds of the different countries where the experiments were carried out), did not present a risk for the environment or health, and were not leached to deep soil horizons or into groundwater. These low risks identified in short-rotation plantations were linked to the fact that willows, poplars, and eucalyptus were able to efficiently extract most soluble/exchangeable metals (Cd, Ni, and Zn) present in the sludge or manure as well as Cr, Pb, Hg, and Cu to a lesser extent.

In general, moderate nitrogen deficiency can improve rooting, although at high levels more energy is required for vegetative growth and especially for leaf expansion [50]. Consequently, carbohydrates are not stored at suitable levels, reducing the C:N ratio. On the other hand, extreme nitrogen deficiency can reduce rooting, as it is necessary for nucleic acid and protein synthesis. Based on the considerations of Hartman and Kester (1983) [51] and of Haissig (1973, 1986) [50,52], a general model for leaf nutrient concentration can be established. The mother plant should be well nourished with macronutrients such as P, K, Ca, and Mg, and should be moderately deficient in nitrogen.

Organic waste compost-based substrates present the highest available contents of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, which is possibly associated with the higher proportions of poultry and cattle manure, coffee husk, and sewage sludge in the mixes. In comparison to the commercial substrates, the levels of  $\text{NH}_4^+$ -N in the organic waste compost-based substrates are higher, and in most cases above the ideal range recommended by Abad et al. (2001) [10]. The clones (*E. grandis* × *E. urophylla*) preferentially absorbed  $\text{NH}_4^+$ -N in relation to  $\text{NO}_3^-$ -N. However, as verified by Atiyeh et al. (2000) [53], the high content of  $\text{NH}_4^+$  in poultry manure could affect plant roots, which may cause detrimental effect in the plant growth.

Studies on the effects of various types of compost on forest production are quite disparate and highly variable. Composted manure did not increase root development or water status in poplars, but increased soil water retention properties. This type of compost caused growth gain. Compost of municipal solid residues stimulated the growth of poplar and eucalyptus. Compost or mulch of Southern fern moderately stimulated the growth of eucalyptus seedlings (+8 to 20%) [49]. In most cases, the spreading of sludge, pretreated wastewater, manure, compost, and industrial effluents actually caused an increase in organic carbon, organic matter, and nitrogen in the soil and increased the productivity of willows, poplars and eucalyptus, filling the deficiencies in N and K of the trees.

In this study, the highest values of eucalyptus growth were obtained with S3, the substrate made with compost derived from corn bare cobs and poultry manure. This might have happened because growth is a function of total pore space [54]. A substrate with low bulk density is economically favorable because it significantly improves the operational capacity of the growing medium, decreasing the cost of transport and manipulation of the materials [55].

Several authors agree with the results found in this study. Oliveira Junior et al. (2011) [56] concluded that the presence of livestock manure in the formulation of substrates gives rise to benefits such as an increase in the supply of nutrients, a reduction in costs in the production of seedlings, and a decrease in the dependence on forestry in relation to commercial substrates. On the other hand, Santos et al. (2000) [57] highlighted that it is difficult to find a material that meets all the requirements of the species that are cultivated. However, Da Silva et al. (2012) [58] obtained the best results for the production of clonal seedlings of *E. urophylla* × *E. grandis* hybrids using coconut husk fiber and carbonized rice husk as pure components of the substrate, indicating that it is possible to find materials that, in a pure state, can be used as substrates. In this sense, Salles et al. (2015) [13] obtained a better agronomic response in hybrid clones of *E. grandis* × *E. camaldulensis* with a substrate based on composted poultry manure (100%). Although this compost had limitations of use due to the high electrical conductivity, limited thermophilic phase, and a certain degree of toxicity [59], when used as a substrate for eucalyptus seedlings they obtained the best results. In this sense, composts of livestock origin, despite the limitations on their use due to their physicochemical properties, could be used as substrates in forestry.

These alternative substrates, when well characterized and corrected by suitable mixtures, make it possible to produce plants with better quality more rapidly and avoid over-exploitation of natural peatlands [60]. The use of those substrates contributes to resolving the problem of local waste generation (e.g., production waste and poultry manure). Alternative substrates must be increasingly used in order to include horticulture in sustainable agricultural systems [7].

An alternative to improve the agronomic performance of organic wastes composted-based substrates in order to reach the performance presented by commercial substrates would be to increase the addition rate of plant residues rather than animal waste, thereby decreasing EC and nutrients contents. The combination of peat with organic waste is another strategy that can reduce high salinity, heterogeneity, and/or high content of contaminants and nutrients in substrates [61].

Marrón (2015) [49] attempted to compile the results of studies about the effects of land applications of various kinds of residues (principally organic), including sewage and industrial sludge, wastewater, manure, compost, ashes, biochar, and landfill leachates in

plantations, under natural conditions, or under controlled pot conditions of the three species mostly used for short-rotation coppice (SRC), namely, willow, poplar, and eucalyptus. Agronomic (at plant and soil levels) and environmental (soil and water contamination) effects were reviewed. The authors found that only 8% of the 288 references were from South America. In contrast to agriculture, where manure is primarily used, in short rotation coppice is used less than one third of the time. In the present study, we found clone preference and synergism for specific substrates. Zaller (2007) [62] highlighted differences of vermicompost effects between crop varieties and provided recommendations on the optimum proportion of vermicompost amendment to horticultural potting substrates.

These results suggest that further research is necessary on the final use of these composts in agricultural applications. This is a strategic way of solving a specific waste problem by recycling it and turning it into a valuable component for a cultivation substrate for specific primary productions. These results suggest that the interactions among substrates and clones are worthy of further investigation.

## 5. Conclusions

The studied substrates showed higher survival than that of the control. *E. grandis* × *E. camaldulensis* hybrid propagation could be carried out in alternative substrates formulated from poultry manure compost. The physicochemical characteristics of substrates composed of pine bark and poultry manure compost provide adequate conditions for the growth of eucalyptus. In this sense, the composting treatment of poultry manure and its use in forest nurseries contributes to the circular economy approach by minimizing negative effects on the environment.

GC8 was the genotype most sensitive to the use of different substrates, showing significant differences in several vegetal growth parameters (roots/callus ratio, radicular dry weight and cutting dry weight). This clone might be a good option to evaluate compost-based substrates for forestry applications.

**Author Contributions:** Conceptualization, P.F.R., D.K. and A.S.; methodology, P.F.R. and L.F.S.; software, V.D.T.; formal analysis, V.D.T.; investigation, P.F.R. and D.K.; resources, P.S.P.; data curation, V.D.T. and M.C.S.; writing—original draft preparation, P.F.R., V.D.T. and M.C.S.; writing—review and editing, P.F.R. and A.S.; visualization, L.F.S., N.I.R. and J.P.D.; supervision, L.F.S., N.I.R. and J.P.D.; project administration, P.F.R.; funding acquisition, P.F.R. and A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by PNNAT 1128042 and 2019-E2-I518-002 from INTA, Argentina.

**Acknowledgments:** Pedro F. Rizzo would like to thank INTA for the PhD research scholarship awarded.

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

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