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# Effects of Mixes of Peat with Different Rates of Spruce, Pine Fibers, or Perlite on the Growth of Blueberry Saplings

**Laima Česonienė [1,*], Ričardas Krikštolaitis [2], Remigijus Daubaras [1] and Romas Mažeika [3]**

1 Botanical Garden, Vytautas Magnus University, Z.E. Zilibero 6, LT-46324 Kaunas, Lithuania
2 Department of Mathematics and Statistics, Vytautas Magnus University, Studentu 10, Kaunas District, LT-53361 Akademija, Lithuania
3 Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Kėdainiai District, LT-58344 Akademija, Lithuania
* Correspondence: laima.cesoniene@vdu.lt

**Abstract:** Investigations of substrates for growing plant saplings is the basis for the search for new components. Currently, large numbers of saplings are grown for blueberry plantations. Studies on the use of various organic and inorganic components in substrates is relevant in order to reduce the amount of excavated peat. The goal of this study was to analyze the effects of mixes of peat with different rates of spruce, pine fibers and perlite on the growth of blueberry saplings. To define the suitability of substrates, plant vigor assessments of the cultivar 'Duke', including plant height and leaf weight, as well as the chlorophyll fluorescence, content of extractable macronutrients and organic carbon in leaves, were investigated. The best effect on the growth of blueberry saplings, the optimal content of macronutrients in the leaves, was shown for substrates in which a part of the peat was replaced by 15–45% $v/v$ of pine wood fiber and by 15–30% $v/v$ of spruce wood fiber. Pine bark fiber in the mix should not exceed 30% $v/v$. The addition of spruce bark fibers in the different rates had a negative effect on the vegetative growth of the saplings. The quantity of peat in the substrates can also be significantly reduced by adding 15–45% $v/v$ of perlite. These results confirm that pine and spruce fibers or perlite in substrates for blueberry sapling growing could reduce the demand for peat and should significantly contribute to the preservation of unique wetland ecosystems.

**Keywords:** blueberry; fiber; peat; substrate; sapling



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## 1. Introduction

Substrates (media) for the cultivation of berry plants are an important component of a sustainable food production chain. The use of suitable growing substrates in modern industrial horticulture meets the needs of plants and ensures their productivity. Peat currently represents 77–80% of the growing substrates used annually in the horticultural industry in Europe [1]. Peat is an extremely important component in substrates; however, its extraction threatens sensitive ecosystems, causes carbon sinks, and increases greenhouse-gas emissions [2–4]. Different studies on bogs have confirmed that these ecosystems can substantially contribute to reducing atmospheric greenhouse gases [5,6].

Therefore, substrates in which peat can be replaced by alternative components of organic or mineral origin are relevant to preserving unique wetland ecosystems. The suitability of various growing substrates in horticulture has been studied, i.e., certain quality parameters have been evaluated, including the degree of decomposition, the content of extractable nutrients, pH, bulk density, electrical conductivity and porosity. Various scientific sources indicated the possibility of using tree or coconut fibers, compost, tree bark, perlite, and other components that can be mixed to create appropriate growing substrates [7,8]. When studying substrate compositions, the vegetative growth of plants should be assessed because the substrate should provide plants with an appropriate amount of water and nutrients [9].

Recently, there has been a rapid increase in interest in blueberries and cranberries in Europe and around the world. Consequently, plantations of species from the Ericaceae Juss. family have increased the demand for large quantities of planting material. Wild species of the genus *Vaccinium* L. grow in areas such as high moors and bogs, where the soil may be peaty and the pH ranges from 2.6 to 6.0 [10]. As Hoover et al. [11] reported, blueberries tolerate a wide range of soils. Notwithstanding, it was found that standard substrates containing higher amounts of fertilizers, especially nitrogen fertilizers, are not suitable for these plants [12]. When searching for substrates for blueberry, it is important to consider the characteristics of its roots. Blueberry roots do not have root hairs, and the thin roots that are responsible for water and nutrient absorption are inhabited by mycorrhiza [13].

In the production of substrates, renewable resources, such as wood chips and tree bark, can be used. Such substrates contribute to the utilization of logging waste [14,15]. Lignin, which is found in plant cell walls, degrades more slowly compared to cellulose or hemicellulose and the degradation process of the substrate also slows down [16]. Meanwhile, the bark of trees is rich in organic compounds (lignin, terpenes, fats, resins, sterols, glycosides, tannins, saccharides, acids, and others), which can change the quality of the substrates and affect the germination of plant seeds or the growth of saplings. It was determined that the quantitative and qualitative chemical composition of the bark of different tree species varies, and it is important to determine these variations. The bark's compounds can affect the chemical characteristics of the substrates differently, for example, approximately 8% mannose has been found in spruce bark and approximately 9% arabinose in pine bark [7,17]. Other studies showed that the phloem and the outer bark are richer in chemical compounds than the wood and also differ significantly among wood species [18]. Kemppainen et al. [19] investigated Norway spruce bark and detected a significant amount of tannins, 10.0%. Other researchers reported that fibrous materials are strong contenders in the replacement of peat in growing media, with a focus on the physical properties [16]. As Vandecasteele et al. [20] indicated, plant fibers have the potential for peat replacement and can provide protection against plant diseases.

Perlite is a non-renewable resource and is used throughout the world in horticultural applications. The physical and chemical properties of perlite as an component of substrates and the effect of this material on human health have been analyzed, and different studies confirmed that perlite can improve porosity and oxygenation to plant roots [21].

Based on these previous studies, we hypothesized that the mix of spruce and pine fibers or perlite additions with peat could ensure the growth and quality of blueberry saplings. In this experiment, the effects of mixes of peat with different rates of spruce or pine fibers and perlite on vegetative growth, the content of extractable macronutrients and chlorophyll fluorescence in the leaves of blueberry saplings were studied.

## 2. Materials and Methods

### *2.1. Plant Material and Substrate Composition*

Five hundred saplings of the highbush blueberry cultivar 'Duke' were purchased from a commercial nursery PLANTIN (Poland). Plants were propagated in in-vitro cultures in the laboratory of this nursery and were replanted to multi-pots after acclimatization. For this experiment, saplings with 1–2 lateral branches reaching a height of 8–12 cm were used. The saplings were transplanted into 2.0 L plastic containers filled with the appropriate substrates. In each substrate variant, thirty saplings were planted.

The substrates were composed of Scots pine *Pinus sylvestris* L. and Norway spruce *Picea abies* (L.) H.Karst. wood or bark fibers and high moor peat, which were used in various proportions. The experiment included 15 treatments: five mixes (peat + fiber of pine wood, peat + fiber of spruce wood, peat + fiber of pine bark, peat + fiber of spruce bark, and peat + perlite), each with three rates (Figure 1, Table 1).

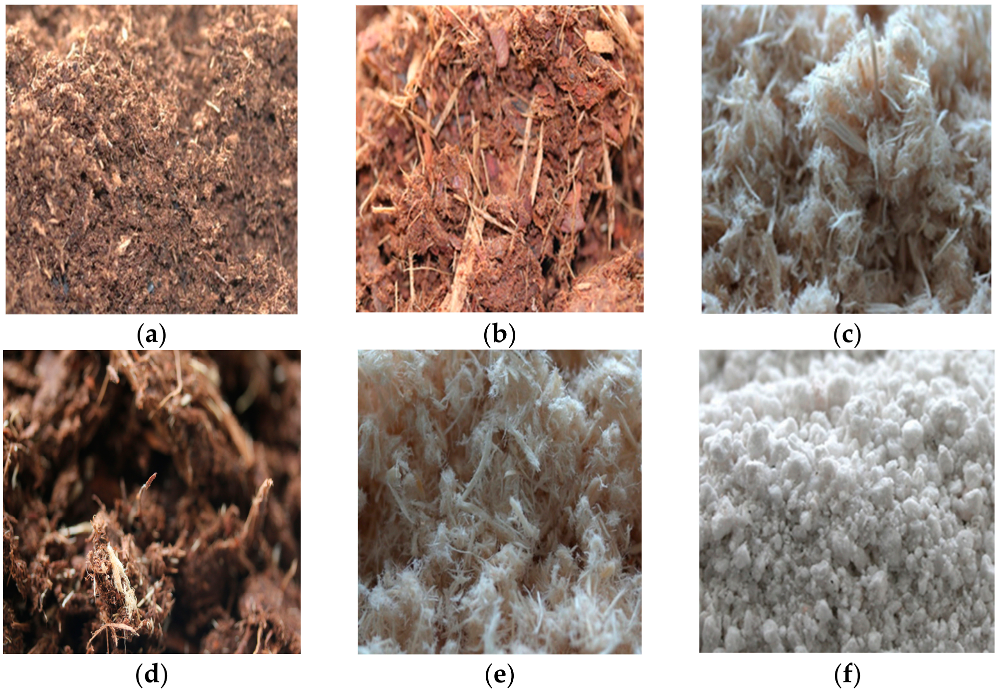

**Figure 1.** Components of the studied substrates: (**a**)—high moor peat, (**b**)—fiber of pine bark, (**c**)—fiber of pine wood, (**d**)—fiber of spruce bark, (**e**)—fiber of spruce wood, and (**f**)—perlite.

**Table 1.** Composition of the substrates.

| Substrate | Substrate Variant | Perlite (%, *v/v*) | Peat (%, *v/v*) | Fiber (%, *v/v*) |
|---|---|---|---|---|
| Peat + fiber of pine wood | 1 | 0 | 85 | 15 |
| | 2 | 0 | 70 | 30 |
| | 3 | 0 | 55 | 45 |
| Peat + fiber of spruce wood | 1 | 0 | 85 | 15 |
| | 2 | 0 | 70 | 30 |
| | 3 | 0 | 55 | 45 |
| Peat + fiber of pine bark | 1 | 0 | 85 | 15 |
| | 2 | 0 | 70 | 30 |
| | 3 | 0 | 55 | 45 |
| Peat + fiber of spruce bark | 1 | 0 | 85 | 15 |
| | 2 | 0 | 70 | 30 |
| | 3 | 0 | 55 | 45 |
| Peat + perlite | 1 | 15 | 85 | 0 |
| | 2 | 30 | 70 | 0 |
| | 3 | 45 | 55 | 0 |

The blueberry saplings were grown under natural light conditions in the greenhouse. The greenhouse temperature and relative humidity were maintained at 25 °C (day) and 15 °C (night) and 60%, respectively.

### *2.2. Content of Extractable Macronutrients and Organic Carbon and Peat Decomposition in Substrates*

Before the planting of the rooted saplings, the content of extractable macronutrients was evaluated in the substrates. Additionally, the content of organic carbon and the degree of peat decomposition were assessed.

The degree of peat decomposition was determined according to LST 1957:2022 [22] and the pH was determined according to LST EN 13037 with the potentiometric method [23].

The pH values of the prepared substrate mixes ranged from 4.5 to 5.4. As Trehane [10] reported, blueberries require a soil pH between 4.0 and 5.2. The content of organic carbon ranged from 27.54% to 41.66%, and the decomposition of peat was 29.1–38.8% (Table 2).

**Table 2.** Chemical composition of the substrates.

| Substrate | Substrate Variant | * pH | ** $N\text{-}NO_3$ $+N\text{-}NH_4$, mg $L^{-1}$ | ** P, mg $L^{-1}$ | *** K, mg $L^{-1}$ | ** Ca, mg $L^{-1}$ | Organic carbon, % | Degree of Peat Decomposition, % |
|---|---|---|---|---|---|---|---|---|
| Peat + fiber of pine wood | 1 | 4.9 | 2.4 | 0.45 | 4.0 | 15.2 | 39.27 | 33.1 |
| | 2 | 4.5 | 1.2 | 0.4 | 5.2 | 11.3 | 37.83 | 32.3 |
| | 3 | 4.9 | 0.6 | 0.48 | 6,2 | 11.0 | 38.17 | 29.1 |
| Peat + fiber of spruce wood | 1 | 4.5 | 3.9 | 0.48 | 3.0 | 9.1 | 37.76 | 34.7 |
| | 2 | 4.7 | 1.3 | 0.52 | 4,0 | 9.3 | 40.39 | 34.4 |
| | 3 | 4.9 | 0.7 | 0.39 | 6,0 | 10.0 | 39.89 | 29.3 |
| Peat + fiber of pine bark | 1 | 4.5 | 1.0 | 0.61 | 8.3 | 12.1 | 37.30 | 35.4 |
| | 2 | 4.9 | 0.7 | 0.51 | 13.2 | 13.3 | 41.66 | 36.5 |
| | 3 | 4.6 | 0.8 | 0.64 | 19.5 | 19.3 | 38.64 | 34.2 |
| Peat + fiber of spruce bark | 1 | 4.5 | 1.5 | 0.23 | 9.2 | 9.0 | 39.50 | 34.5 |
| | 2 | 4.7 | 0.9 | 0.31 | 21.6 | 21.5 | 38.71 | 32.8 |
| | 3 | 4.9 | 0.7 | 0.4 | 38.1 | 38.3 | 38.86 | 34.7 |
| Peat + perlite | 1 | 4.9 | 3.3 | 0.15 | 2.0 | 22.2 | 37.30 | 35.4 |
| | 2 | 5.1 | 2.0 | 0.41 | 2.1 | 2.1 | 29.89 | 37.4 |
| | 3 | 5.4 | 1.7 | 0.16 | 2.0 | 2.4 | 27.54 | 38.8 |

* Using potentiometric method with an error ±0.2; ** using spectrometric analysis method with an error ±10%; *** using flame photometry method with an error ±20%.

The detection of elements available for plants (K, P, Ca, and Mg) was accomplished according to LST EN 13652 [24]. In the water extracts, the phosphorus (P) concentration was detected using the spectrometric method with ammonium molybdenum complexes in Shimadzu UV 1800; the concentration of potassium (K) was measured using the flame photometric method with a Flame Photometer Sherwood M410; and the calcium (Ca) and magnesium (Mg) concentrations were determined using the atomic absorption spectrometric method using Atomic Absorption Spectrometer (Perkin Elmer AAnalyst 200, Waltham, MA, USA) (Table 2).

Mineral nitrogen (N) was extracted in a 1:5 (wt./vol) substrate suspension of 1 M KCl solution. The suspension was shaken for 60 min at 20 ± 2 °C. After shaking, the suspension was filtrated and analyzed using a flow injection analysis (FIA) system with FIASTAR 5000 analyzer. After the addition of an acidic sulfanilamide solution, the nitrates in the substrate extract were converted to nitrites in the cadmium column. They, then, reacted with N-(1-naphthyl) ethylenediamine dihydrochloride to form a purple azo dye whose absorbance can be measured at 540 nm and 720 nm. The substrate extract was injected into a flowing carrier solution, where ammonium was mixed with sodium hydroxide to form gaseous ammonia, which passed through a gas-permeable membrane into the indicator stream. Acidic indicators changed color in this stream when they reacted with ammonium gas. Photometric measurements were performed at 540 nm and 720 nm. The calculation of mineral nitrogen involved adding the combined amounts of nitrate and nitrite nitrogen to the ammonia nitrogen.

The organic carbon content was determined using dry combustion, where the sample was heated to 900 °C in a stream of air, and the carbon dioxide formed was measured using infrared spectroscopy. The amount of organic carbon in the substrate was determined according to the standard ISO 10694:1995 with an organic carbon analyzer multi-EA 4000 Analytik Jena [25].

### *2.3. Growth of Blueberry Saplings and Content of Extractable Macronutrients in the Leaves*

Saplings' height and fresh leaf weight per plant were determined for all variants of substrates by evaluating all thirty saplings. Leaves were collected from each plant separately and the average weight was determined at the end of the first growth flush of vegetative shoots, i.e., at 90–95 days after transplanting, in the last decade of July. Plant height was measured with a measuring tape. To determine the nutrient concentration, samples of fully expanded leaves from the current season shoots were prepared. From five to ten leaves were collected per plant and mixed before being sent to the laboratory. A total of 200 g of raw material per substrate was prepared. The leaves of the blueberry saplings were air dried, then ground and analyzed using the appropriate methods: nitrogen (N) with the Kjeldahl method, potassium (K) with the flame photometric method, phosphorus (P) with the photometric method with molybdovanadate, calcium (Ca) with the oxalic acid method, and magnesium (Mg) with atomic absorption spectrophotometry at 285·2 nm [26–29]. The amounts of organic carbon in the blueberry leaves were determined according to the standard ISO 10694:1995 with the organic carbon analyzer multi-EA 4000 Analytik Jena [25]. All analyses were performed in triplicate.

### 2.4. Determination of Chlorophyll Fluorescence

For the investigation of the maximum photochemical efficiency of Photosystem II (Fv/Fm), the leaves were fully dark, adapted, prior to the measurement. Dark-adapted leaf areas were achieved using lightweight leaf clips for 20 min. The chlorophyll fluorescence was measured with a chlorophyll fluorimeter (Pocket PEA Chlorophyll Fluorimeters, Hansatech Instruments Ltd., Norfolk, UK) with a Fv/Fm test duration of 1 s. A total of 5 measurements per plant were taken from leaves located in different directions, at an average height of 0.3–0.4 m, using leaf clips. Ten replications for each substrate variant were performed. The maximum photochemical efficiency of photosystem II was quantified (Fv/Fm) using the following relationship proposed by Maxwell and Johnson [30].

According to Murchie and Lawson [31], the Fv/Fm of non-stressed plant material should be in the range of 0.81–0.83. A much smaller relative interval ($0.79 \leq Fv/Fm \leq 0.84$) was indicated by Maxwell and Johnson [30].

### 2.5. Statistical Analysis

In the evaluation of the average height and leaf weight of saplings, and chlorophyll fluorescence, a non-parametric Kruskal–Wallis test comparing the ranks of the samples was used. For all hypotheses, statistically significant differences were evaluated at a significance level of $p = 0.05$. The same level of significance was used in testing for differences between means by employing a one-way ANOVA with a multiple (pairwise) comparison procedure using Duncan's test. The statistical calculations were carried out using the IBM SPSS Statistics 27 software application.

## 3. Results and Discussion

### 3.1. Effect of Different Substrate Mixess on the Growth of Blueberry Saplings

As presented in Table 3 and Figure S1, the height of blueberry saplings grown in mixes of peat with different amounts of pine wood fiber were not significantly different.

**Table 3.** Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the height of blueberry saplings.

| Substrates | Height, cm | | |
|---|---|---|---|
| | **1 *** | **2 *** | **3 *** |
| Peat + fiber of pine wood | 44.26 ± 10.45 *ab* | 48.09 ± 7.69 *a* | 47.41 ± 8.50 *a* |
| Peat + fiber of spruce wood | 52.59 ± 8.28 *a* | 44.02 ± 8.29 *b* | 40.57 ± 5.52 *b* |
| Peat + fiber of pine bark | 44.31 ± 6.95 *a* | 31.48 ± 9.41 *b* | 27.74 ± 5.32 *c* |
| Peat + fiber of spruce bark | 38.51 ± 4.59 *a* | 26.97 ± 7.98 *b* | 19.90 ± 3.28 *c* |
| Peat + perlite | 44.95 ± 9.54 *ab* | 46.51 ± 7.62 *ab* | 50.41 ± 8.41 *a* |

* Substrate variants: 1—15% $v/v$; 2—30% $v/v$; 3—45% $v/v$. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

The same trend was found for the average weight of leaves per plant (Table 4, Figure S2). Among the substrates with the spruce wood fiber, significantly higher blueberry saplings (52.59 ± 8.28 cm) were detected for the substrate with the lowest amount of fibers (15% *v*/*v*), while the larger fiber content (30% *v*/*v*) led to the significantly lower height in plants (44.02 ± 8.29 cm). Accordingly, the minimum leaf weight was determined for plants growing in the substrate with 45% *v*/*v* of spruce wood fiber (9.31 ± 2.89 g). In substrates with pine bark fiber, blueberry saplings reached the maximum height when fibers made up 15% *v*/*v* of the total capacity. It was determined that the height of the blueberry saplings decreased significantly as the amount of spruce bark fiber increased. When assessing the influence of perlite on plant growth, it was established that the plants reached a height ranging from 44.95 ± 9.54 cm (15% *v*/*v* of perlite) to 50.41 ± 8.41 cm (45% *v*/*v* of perlite). No differences in leaf weight were found among substrates with this mineral addition.

**Table 4.** Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the leaf weight of blueberry saplings.

| Substrates | Leaf Weight, g/Plant | | |
|---|---|---|---|
| | **1 *** | **2 *** | **3 *** |
| Peat + fiber of pine wood | 14.55 ± 2.29 *ab* | 13.26 ± 2.67 *a* | 13.08 ± 2.55 *a* |
| Peat + fiber of spruce wood | 16.41 ± 3.14 *a* | 13.70 ± 1.71 *a* | 9.31 ± 2.89 *b* |
| Peat + fiber of pine bark | 13.43 ± 2.00 *a* | 4.17 ± 1.93 *b* | 3.03 ± 1.45 *b* |
| Peat + fiber of spruce bark | 7.05 ± 3.11 *a* | 3.33 ± 1.44 *b* | 1.07 ± 0.35 *cd* |
| Peat + perlite | 13.78 ± 3.09 *ab* | 13.50 ± 2.25 *ab* | 14.63 ± 2.52 *a* |

* Substrate variants: 1—15% *v*/*v*; 2—30% *v*/*v*; 3–45% *v*/*v*. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

It can be summarized that blueberry saplings grown in substrates with pine and spruce wood fiber or perlite additions reached a height ranging from 40.57 ± 5.52 cm to 52.59 ± 8.28 cm during the first year of vegetation, which is important in order to produce high-quality planting material for blueberry plantations [10,11].

Corresponding differences were determined in terms of leaf weight when the average leaf weight per plant was only 1.07 ± 0.35 g at 45% *v*/*v* of spruce bark fiber in the substrate (Figure S2, Table 4). The blueberry plants were more vigorous in the substrate with even 45% *v*/*v* of perlite compared to the substrate with 15% *v*/*v* of perlite. Consequently, leaf weight per plant did not differ significantly among the substrates with different perlite additions. Perlite is widely preferred because it reduces the risk of damping off, provides a balance between air and water in root zone and stimulates faster root growth [31]. Comparison of equal amounts of different additions confirmed that plant growth was very poor in substrates with 15% *v*/*v*, 30% *v*/*v*, and 45% *v*/*v* of spruce bark fiber (Tables S1 and S2). Accordingly, the saplings achieved the minimum leaf weight per plant in these substrates. Statistically significant differences found when comparing the same amount of different additions confirmed the necessity of choosing the most suitable variants and quantities of the additions.

### *3.2. Effect of Different Substrate Mixes on the Chlorophyll Fluorescence in the Leaves of Blueberry Saplings*

The determined values of chlorophyll florescence showed no significant differences among 1–3 variants of each substrate. Therefore, the various amounts of pine and spruce wood or bark fiber and perlite did not significantly affect the Fv/Fm ratio (Table 5, Figure S3). On the other hand, the significant differences among substrates with the same amount of particular additions were determined (Table S3). The lowest values of Fv/Fm were detected for mixes of peat with 15% *v*/*v*, 30% *v*/*v*, and 45% *v*/*v* of spruce bark fiber. The growth of saplings was also lower in these substrates (Tables S1 and S2). In this study, the highest Fv/Fm ratio was determined for the pine and spruce substrates with 15% *v*/*v* and 30% *v*/*v* of wood fiber. Moreover, the Fv/Fm values were close to or lower than 0.80. As other authors have reported, the time of measurement during the day may have influenced the chlorophyll fluorescence [32]. In the study of Björkman and Deming [33], it was stated that Fv/Fm is virtually constant when measured under no-stress conditions, being in the range of $0.75 \leq Fv/Fm \leq 0.86$.

**Table 5.** Effect of mixes of peat with different amounts of wood and bark fibers and perlite on the chlorophyll fluorescence (Fv/Fm) of the blueberry saplings leaves.

| Substrates | Fv/Fm | | |
|---|---|---|---|
| | 1 * | 2 * | 3 * |
| Peat + fiber of pine wood | 0.784 ± 0.020 *a* | 0.779 ± 0.021 *a* | 0.759 ± 0.035 *ab* |
| Peat + fiber of spruce wood | 0.778 ± 0.024 *a* | 0.797 ± 0.010 *a* | 0.791 ± 0.022 *a* |
| Peat + fiber of pine bark | 0.774 ± 0.033 *a* | 0.771 ± 0.018 *a* | 0.711 ± 0.085 *ab* |
| Peat + fiber of spruce bark | 0.689 ± 0.090 *ab* | 0.738 ± 0.014 *a* | 0.711 ± 0.039 *ab* |
| Peat + perlite | 0.760 ± 0.044 *ab* | 0.773 ± 0.018 *a* | 0.758 ± 0.032 *ab* |

* Substrate variants: 1—15% *v/v*; 2—30% *v/v*; 3—45% *v/v*. Values followed by different lowercase letters, within the line, indicate statistically significant differences by Duncan's test, $p = 0.05$.

A non-invasive measurement of the chlorophyll-fluorescence parameter photochemical efficiency of PSII (Fv/Fm) is a commonly used technique in plant physiology. It has been confirmed that the determination of the Fv/Fm ratio can be used to identify nitrogen deficiency [33,34]. Previous studies also showed a significant correlation between nitrogen concentration and the leaves' Fv/Fm ratio. It was determined that the Fv/Fm ratio correlates not only with low nitrogen amounts but also with low chlorophyll levels and low biomass growth [35]. Different soil pH treatments had various effects on the photosynthetic characteristics [34]. The chlorophyll fluorescence Fv/Fm ratio is correlated with the efficiency of leaf photosynthesis, and a decline in this ratio is a good indicator of photoinhibition damage when plants suffer from a wide range of environmental stresses [36]. In this study, the Fv/Fm values of plants in all substrates with the spruce bark fiber showed a decrease in the Fv/Fm ratio, which confirms that plants may have suffered from stress in some of the substrates studied [33,37].

### *3.3. Effect of Different Substrate Mixes on the Content of Extractable Macronutrients*

The nutritional status of blueberry plants is mainly assessed on the basis of studies on the chemical composition of leaves [38]. The obtained results on the content of extractable macronutrients in blueberry leaves showed significant variation among saplings grown in different substrates (Table 6). Attention was paid to whether our data corresponded to the proper foliar concentrations of nutrients for blueberry indicated in the studies of other researchers [39–42]. In our study, the amount of nitrogen (N) in blueberry leaves ranged from 0.78% (substrate with 45% *v/v* of spruce bark fiber) to 1.98% *v/v* (substrates with 15% *v/v* of pine wood fiber and with 30% *v/v* of perlite). The data presented in Table 6 shows that the leaves of the saplings grown in substrates with 45% *v/v* of spruce bark fiber were distinguished by lower nitrogen content than the limits indicated in the above-mentioned references. Glonek and Komosa [43] reported that the optimum ranges of N in leaves collected in the middle of the summer should be 1.52–2.17%. Studies with other plants have shown that N-immobilization can cause nutritional imbalance on young seedlings grown in organic substrates with wood fiber. In such cases, the use of N-impregnated media and an additional supply of nutrients is necessary [44].

Compared with the sufficient or normal concentration of phosphorus in blueberry leaves determined by Hart et al. [41] and Fugua et al. [42], the results of our research showed the proper content of phosphorus (P) in all variants of substrates, while the amounts of phosphorus ranged from 0.11% (45% *v/v* of pine bark fiber) to 0.22% (45% *v/v* of spruce bark fiber). The obtained leaf N:P ratio ranged from 3.55 to 13.67 (Table 6). Dibar et al. [45] reported that plants with a higher nitrogen concentration and a low N:P ratio, especially in the photosynthetic active organs, are the best-adapted to the environment. In our study, blueberry saplings grown in substrates with 30% *v/v* and 45% *v/v* of spruce bark fibers showed a particularly weak growth, and, in addition, not only low amounts of nitrogen but also the lowest N:P ratio were determined in the leaves. Xia et al. [46] presented the possibility of using N:P ratio as an effective indicator for the health condition and growth status of plants.

**Table 6.** Content of extractable macronutrients and organic carbon in the leaves of blueberry saplings according to the different additions of fiber and perlite.

| Substrates | Substrate Variant | N, % | P % | K, % | Ca, % | Mg, % | Organic C, % | N:P |
|---|---|---|---|---|---|---|---|---|
| Peat + fiber of pine wood | 1 | 1.98 ± 0.11 *a* | 0.16 ± 0.02 *b* | 0.65 ± 0.02 *b* | 0.98 ± 0.01 *b* | 0.25 ± 0.01 *cd* | 39.01 ± 2.03 *c* | 12.37 |
| | 2 | 1.64 ± 0.10 *c* | 0.14 ± 0.02 *c* | 0.55 ± 0.03 *c* | 1.01 ± 0.04 *a* | 0.26 ± 0.01 *c* | 36.59 ± 3.02 *cd* | 11.71 |
| | 3 | 1.49 ± 0.11 *cd* | 0.14 ± 0.02 *c* | 0.58 ± 0.03 *c* | 1.00 ± 0.09 *a* | 0.25 ± 0.01 *cd* | 39.07 ± 3.56 *c* | 10.64 |
| Peat + fiber of spruce wood | 1 | 1.92 ± 0.09 *a* | 0.17 ± 0.02 *b* | 0.59 ± 0.01 *c* | 0.91± 0.05 *bc* | 0.25 ± 0.02 *cd* | 41.44 ± 3.11 *b* | 11.29 |
| | 2 | 1.56 ± 0.10 *c* | 0.15 ± 0.01 *bc* | 0.58 ± 0.04 *c* | 1.00 ± 0.03 *a* | 0.26 ± 0.03 *c* | 28.41 ± 1.25 *e* | 10.44 |
| | 3 | 1.3 ± 0.06 *d* | 0.12 ± 0.02 *d* | 0.56 ± 0.03 *c* | 0.95 ± 0.01 *b* | 0.25 ± 0.02 *cd* | 40.22 ± 2.96 *b* | 10.8 |
| Peat + fiber of pine bark | 1 | 1.43 ± 0.04 *cd* | 0.12 ± 0.02 *d* | 0.58 ± 0.02 *c* | 0.88 ± 0.01 *d* | 0.25 ± 0.01 *cd* | 36.0 ± 2.35 *cd* | 12.03 |
| | 2 | 1.15 ± 0.05 *e* | 0.12 ± 0.01 *d* | 0.72 ± 0.03 *b* | 0.91 ± 0.05 *bc* | 0.31± 0.02 *b* | 35.81 ± 3.08 *cd* | 11.07 |
| | 3 | 1.13 ± 0.04 *e* | 0.11 ± 0.01 *d* | 0.61 ± 0.03 *bc* | 0.93 ± 0.03 *bc* | 0.28 ± 0.01 *c* | 33.04 ± 2.85 *d* | 10.27 |
| Peat + fiber of spruce bark | 1 | 1.21 ± 0.02 *d* | 0.12 ± 0.03 *d* | 0.64 ± 0.02 *b* | 0.88 ± 0.01 *d* | 0.25 ± 0.01 *cd* | 41.40 ± 3.74 *b* | 10.08 |
| | 2 | 1.09 ± 0.02 *e* | 0.14 ± 0.02 *c* | 0.88 ± 0.02 *b* | 0.90 ± 0.01 *bc* | 0.28 ± 0.03 *c* | 41.06 ± 3.96 *b* | 7.79 |
| | 3 | 0.78 ± 0.01 *f* | 0.22 ± 0.03 *a* | 1.75 ± 0.03 *a* | 0.84 ± 0.02 *d* | 0.37 ± 0.02 *a* | 46.83 ± 2.87 *a* | 3.55 |
| Peat + perlite | 1 | 1.82 ± 0.11 *b* | 0.16 ± 0.01 *b* | 0.57 ± 0.01 *c* | 0.84 ± 0.01 *d* | 0.24 ± 0.01 *d* | 38.38 ± 3.01 *cd* | 11.38 |
| | 2 | 1.98 ± 0.09 *a* | 0.16 ± 0.01 *b* | 0.50 ± 0.02 *d* | 0.83 ± 0.01 *d* | 0.22 ± 0.01 *e* | 37.74 ± 3.25 *cd* | 12.38 |
| | 3 | 1.64 ± 0.09 *c* | 0.12 ± 0.02 *d* | 0.49 ± 0.01 *d* | 0.83 ± 0.02 *d* | 0.22 ± 0.02 e | 39.07 ± 2.56 *c* | 13.67 |

Values followed by different lowercase letters, within the column, indicate statistically significant differences by Duncan's test, $p = 0.05$.

A high amount of potassium (K) was found in the leaves of blueberry saplings grown in the substrates with the addition of spruce bark fiber. Even the plants grown in the substrate with 45% $v/v$ of spruce bark fiber had exceptionally high amounts of potassium, 1.75%, compared to the proper amounts of potassium in blueberry leaves determined by other authors [39–42].

A high content of calcium (Ca) in the leaves was found in both saplings grown in the mixes of peat with pine and spruce wood fibers, while adequate calcium amounts were determined in the leaves of plants in all variants with spruce bark fiber and in substrates with perlite additions. The content of magnesium (Mg) varied significantly in the leaves of all studied plants, and high amounts for substrates with 30% $v/v$ of pine bark fiber and 45% $v/v$ of spruce bark fiber were determined.

Leaf organic carbon (C) content was significantly higher in saplings grown in substrates with additions of spruce bark fiber and in substrates with 15% $v/v$ and 45% $v/v$ of spruce wood fiber (Table 3).

The bark of various tree species has been evaluated, highlighting not only physical-chemical properties but also the different methods of medical, energetic, and industrial utilization [18,47]. In this research, substrates of peat with mixes of pine and spruce wood and bark fibers were studied. The use of tree bark in the production of substrates could be a novelty; however, the use of tree bark for the production of peat substrate mixes needs to be investigated in more detail. This study confirmed that mixes of peat with 15–45% $v/v$ of spruce fibers had a negative effect on the growth of blueberry saplings. As other authors have reported, it is necessary to study what toxic substances are released from the new components, which could inhibit plant growth [48,49]. In the mixes of peat with fiber additions, microorganisms that need mineral nitrogen must be also evaluated [20].

The challenges presented by climate change require a new approach to the conservation of natural resources, including peat. The search for innovative substrates, evaluating the possibilities of using renewable materials, has great potential [50,51]. This study confirmed the possibility of reducing the amount of peat in substrates using tree fibers and perlite. In the continuation of this research, it would be promising to investigate substrate compositions with mixtures of organic and mineral additions.

## 4. Conclusions

In this study, mixes of peat with various rates of wood and bark fibers or perlite were compared to evaluate the possibility of reducing the amount of peat. The research carried out on the growth of blueberry saplings showed that the best characteristics of plants were achieved for substrates with 15–45% $v/v$ of pine wood fiber and with 15–30% $v/v$ of spruce wood fiber. Different amounts of spruce bark fiber had the strongest negative effect on vegetative growth and the lowest values of chlorophyll fluorescence Fv/Fm (0.689–0.738) in the leaves of the saplings were determined. Investigations of extractable macronutrients in the leaves confirmed the qualitative and quantitative composition of peat mixes suitable for the cultivation of blueberry saplings. Nitrogen and potassium levels did not meet the accepted standards and a low N:P ratio was found in the leaves of plants grown in substrates with 30–45% $v/v$ of spruce bark fibers. The results of the investigations corroborated that 15–45% $v/v$ of perlite in peat substrates is suitable for the purpose of growing blueberry saplings.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020151/s1, Figure S1: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the height of blueberry saplings; Figure S2: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the height of blueberry saplings; Figure S3: Effect of mixes of peat with different additions of wood and bark fibers and perlite on the chlorophyll fluorescence (Fv/Fm) of blueberry-saplings leaves; Table S1: Effect of mixes of peat with the same percentage of components on the height of blueberry saplings; Table S2: Effect of mixes of peat with the same percentage of components on the leaf weight of blueberry saplings; Table S3: Effect of mixes of peat with the same percentage of components on the chlorophyll fluorescence (Fv/Fm) of blueberry-saplings leaves.

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