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# Composted Solid Digestate and Vineyard Winter Prunings Partially Replace Peat in Growing Substrates for Micropropagated Highbush Blueberry in the Nursery

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**Abstract:** The "soilless" cultivation of blueberry (*Vaccinium corymbosum* L.) in containers with peat as substrate allows overcoming the problem of unsuitable soils, thus enhancing the spread of this crop in new areas. Since the use of peat presents several critical environmental and economic sustainability issues, the evaluation of alternative solutions is required. The effectiveness of compost produced with solid digestate and residues from the vine-wine chain to replace part of the peat was therefore tested. Micropropagated plants of cultivar Duke grown in three substrates consisting of a mixture of commercial peat with three compost fractions (10, 20, 40%) were compared with plants grown in 100% unfertilized or fertilized peat (0.3 g of Osmocote per pot). Plant height did not significantly differ between the five theses at the end of the trial, whereas the total number of nodes per plant was higher than in the control theses, due to a greater development of secondary shoots. The nutritional status of the plants, monitored with Dualex, during the growing season, was generally not significantly different in the innovative substrates compared to peat alone. In mid-summer the plants grown in substrates with compost showed the best nitrogen balance index (NBI values). Plants cultivated with medium-high percentages of compost (20–40%) showed a lower degree of defoliation at the end of the trial, dependent on a slower decline of vegetative activity. The final destructive measures of fresh and dry weight of biomass and of its partitioning between roots and shoots highlight that the use of compost did not negatively affect the production of biomass, but rather, in the theses with the highest percentages of compost (20–40%), root development was stimulated.

**Keywords:** compost; *Vaccinium corymbosum*; by-products; smart agriculture; sustainability



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## 1. Introduction

The highbush blueberry (*Vaccinium corymbosum* L.) is becoming the most popular among the cultivated berries, because of consumer interest in the health properties and sensory attributes of its fruits [1–3]. Since the market of fresh and processed fruits and extracts is very receptive to this "superberry", production is constantly growing worldwide, providing in many countries the opportunity of a differentiation of the fruit offer and potential alternative non-food uses.

However, the species need acidic and limestone-free soils that might limit the possibility of extending its cultivation. Although a large part of the arable land in the world is acidic [4], most of it is not suitable for the cultivation of blueberry due to the geographical distribution of these areas in inappropriate latitudes and because the acid reaction may be the effect of acid rain and poor farming practices, conditions that prevent a successful

production activity. For this reason, there is a growing interest in the "soilless" cultivation of blueberry in containers, using proper substrates to overcome the problem of unsuitable traditional soils [5,6]. This production system is traditionally used in nurseries, but it is nowadays becoming important for the production cycle in the world's largest producer country (USA) as in other parts of the world and is playing an important role in enhancing the spread of this crop in new areas. Currently peat is the only or preeminent component of these substrates due to its water retention capacity, high ion exchange capacity, and acid pH [5]. Nevertheless, the use of peat presents several critical environmental and economic sustainability issues. Peat rarity and cost, and the environmental impact of using a resource renewable only in an extremely long time, makes it important to identify more sustainable alternative solutions. Coir is used as a substitute for peat [5], but it is a fairly expensive and not indefinitely available resource. Composts of different origin, starting from a wide range of feedstocks, such as manure solids, chipped woody prunings, plant materials from urban landscape maintenance, bark, etc., have been tested on highbush blueberry and chemical characteristics that indicate potential suitability for blueberry have been suggested [7,8]. The trials of Sullivan et al. [7] have shown that composts can be used to increase soil organic matter for blueberry, but limits to N content (total N < 20 g $kg^{-1}$) were considered necessary in order to avoid problems with high pH, EC, and excess K. The kind of feedstock composted is then a crucial factor in obtaining a custom compost for blueberry, which must balance the need to supply nutrients and avoid high pH and EC values.

Among the potential feedstocks for composting, digestates from anaerobic digestion have attracted attention for the positive effects on the physical properties of the soil, the good fertilizer and biostimulating potential, due to the content of nutrients (N and P) and humic and fulvic acids [9]. Digestates have been recently proposed as components of substrates for different horticultural and fruit tree species [10–12], also together with other materials, such as vineyard prunings [11]. However, the effect of these matrices as components of substrates for blueberry cultivation is unknown. Therefore, this research was aimed at evaluating the response of blueberry plants growth to innovative substrates in which peat has been partially replaced by compost obtained with solid digestate and vineyard pruning wood. The choice of these materials was linked to a wide availability in the area where the research has been carried out, in order to ensure the sustainability of a compost production process strongly related to criteria of circular economy and valorization of agro-food by-products.

The high demand of highbush blueberry plants for new cultivations has stimulated the use of micropropagation to make a large number of plants available in a short time [13,14]. Micropropagated plants of highbush blueberry are currently available and largely used in Italy and other countries in order to quickly establish new plantations. For this reason, in this trial, micropropagated plants were used and their growth response to the experimental substrates was evaluated.

## 2. Materials and Methods

### *2.1. Compost Production and Analysis*

Solid digestate was composted with chips (1 cm in length) of vineyard winter prunings mixed at a ratio of 15.0–83.3% in dry weight, respectively. Solid digestate was conferred by a local biogas plant (CAT, Correggio, Italy). Feedstocks used in the anaerobic digestion were maize (*Zea mays* L.) silage (43%), triticale (X Triticosecale Wittmack) silage (22%), cow slurry (27%), and grape stalks (8%), derived from Lambrusco's vineyards surrounding the biogas plant. Pruning wood was taken from a vineyard of cultivar "Lambrusco Salamino", grafted on "Kober 5 BB" rootstock, located in Reggio Emilia, Italy.

The composting process was carried out at the University of Modena and Reggio Emilia, located at Reggio Emilia, Italy, through a static pile on a farm composting for 115 days, turned weekly. An aliquot of 1.7% dry weight of mature compost was added to the 1 $m^3$ pile as a composting starter. The pile was periodically irrigated when the pile

gravimetrically determined relative humidity (RH) was <50%. The composting trend was checked by measuring the temperature in different points of the mass by thermoresistance sensors (PT100, Gandolfi, Parma, Italy).

The compost was analyzed at the laboratory EST (Bergamo, Italy). The content of the following components was detected according to the respective procedures: total organic carbon (C) DM 21/12/2000 GU n° 21 26/01/2001 suppl. N° 6 (UNI EN 13137:2002); total nitrogen (N) (UNI EN 13654–1:2001 and ISO 11261:1995); Mg, Fe, NaO, Ca, $P_2O_5$, and $K_2O$ contents (UNI EN 13650:2002 and UNI EN ISO 11885:2009); water content (UNI EN 13040:2008); Pb, Zn, Cu, Cd, Ni (UNI EN 13650:2002 and UNI EN ISO 11885:2009); Hg (ISO 16772:2004); Cr (ANPA Met.16 Man 3 2001); pH (UNI EN 13037:2012); electrical conductivity (EC) was determined on wet material (1:5 ratio) using a CRISON GLP 31 EC meter (Crison Instrument, Barcelona, Spain).

### 2.2. Phytotoxicity Characterisations of Compost

Compost phytotoxicity as germination index (GI) was determined according to Zucconi et al. [15]. Total of 4 mL of water compost extract (50 g $L^{-1}$) was applied over sterile filter paper in Petri dishes and 20 seeds of garden cress (*Lepidium sativum* L.) were then placed on the filter paper and incubated at 25 °C for 5 days. The analysis was run in triplicate. The germination index percentage (GI%) was calculated, according to the following formula for roots (1) and shoots (2):

$$GI\% = 100 \times (G1/G2) \times (R1/R2) \quad (1)$$

$$GI\% = 100 \times (G1/G2) \times (S1/S2) \quad (2)$$

where G1 and G2 are germinated seeds in the sample and control; R1 and R2 are the mean root length for the sample and for the control, respectively (1), and S1 and S2 are the mean shoot length of the sample and control, respectively (2).

### 2.3. Microbiological and Suppressiveness Characterizations

The abundance of culturable filamentous fungi, total bacteria, and spore-forming bacteria in the compost produced was evaluated by a serial ten-fold ($10^{-1}$ to $10^{-7}$) dilution method in triplicate. Fungi were counted on the potato dextrose agar (PDA, Oxoid, UK) pH 6, supplemented with 150 mg $L^{-1}$ of nalidixic acid and 150 mg $L^{-1}$ of streptomycin. Total bacteria were counted on the selective medium (glucose 1 g $L^{-1}$, protease peptone 3 g $L^{-1}$, yeast extract 1 g $L^{-1}$, potassium phosphate buffer 1 g $L^{-1}$, agar 15 g $L^{-1}$) supplemented with 100 mg $L^{-1}$ of actidione. Spore-forming bacteria were counted by plating ten-fold dilutions of the compost on nutrient agar previously heated at 90 °C for 10 min. Population densities were expressed as a colony forming unit (CFU) $g^{-1}$ dry weight of compost. Coliform, *Escherichia coli*, and *Salmonella* spp. detection was performed following the methods reported by Cekmecelioglu et al. [16].

The suppressiveness capacity of this compost on *Rhizoctonia solani* and *Sclerotinia minor* was assessed using garden cress as a host plant. Potting mixes were done by amending commercial peat with compost at a rate of 20% by vol. [17]. The bioassays were performed on ten pots per treatment, and the pots filled up only with non-amended peat were used as the control. Pathogen inoculations and pot assessment were performed as reported by Pane et al. [18]. The bioassay was done in duplicate.

### 2.4. Plant Materials and Nursery Greenhouse Trial

Plants micropropagated and acclimatized of *Vaccinium corymbosum* cultivar *Duke* were provided by Battistini Nursery (Cesena, Italy). The plants were transplanted manually at the beginning of April (one plant per pot) in 1.5 L plastic pots and placed in a nursery greenhouse, located at Reggio Emilia, Italy, with programmed temperature ranging from 19 to 28 °C (day/night), within the range of temperatures not detrimental to growth for blueberry cv. Duke (Zheng et al., 2017) [19], relative humidity ranging from 50% to 70%,

and with a natural photoperiod and solar radiation. Three experimental formulations consisting of commercial peat mixed with three different percentages of compost (T1: 10%; T2: 20%; T3: 40%) were compared with two reference substrates: T4, consisting of 100% unfertilized peat, and T5, consisting of 100% peat fertilized with commercial nitrogen fertilizer: 0.3 g of Osmocote top dress (22-5-6 + 2MgO) per pot, applied at the end of May. The commercial peat (TERCOMPOSTI Acid, Tercomposti Spa, Calvisano (BS), Italy) was characterized by pH 4, EC 0.4 dS $m^{-1}$, bulk density 140 kg $m^{-3}$, total porosity 93%.

The plants were kept inside the greenhouse until the end of July and then moved outside under shade nets, where they were irrigated every night by spinner-type sprinklers in order to maintain the substrate at water capacity. The average outdoor temperature was 26.1 °C in August (with an average maximum month temperature of 30 °C), 20.7 °C in September, 16.6 °C in October, and 10.1 °C in November. Rainfall in the August-November period was 380 mm, with 19.2 mm in August.

Due to the absence of pests no phytosanitary treatments were done. Pots were arranged in a completely randomized design with twelve replicates.

### *2.5. Recorded Parameters*

During the growing season, plant growth was monitored periodically measuring the maximum plant height and the number of shoot nodes per plant.

In mid-summer (193 DOY), when shoot elongation was almost completed, for each thesis a total of 36 leaves, divided into groups of 12 for small, medium, and large sizes, were sampled on which length and width were measured and the leaf area was calculated, according to the following equation defined for *Vaccinium corymbosum* cultivar *Duke* by Fallovo et al. [20]:

Leaf area = 0.54 + 0.68 × L × W, where L = length of leaf blade; W = width of leaf blade.

The total number of leaves *per* plant was counted and the total leaf area was then calculated.

Physiological status of the plants was non-destructively assessed measuring at 185, 206, 225, and 285 DOY, the following parameters: leaf chlorophyll (CHL) and flavonoid content (FLAV), nitrogen balance index (NBI) (the ratio between CHL and FLAV), leaf anthocyanin content (ANT), by means of the optical leaf-clip meter Dualex 4 Scientific (Dx4, FORCE-A, Orsay, France) [21].

At the end of the trial (327 DOY), the plants were extracted from the pots and the substrate was accurately removed from the roots. The plants were then dissected into aboveground part (A) and roots (R) and fresh weight of the above ground part of the plant (AFW) and root fresh weight (RFW) were detected and the total fresh weight (TFW) of the plant was calculated. Then, each part was oven-dried at 70 °C, to a constant weight, and the aboveground part dry weight (ADW), root dry weight (RTDW), and total dry weight (TDW) were measured, and the fractions of total dry weight allocated to the aboveground part of the plant (FDWA) and to roots (FDWR) were recorded.

### *2.6. Data Analysis*

The results were expressed as means ± standard deviations (SD). Analysis of variance (ANOVA) was performed on the data collected during the experiment and Duncan's test and (at $p < 0.05$) used to compare treatment. All data processing was carried out using the GenStat 17th software (VSN International, Hemel Hempstead, UK).

## 3. Results and Discussion

### *3.1. Compost Characterization and Assessment*

The analytical data reported in Table 1 reveal quite high contents of N, P, K, and Mg in the compost produced from digestate and grapevine prunings. The content of heavy metals was within the limits admitted by the European Regulation CE 2003/2003 for commercial amendment in the European Community. A pH value of 7.0 and an EC of 4.5 dS $m^{-1}$ were detected. The C/N ratio of 12.9 indicates the condition of compost maturity [22,23]. This C/N ratio corresponds to the threshold below which the compost is probably unsuitable for

high-rate application to blueberry because of the high content of soluble nutrients and high pH [7]. Negative linear responses were observed between the plant growth parameters of foliage volume and cumulative water uptake and pH values in the range of 4.9 to 6.8 [24]. Similarly, the total K > 10 g $kg^{-1}$ and EC > 4 dS $m^{-1}$ must be considered prudentially at risk for short-term plant damage when applied at high rates, according to the observation of Sullivan et al. [7] in trials on blueberry grown in pots. As a consequence, the composition of the compost obtained from digestate and vineyard pruning, as a complex, has potentiality for growing blueberry, but these negative traits with respect to the needs of the species seem to preclude the use of pure compost. Based on these considerations and on observations obtained also in other experiments on different species [25,26], in the present study compost was used as fractions from 10% to 40% vol. in a peat-based mixture, to mitigate these negative traits.

**Table 1.** Chemical characteristics of the compost. All values as dry weight.

| Compost Characteristics | Value |
|---|---|
| pH | 7.03 |
| EC (dS $m^{-1}$) | 4.15 |
| Moisture (%) | 69.5 |
| CEC (meq 100 $g^{-1}$) | 54.3 |
| Organic Carbon (%) | 34.5 |
| Total Nitrogen (%) | 2.67 |
| Organic Nitrogen (%) | 99.45 |
| Humic and Fulvic Carbon (%) | 16.4 |
| Cr VI (mg $kg^{-1}$) | <0.50 |
| Pb (mg $kg^{-1}$) | 11.7 |
| Zn (mg $kg^{-1}$) | 191.1 |
| Cu (mg $kg^{-1}$) | 48.2 |
| Hg (mg $kg^{-1}$) | <0.20 |
| Cd (mg $kg^{-1}$) | 0.5 |
| Ni (mg $kg^{-1}$) | 9.8 |
| C/N Ratio | 12.9 |
| P (%) | 0.74 |
| K (%) | 1.58 |
| Al (mg $kg^{-1}$) | 4597.0 |
| Mg (%) | 0.54 |
| Fe (mg $kg^{-1}$) | 6253.0 |
| Ca (%) | 3.00 |
| Na (mg $kg^{-1}$) | 607.1 |
| S (%) | 0.469 |
| Mn (mg $kg^{-1}$) | 215.0 |

The germination assays indicated no phytotoxicity problems. In fact, the germination index displayed values higher than 50%, which is considered the threshold value for phytotoxicity [15]. In particular, the germination index showed higher values for the root and shoot of the sensitive reference species "garden cress" when treated with a water extract of compost (Table 2).

The microbiologically induced suppression of soilborne plant pathogens is one of the beneficial properties often attributed to compost [27]. Research on the suppressiveness of composts has revealed in general a positive or no effect on disease suppression, depending on the type of compost [28]. A key role to the biological control of organic matrices has also been attributed to the fungal population [29]. Blueberry forms symbiosis with ericoid mycorrhizal fungi able to decompose the organic substance. Research on the effects on *Vaccinium corymbosum* of organic fertilizer, including compost, showed an increase of soil biota activity, an improvement of mycorrhizal colonization, and tolerance to soil pathogens with respect to conventional N source [30].

**Table 2.** Phytotoxicity assessment and microbiological and suppressiveness characterization of Compost. * = statistically significant; CFU = colony forming unit. Data are reported as mean values ($n$ = 5) ± SD.

| Index | Value |
|---|---|
| C-GI root (%) | 60.01 * ± 15.01 |
| C-GI shoot (%) | 136.51 * ± 13.21 |
| *Pseudomonas* (CFU $g^{-1}$) | $2.3 \times 10^4$ ± 3011.04 |
| *Bacillus* (CFU $g^{-1}$) | $6.9 \times 10^5$ ± 2807.02 |
| Fungi (CFU $g^{-1}$) | $5.0 \times 10^4$ ± 1905.45 |
| Bacteria (CFU $g^{-1}$) | $1.0 \times 10^7$ ± 4708.19 |
| Total *Coliform* bacteria (CFU $g^{-1}$) | Absent |
| *Faecal coliform* (CFU $g^{-1}$) | Absent |
| *Yeasts* (CFU $g^{-1}$) | Absent |
| *Streptococci* (CFU $g^{-1}$) | Absent |
| *Escher coli* (CFU $g^{-1}$) | Absent |
| *Salmonella* spp. (CFU $g^{-1}$) | Absent |
| *Clostridia* spp. (CFU $g^{-1}$) | Absent |
| *Rhizoctonia solani* damping-off (%) | 87.6 * ± 15.12 |
| *Sclerotinia minor* damping-off (%) | 40.3 ± 3.26 |

Table 2 shows the level of microbial populations in the produced compost. Populations of total fungi and total bacteria were $5.0 \times 10^4$, $1 \times 10^7$ (CFU $g^{-1}$), respectively. *Coliform bacteria*, *Escherichia coli*, *Clostridia* spp., and *Salmonella* spp. were completely absent, complying with the requirement of Decision 2001/688/CE for assigning a community eco-label to soil amendments and cultivation substrates.

The suppressive bioassays revealed that garden cress dumping-off in samples inoculated with *Rhizoctonia solani* has been slightly reduced by compost, while was significantly cut down in presence of *Sclerotinia minor* (Table 2). As observed more generally for composts by Avilés et al. [31], this suppressive capacity can be considered a very positive feature that gives compost a plus value for use as a component of growing media.

### 3.2. Vegetative Growth and Physiological Indices

Plant height was uniform in the compared theses at the end of April, at the start of the experiment (DOY 113), but in the subsequent surveys, at the beginning of the summer, the length of the main shoot became significantly shorter in T2 (20% compost–80% peat) with respect to the other theses (Table 3). Subsequently, in August (DOY 225) and at the end of September (DOY 266), the plants reached a very similar maximum height on the different substrates in comparison (Table 3). In particular, the plants of T2 were those with the highest growth rate (+143% from June to November), reaching final heights similar to those of the other theses. The lower growth in height of the plants of thesis 2 in the first months of vegetative activity is, as mentioned, temporary and refers only to the elongation of the main shoot. Therefore, the different behavior of thesis 2 plants was interpreted by evaluating the total number of nodes per plant that best expresses the overall growth of the canopy. As can be seen in Table 4, the total number of nodes did not differ between theses in April (DOY 113), it became significantly higher in early July in plants grown on fertilized peat (thesis 5), but it was significantly higher in plants of the thesis 2 than in the control plants at the end of vegetative growth. The initial reduction of the elongation of the main shoot therefore seems to go along with a greater development of lateral shoots, which leads to a greater complexity of the plant structure and to a higher number of nodes. The promoting action of these development differences may have been determined by hormone-like actions of the compost, which have also been hypothesized for other composted matrices [32–34].

**Table 3.** Effects of substrates with different compost ratio (0–10–20–40%) on plant height during the growth season. Means followed by the same letter do not significantly differ at $p < 0.05$. DOY = day of year. Data are reported as mean values ($n = 12$) ± SD, n.s. = not significant.

| | Plant Height (cm) | | | | | |
|---|---|---|---|---|---|---|
| **Treatment DOY** | **113** | **144** | **173** | **193** | **225** | **266** |
| T1 | 13.9 ± 2.56 n.s. | 16.2 ± 3.07 n.s. | 24.9 ± 5.68 bc | 40.1 ± 8.13 a | 46.0 ± 9.33 n.s. | 48.3 ± 6.17 n.s. |
| T2 | 13.9 ± 2.81 n.s. | 14.8 ± 2.99 n.s. | 22.3 ± 5.47 c | 29.3 ± 11.23 b | 44.6 ± 10.27 n.s. | 54.2 ± 11.64 n.s. |
| T3 | 12.6 ± 1.98 n.s. | 16.0 ± 2.17 n.s. | 26.9 ± 3.52 ab | 38.1 ± 7.37 a | 48.2 ± 8.60 n.s. | 51.6 ± 10.63 n.s. |
| T4 | 12.9 ± 2.73 n.s. | 15.3 ± 3.07 n.s. | 31.0 ± 6.86 a | 44.7 ± 9.71 a | 52.1 ± 9.13 n.s. | 54.0 ± 9.15 n.s. |
| T5 | 12.8 ± 2.21 n.s. | 16.0 ± 2.76 n.s. | 31.3 ± 4.15 a | 44.5 ± 8.93 a | 54.0 ± 16.17 n.s. | 53.3 ± 17.48 n.s. |

T1 = commercial peat (P) 90% + compost (C) 10%; T2 = P 80% + C 20%; T3 = P 60% + C 40%; T4 = commercial peat (P) 100%; T5 = P 100% + Osmocote.

**Table 4.** Total number of nodes per plant and estimated leaf area in the compared treatments. Means followed by the same letter do not significantly differ at $p < 0.05$. Total number of nodes and leaf area. DOY = day of year. Data are reported as mean values ($n = 12$) ± SD, n.s. = not significant.

| **Treatment** | **Total Number of Nodes (cm)** | | | **Leaf Area ($cm^2$)** |
|---|---|---|---|---|
| **DOY** | **113** | **193** | **324** | **193** |
| T1 | 20.4 ± 7.12 n.s. | 50.0 ± 15.79 b | 130.6 ± 26.0 ab | 669.3 ± 249.66 b |
| T2 | 18.6 ± 6.15 n.s. | 38.9 ± 14.13 b | 139.3 ± 17.93 a | 378.4 ± 224.21 c |
| T3 | 20.0 ± 5.56 n.s. | 46.3 ± 12.03 b | 125.0 ± 12.49 ab | 506.9 ± 205.58 bc |
| T4 | 16.4 ± 6.32 n.s. | 45.8 ± 4.92 b | 113.4 ± 31.06 b | 684.4 ± 218.15 ab |
| T5 | 20.8 ± 6.48 n.s. | 64.9 ± 15.01 a | 115.8 ± 16.92 b | 865.7 ± 234.08 a |

T1 = commercial peat (P) 90% + compost (C) 10%; T2 = P 80% + C 20%; T3 = P 60% + C 40%; T4 = commercial peat (P) 100%; T5 = P 100% + Osmocote.

These results suggest that the plants grown on compost-based substrates showed on the whole a greater development of secondary shoots, often even small ones, which inevitably raised the average number of nodes and total leaf area per plant (Table 4).

The plants of T5 initially responded to the fertilization carried out at the end of May with a marked increase in the growth of the leaf surface, rather than with an increase in the height of the main shoot (Tables 3 and 4). As for the remaining theses, T1 shows intermediate, and not statistically different, leaf area values between T2–T4 and T5.

The nutritional status of the plants grown in the innovative substrates, monitored with Dualex during the growing season, was generally not significantly different compared to peat alone (Table 5). The chlorophyll index was significantly higher in plants grown on substrates containing 10 and 20% of compost with respect to 100% peat (T5) and 40% compost (T3) at the beginning of July, whereas the values were not significantly different in the following measurements. On the same date the highest flavonoid index was observed in plants on substrate with 10% of compost, with values not significantly different from T2 and T5. In mid-summer, the plants grown in substrates with compost showed the highest NBI values.

Chlorophyll, flavonoids, and anthocyanin indices are assumed as references of the plant nutritional status and vigor, in particular as regard to nitrogen content and the response to growing media [35]. The satisfactory nitrogen nutritional status of plants on substrates with compost could be due to its good content of organic nitrogen (Table 1). Good availability of organic N in the soil provides $NH_4^+$ to the roots, the preferred form absorbed by highbush blueberries, that was indicated as a determinant of an increase of leaf nitrogen concentration and gas exchange and a higher vegetative growth in 1-year plants of "Emerald" blueberries [36].

**Table 5.** Effects of substrates with different compost ratios (0–10–20–40%) on chlorophyll (CHL), flavonoids (FLA) and anthocyanins (ANTH) content, and nitrogen balance index (NBI) at different times during the growing season. Means followed by the same letter do not significantly differ at $p < 0.05$. DOY = day of year. Data are reported as mean values ($n = 12$) ± SD, n.s. = not significant.

| DOY | Treatment | CHL (−) | FLAV (−) | NBI (−) | ANTH (−) |
|---|---|---|---|---|---|
| 185 | T1 | 31.00 ± 3.04 a | 1.60 ± 0.19 a | 19.44 ± 2.86 n.s. | 0.25 ± 0.03 n.s. |
| | T2 | 31.47 ± 1.88 a | 1.47 ± 0.07 ab | 21.46 ± 1.08 n.s. | 0.24 ± 0.01 n.s. |
| | T3 | 26.78 ± 2.29 b | 1.30 ± 0.09 c | 20.72 ± 1.51 n.s. | 0.25 ± 0.02 n.s. |
| | T4 | 26.93 ± 1.49 b | 1.38 ± 0.05 bc | 19.80 ± 1.82 n.s. | 0.27 ± 0.01 n.s. |
| | T5 | 29.13 ± 2.06 ab | 1.45 ± 0.03 ab | 20.16 ± 1.75 n.s. | 0.25 ± 0.01 n.s. |
| 206 | T1 | 26.60 ± 2.27 n.s. | 1.48 ± 0.09 n.s. | 18.04 ± 2.61 abc | 0.27 ± 0.01 n.s. |
| | T2 | 25.79 ± 4.12 n.s. | 1.31 ± 0.15 n.s. | 19.70 ± 2.48 ab | 0.25 ± 0.02 n.s. |
| | T3 | 26.08 ± 4.91 n.s. | 1.28 ± 0.11 n.s. | 20.42 ± 3.17 a | 0.27 ± 0.03 n.s. |
| | T4 | 22.95 ± 1.63 n.s. | 1.38 ± 0.07 n.s. | 16.81 ± 1.82 bc | 0.27 ± 0.02 n.s. |
| | T5 | 22.43 ± 1.82 n.s. | 1.39 ± 0.16 n.s. | 16.31 ± 1.88 c | 0.28 ± 0.01 n.s. |
| 225 | T1 | 24.17 ± 2.63 n.s. | 1.49 ± 0.01 n.s. | 16.41 ± 2.49 n.s. | 0.27 ± 0.02 n.s. |
| | T2 | 26.85 ± 4.06 n.s. | 1.42 ± 0.08 n.s. | 19.25 ± 3.45 n.s. | 0.27 ± 0.03 n.s. |
| | T3 | 24.35 ± 3.70 n.s. | 1.56 ± 0.01 n.s. | 15.84 ± 3.06 n.s. | 0.29 ± 0.02 n.s. |
| | T4 | 25.76 ± 4.10 n.s. | 1.38 ± 0.02 n.s. | 18.83 ± 3.68 n.s. | 0.25 ± 0.02 n.s. |
| | T5 | 28.44 ± 3.51 n.s. | 1.33 ± 0.01 n.s. | 21.85 ± 2.82 n.s. | 0.27 ± 0.03 n.s. |
| 255 | T1 | 28.19 ± 4.81 n.s. | 1.64 ± 0.28 n.s. | 17.20 ± 5.16 n.s. | 0.27 ± 0.03 n.s. |
| | T2 | 29.29 ± 3.28 n.s. | 1.81 ± 0.12 n.s. | 16.19 ± 2.21 n.s. | 0.26 ± 0.04 n.s. |
| | T3 | 28.22 ± 4.32 n.s. | 1.78 ± 0.11 n.s. | 15.90 ± 2.67 n.s. | 0.27 ± 0.01 n.s. |
| | T4 | 25.55 ± 3.83 n.s. | 1.61 ± 0.10 n.s. | 16.51 ± 2.38 n.s. | 0.26 ± 0.03 n.s. |
| | T5 | 28.03 ± 2.75 n.s. | 1.71 ± 0.29 n.s. | 16.93 ± 4.02 n.s. | 0.27 ± 0.01 n.s. |

T1 = commercial peat (P) 90% + compost (C) 10%; T2 = P 80% + C 20%; T3 = P 60% + C 40%; T4 = commercial peat (P) 100%; T5 = P 100%+ Osmocote.

Plants cultivated with medium-high percentages of compost (20–40%) showed a lower degree of defoliation at the end of the trial, dependent on a slower decline of vegetative activity (Table 6). The thesis 4 (100% peat) showed the highest number of plant totally defoliated, followed by thesis 1, (10% of compost). The plants grown on higher percentages of compost, i.e., T2 and T3, and those fertilized with nitrogen fertilizer, on the other hand, showed a medium-low degree of defoliation, an indication of good vigor and good nutritional status. In theses with compost, the low degree of defoliation also coincided with a lower lignification of the stems, a characteristic that can be negative in environments with harsh winters, but which can instead be interesting in mild climates or in conditions of protected cultivation in containers.

**Table 6.** Qualitative data about the percent of plant at different levels of defoliation in the compared theses in mid-November. DOY = day of year. Data are reported considering the total plant and without replicates ($n = 12$).

| | Degree of Leaf Fall—DOY 318 | | | |
|---|---|---|---|---|
| **Treatment** | **Total** | **High** | **Medium** | **Low** |
| T1 | 0.56 | 0.22 | 0.11 | 0.11 |
| T2 | 0.00 | 0.00 | 0.22 | 0.22 |
| T3 | 0.11 | 0.22 | 0.56 | 0.56 |
| T4 | 0.89 | 0.11 | 0.00 | 0.00 |
| T5 | 0.00 | 0.22 | 0.67 | 0.67 |

T1 = commercial peat (P) 90% + compost (C) 10%; T2 = P 80% + C 20%; T3 = P 60% + C 40%; T4 = commercial peat (P) 100%; T5 = P 100% + Osmocote.

### *3.3. Fresh and Dry Biomass Partitioning*

The final destructive measures of plant fresh and dry weight and of its partitioning between roots and above ground part of the plant highlight that the use of compost did

not negatively affect the production of biomass, but rather, in the theses with the highest percentages of compost (20–40%); there was a high dry biomass weight and a tendency of a greater root development (Table 7). The total annual growth, expressed by total fresh and dry weight, on substrates with 20 and 40% compost (T2 and T3), without additional N fertilizer, was not significantly different from that obtained on fertilized peat. Plants grown in soil with 10% compost and in not fertilized peat accumulated a lower quantity of biomass with respect to the other thesis. It can be assumed that the nitrogen needs of plants T2 and T3 were supported in the first-year growth by the nitrogen present in the substrate and derived from the mineralization of the organic substance, considering the reserves in the plant at the time of transplantation to be negligible. This tendency to better use the resources available in the container could be exploited in organic nursery systems. Bañados et al. [37] found that in field-grown blueberries the application of N fertilizer leads to a decrease of 56% of nitrogen derived from these sources and from the pre-planting application.

**Table 7.** Values at DOY 327 of fresh and dry weight allocated in the above ground part and in the roots of blueberry plants cv Duke grown on substrates with different percentage of peat and compost from solid digestate and grapevine pruning woods. Means followed by the same letter do not significantly differ at $p < 0.05$. Data are reported as mean values ($n = 12$) ± SD.

| Treatment | AFW (g plant$^{-1}$) | | RFW (g plant$^{-1}$) | | TFW (g plant$^{-1}$) | | ADW (g plant$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| T1 | 14.37 ± 5.91 | ab | 8.26 ± 6.51 | b | 24.63 ± 5.02 | b | 6.87 ± 2.78 | b |
| T2 | 16.69 ± 5.61 | ab | 30.52 ± 7.37 | a | 49.21 ± 6.56 | a | 8.36 ± 2.76 | ab |
| T3 | 12.90 ± 5.43 | b | 22.06 ± 5.39 | ab | 36.96 ± 4.86 | ab | 6.51 ± 2.77 | b |
| T4 | 13.54 ± 4.31 | b | 15.58 ± 4.98 | b | 31.12 ± 4.13 | b | 6.23 ± 2.99 | b |
| T5 | 21.91 ± 4.51 | a | 15.95 ± 7.71 | b | 39.87 ± 5.93 | ab | 11.07 ± 3.01 | a |
| **Treatment** | **RDW (g plant$^{-1}$)** | | **TDW (g plant$^{-1}$)** | | **FDWA (%)** | | **FDWR (%)** | |
| T1 | 3.22 ± 2.41 | b | 10.08 ± 5.08 | b | 71 ± 9.19 | a | 29 ± 8.45 | b |
| T2 | 7.38 ± 2.65 | a | 15.74 ± 3.46 | ab | 53 ± 8.95 | b | 47 ± 9.12 | a |
| T3 | 6.98 ± 2.27 | ab | 13.49 ± 4.11 | ab | 49 ± 7.43 | b | 51 ± 7.78 | a |
| T4 | 4.45 ± 2.62 | ab | 10.78 ± 5.67 | b | 58 ± 8.14 | ab | 42 ± 8.32 | ab |
| T5 | 5.90 ± 2.38 | ab | 16.97 ± 3.92 | a | 66 ± 9.01 | a | 34 ± 8.88 | ab |

T1 = commercial peat (P) 90% + compost (C) 10%; T2 = P 80% + C 20%; T3 = P 60% + C 40%; T4 = commercial peat (P) 100%; T5 = P 100% + Osmocote. AFW = shoot fresh weight; RFW = root fresh weight; TFW = total fresh weight; ADW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; FDWA = fraction dry weight to aboveground; FDWR = fraction dry weight to root.

As regards the distribution of biomass between above ground and below ground parts of the plant, in the young blueberries of this experiment the allocation of biomass was different in the substrates under assessment. The largest part of dry matter was allocated to the crown, with the exception of T3 (40% compost). The prevailing distribution of dry weight in the crown was also detected in mature plants of different cultivars of *Vaccinium corymbosum* [38]. The general characteristics of the root system were similar in all theses and corresponding to what is known for the blueberry: very thin and dense roots [39], but the results of dry biomass partitioning between above and below ground part of the plants suggest a promoting activity of medium and high rates of compost on root growth, leading to higher values of root dry weight fraction to total plant dry weight. A similar promoting function on biomass production of plants of different species grown in pots under greenhouse conditions has been observed for composts obtained starting from different vegetal matrices including pomace [11,26]. In previous vineyard trials it was highlighted that root growth of grapevines was stimulated by the distribution of composted vine prunings under the row [40]. In blueberries young plants with large canopies and small root systems are considered less tolerant to environmental and cultural limitation [41], therefore a higher root/crown ratio is to be considered a positive factor. However, it should be considered that in the present research the results obtained on blueberries currently concern cultivation on the non-acidified compost from digestate and vineyard prunings

for a relatively shorter period (8 months). This system therefore proves to be suitable for nursery activities but must be tested over a longer period for use in soil-less cultivation or as a soil amendment for field production. Multi-year tests in the field have shown that unmodified yard debris compost added as a pre-plant amendment caused an increase in the pH of the soil above the critical threshold for blueberry [42].

It is not easy to discriminate the direct and indirect effects and the interactions that the compost component of a substrate can determine on growth and physiological processes of blueberry. It is known that compost modifies the physical and chemical characteristics of the soils and substrates to which it is added. Considering the results of the present study, the beneficial effects on vegetative growth of substrates containing this new compost seem attributable in large part to the nutritional enrichment, particularly N, compared to the peat alone, in the absence of fertilizer inputs. Nitrogen is the primary nutrient applied to blueberry crops for successful growth and production and is particularly important for young establishing plants [43]. Among the macronutrients, even good supply of K provided by the compost can have a positive effect. The demand for K in blueberry is considered very low compared to other crops, nevertheless too low concentrations of this element have been found by Voogt et al. [44] in the leaves of soilless grown blueberries, making it necessary to adjust the content of this element in the nutrient solution.

The neutral pH of the compost, which initially was assumed as a critical aspect, does not seem to have any negative effects on the young blueberries in the ratios with peat used in the experiment. Chlorophyll synthesis and function were not impaired, nor biomass production reduced. This seems to testify that the absorption of microelements Mn and Fe, which are correlated with the concentration and functionality of the chlorophyll [24], were maintained at adequate levels during the growing season.

## 4. Conclusions

The partial replacement of peat with compost obtained using vineyard pruning wood and solid digestate, has produced satisfactory results in the cultivation of Vaccinium corymbosum in containers. In fact, the young plants grown on these innovative substrates did not show excessive slowdowns in vegetative growth and even nutritional deficiencies, but on the contrary, positive effects were obtained in terms of total biomass, development of the root system, and as regards the length of the vegetation period, in particular with an addition of 20% compost. Furthermore, even in plants grown with the highest percentage of compost (40%), no symptoms of phytotoxicity and chlorosis were detected, and a good vigor of the plant and a high production of dry matter were found, a characteristic that predisposes to a satisfactory recovery and active growth after planting in open fields or in larger containers.

From an economic and environmental point of view, these results are very encouraging, as they show how blueberries can be cultivated in containers using substrates that contain much lower percentages of peat than those currently in use, while offering a valuable contribution to the re-use of unexploited agro-industrial by-products and a powerful incentive for a soilless cultivation of blueberries even in areas with soil limitations.

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