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# Growth dynamic of three different white willow clones used in a zero-discharge wastewater treatment system in the sub-Mediterranean region – an early evaluation

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**ABSTRACT**

An evapotranspirative willow system (EWS) is a zero-discharge wastewater treatment system in which all influent water is used for growing willows and evaporation. Willow clones used in EWS may significantly affect the performance of EWS; therefore, the clones with high biomass production and resilience to permanent flooding, increased nutrient concentrations, and salinity must be selected. In the presented study, a 27 m² pilot EWS was set up in November 2015, enabling the testing of three different willow clones of *Salix alba* (L.): indigenous white willow 'V 160' (*S. alba*) and two of its hybrids: 'V 052' (*S. alba* var. *calva* × *S. alba*) and 'V 093' (*S. alba* × *S. alba* var. *vitellina*) × *S. alba*. The stem height, diameter, and number of shoots per stump were measured weekly in the first year of growth on site, along with the water quality parameters and water levels in the test beds. There were no statistically significant differences in stem height and stem diameter between the three tested clones at the end of the vegetation season; however, the indigenous clone indicated better adaptability to conditions in EWS but somewhat lower biomass production in comparison with the hybrids. For all clones, the willows growing in the EWS outgrew the control willows, showing the positive effects of high water availability and wastewater on willow growth. Investigations in the following vegetation season will further evaluate the water demand and the biomass yield, estimate the efficiency of nutrient transfer from wastewater to wood biomass, and define the differences for the selected clones.

*Keywords:* Closed material loop; Wastewater reuse; Evapotranspiration; Willows; On-site wastewater treatment

## 1. Introduction

Evapotranspirative willow systems (EWS) enable wastewater treatment and the recycling of water and nutrients through the willow biomass. They are zero-discharge systems with no direct impact on the environment in terms of pollutant emissions, because all the water and nutrients are used for processes by the system; moreover, EWS enable the production of biomass as a renewable energy resource. Wastewater reuse has a great potential for a positive shift towards the circular economy, in which all materials are recycled as resources. EWS are most appropriate for on-site treatment of domestic wastewaters in places where requirements for wastewater discharge are strict or where soil infiltration

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is not possible. EWS are also attractive for users interested in biomass production for energy use (e.g., for house heating). EWS consists of an impermeable bed with no outflow with which all the water is used for willow growth and evaporation to the atmosphere; however, the efficiency greatly depends on climate condition, for example, temperature, solar radiation, wind, humidity and annual rainfall distribution. In the last 15 years, most EWS were constructed in the areas with strict requirements for effluent discharge and/or areas of low permeability soils, for example, Denmark and Ireland [1–3]; however, the challenge remains to define a performance of EWS for different climate conditions such as sub-Mediterranean area with unevenly distributed annual precipitation and extreme rainfall events, as well as for different willow species and clones.

According to Gregersen and Brix [1] and Börjesson and Berndes [4], the composition of domestic wastewater corresponds to the willows' nutrient requirements; moreover, the wastewater can act as a fertilizer and can significantly increase the willow yield. However, the efficiency in pollutant uptake from domestic wastewater to wood biomass and their accumulation in the system is poorly known but of crucial importance in the design and management of the system. Many studies have been done on short rotation willow coppices for wood biomass production, which are irrigated with partially treated wastewater, and the excess water is discharged to the underground or surface water bodies [5–7]. In these systems, the water and nutrient loadings are important to define the performance efficiency and to meet effluent requirements. In contrast, the EWS have no outflow, meaning that the water and nutrient balance depend only on assimilation in willow biomass, accumulation in the soil, and evaporation to the atmosphere. The nutrients in sewage treated by the EWS can be removed only by harvesting wood biomass and by the reuse of nutrients accumulated in the soil for agriculture purposes at the end of the operation of EWS. High evapotranspiration rates and water usage are also beneficial characteristics when designing EWS, since the area footprint of a treatment plant can be smaller. Consequently, the costs of construction also can be reduced.

According to Swedish research [5], biomass production in commercial short rotation willow coppice is ca. 10 tonnes of dry matter per hectare per year (t DM $ha^{-1}$ $a^{-1}$). Similar to this, a North American study on fertilized short rotation willow coppice reports annual biomass production of 15–22 t DM $ha^{-1}$ $a^{-1}$ [8]. On the other hand, a study on Mediterranean climatic conditions reports that the aboveground biomass production of fertilized willow stand can reach up to 64 t DM $ha^{-1}$ in 2 years after planting unrooted cuttings [9], indicating that climate may have a significant effect on the system performance.

The studies on biomass production in short rotation willow coppices report that appropriate clones have to be selected because there are significant differences between them regarding growth, nitrogen, salt tolerance and water use efficiency [10,11]. From previous research in phytoremediation, it is known that autochthonous plant species are more resistant and productive in comparison with fast growing clones acquired in other climatic environments [12]. Furthermore, there is also a difference between autochthonous plants; for example, the performance of poplars in short rotation coppices showed lower biomass production yields, lower evapotranspiration and less accumulation of nutrients compared with willows [9,13]. Despite the better performance of willow in comparison with poplar, a significant willow biomass production in EWS can be reached only with the usage of specific willow species, hybrids or clones with high biomass production, high water uptake, and high tolerance to salt, permanent flooding and increased nutrient concentrations in domestic wastewater. In contrast, and according to Guidi et al. [9], transpiration depends mainly on development stage, plant nutritional status, and climatic characteristics rather than on the willow species.

The differences in biomass production by different species of genus *Salix* (L.) have been investigated in short rotation willow coppice treated with landfill leachate: *S. purpurea* [14], *S. alba*, *S. nigra* [15] and *S. amygdalina* [16]. *S. viminalis* was studied in EWS [1] and *S. matsudana*, *S. jessoensis*, *S. fragilis* and *S. alba* were studied in terms of biomass production for production of bioenergy [11]. The later study showed the highest biomass production for clone SE03–001 (*S. babilonica* × *S. alba*) × *S. matsudana* f. *lobatoglandulosa*. The results of these studies are difficult to compare since the willows were grown under different conditions regarding water and nutrient availability and different climates, but it is obvious that the appropriate selection of a willow species, hybrids or clones plays an important role in biomass production.

This study presents the results of the performance and efficiency of a newly designed EWS in the sub-Mediterranean region in terms of willow growth, biomass production and differences between three clones of *S. alba* (L.). It gives an important comparison of an indigenous clone of *S. alba* with two *S. alba* hybrids in terms of adaptation to the conditions in the EWS during first vegetation season.

## 2. Materials and methods

The 27 $m^2$ pilot EWS is in Ajdovščina, Slovenia (45°52'32"N 13°54'20"E), next to a municipal wastewater treatment plant (WWTP) and has been in operation since March 2016. The pilot plant consists of nine impermeable test beds (each 3 m long, 1 m wide and 1.8 m deep), filled with local soil up to 1.5 m; the soil is loamy with high organic and phosphorous content. A pilot EWS was designed based on willow systems that are in operation in Denmark; however, a design modifications were done according to the higher, intense and time concentrated precipitation of sub-Mediterranean climate, that is, inclination of the test beds' top soil layer towards a rainwater drainage pipe installed on the surface at the end of each bed. In addition, a clay layer was integrated 0.1 m underneath the soil surface in order to reduce the percolation of rainwater into the bed. Each test bed of EWS was planted with three willow trees, resulting in plant density of 1 $m^{-1}$ (Fig. 1).

In the present study, three clones of *S. alba* from a selection of Croatian arborescent willows were tested. Clone 'V 052' originates from the interracial hybrids of the indigenous white willow and the English 'cricket-bet' willow (*S. alba* L. var. *calva* G.F.W. Mey × *S. alba* L.), 'V 093' originates from backcross hybrids of the indigenous white willow and the golden willow (*S. alba* L. × *S. alba* var. *vitellina* (L.) Stokes)

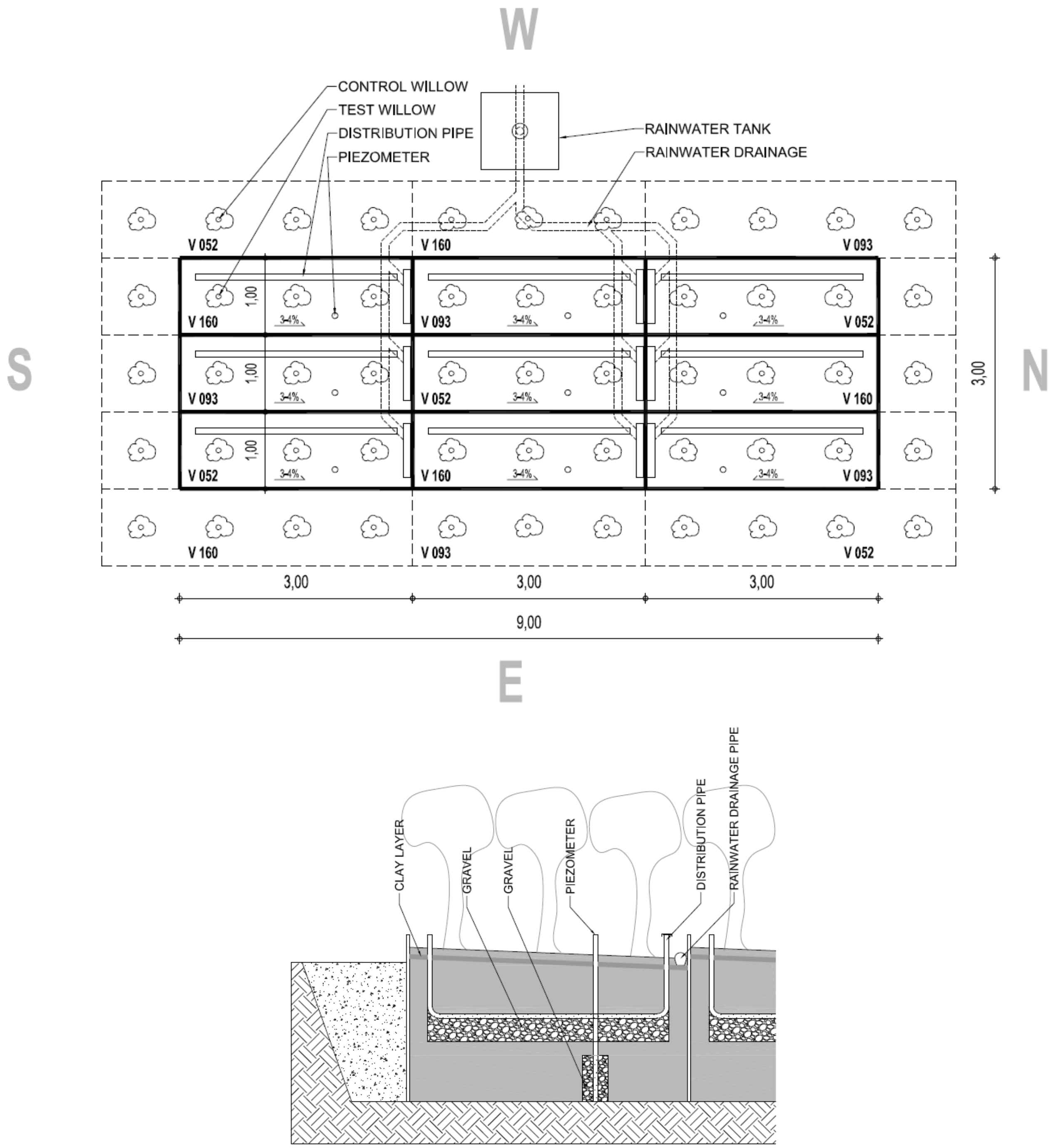


Fig. 1. Design of a pilot evapotranspirative willow system consisting of nine impermeable test beds and control willows planted around the pilot system (top-down view of the whole system and a cross-section of a test bed; units in meters).

× *S. alba* L.) and the 'V 160' represents the clone of the indigenous white willow (*S. alba* L.). Indigenous white willows were selected from natural populations in Croatia [17]. The studied clones were produced by the registered forest nursery Topolje (Forest Administration Osijek) in eastern Croatia and were provided as 1-year-old seedlings. The willows were immediately planted in November 2015; each clone in three test beds (parallels) distributed in a Latin square and were cut back (coppiced) to 10 cm above ground level in February 2016 to encourage the development of a multi-stemmed coppice.

Each test bed was equipped with an inlet pipe and piezometers, which enabled monitoring of water level in the bed. The water level in test beds was monitored weekly by a pumice that floated on the water surface in each piezometer and a Leica DISTO™ A5 laser distance meter. The water level was calculated from the piezometer height deduced for the measurement and the pumice height above the water. The decrease in water level was levelled up to 1 m on a weekly basis. The volume of added water, therefore, corresponded to the water needs of the willows. The detailed results on water level fluctuation will be presented elsewhere. The inflow water was mechanically pre-treated domestic wastewater from adjacent WWTP. The volume of inflow water was controlled by a flow meter and manually operated valve. There were no outlet pipes in the test beds.

The pilot EWS is positioned perpendicular to the dominant wind direction with the purpose of increasing evapotranspiration. Besides this, the EWS is located on a plane area deprived of higher vegetation and shading, which additionally increases wastewater usage. With the intention of avoiding the borderline effect of a relatively small pilot plant, the same willow clones were also planted along each side of the EWS, as shown in Fig. 1. The willows planted outside of the test beds were monitored as control willows. They grew in the same soil as the test willows did but received only rainwater and were, as the first line of trees, more exposed to the wind and sun.

The volume and quality of inflow wastewater were monitored (biochemical oxygen demand (BOD), chemical oxygen demand (COD), $PO_4$–P, total phosphorous (TP), $NH_4$–N, $NO_3$–N, total nitrogen (TN)) as well as dissolved oxygen (DO), pH, electric conductivity (EC) and temperature (T) of the influent and water in the test beds. Water quality parameters were measured according to standard methods [18]. DO, pH, EC, and T were measured using WTW Multiline/F portable meters. All willows in the pilot EWS were measured weekly from April to October 2016 for height and number of shoots, and from June to October 2016 also for the diameter 20 cm above stump. The water level, DO, pH, EC, and T in the beds were measured weekly from April to December 2016 in order to monitor the parameters also after the vegetation season. Biomass production in the pilot plant was estimated at the end of the growing season (October) based on biomass production of the control willows. Specifically, for each tested clone, 20 shoots of different sizes from the control willows were cut, measured for height and stem diameter and analysed for average productivity in DM of cut shoots (dried at 105°C until at a constant weight) to define the trendlines comparing DM content with stem height or stem diameter for the control willows. Next, all shoots of experimental willows were measured for stem height and diameter, and their biomass was calculated based on the trendline formulas. The calculated biomass produced for each clone in the pilot EWS presented a biomass produced on 9 m² (three test beds of 3 m²). The results were therefore extrapolated to one hectare per year.

The statistical analyses of the data were carried out using Microsoft Office Excel 2016 software. The results are presented as means ± standard deviation of the mean. One-way analysis of variance was used to identify significant differences at the 5% probability level between means of height, number and diameter of shoots and biomass production of the test clones in the EWS as well as between the test and control willows of individual clones.

The temperature and precipitation have been measured on site while sun radiation, relative humidity, and wind direction and strength were obtained from the Slovenian Environmental Agency for the nearest meteorological station for the year 2016.

## 3. Results

### 3.1. Climate and water characteristics

This paper presents the results of willow growth in a pilot EWS during the first vegetation season (March–October 2016). The yearly precipitation at the location of the pilot EWS in 2016 was 1,485 mm, which is in line with the average yearly precipitation of 1,428 mm for the period from 2006 to 2015. The average yearly temperature was 13.3 °C with a minimum of –8.0 and maximum of 35 °C. The nine test beds of EWS were filled with mechanically pre-treated domestic wastewater at the beginning of the experiment in March 2016. The first loading of water satisfied the water needs of willows until June, following which the water was added weekly until October up to a level of 1 m. The volume of added water varied according to the plants' needs and covered the water use in 1 week. The characteristics of the added water were typical for domestic wastewater (Table 1). The EC, pH, DO, and T were monitored in each bed (Table 2) and generally did not differ significantly between the beds with different clones showing similar growing conditions for all willows. Average EC and DO were declining throughout the experiment, while pH remained constant. T shows typical seasonal variation (Fig. 2).

The monitoring of water level in the beds showed that in vegetation season 2016, the willows used 4.53, 4.95, and 5.08 m of water for 'V 052', 'V 160', and 'V 093', respectively; indicating that there was no significant difference between the clones.

Table 1
Characteristics of mechanically pre-treated wastewater added to the test beds of pilot evapotranspirative willow system throughout the experiment ($N$ = 11)

| | Mean | SD |
|---|---|---|
| $BOD_5$, mg/L | 483 | 191 |
| COD, mg/L | 743 | 251 |
| TP, mg/L | 7.6 | 1.8 |
| $PO_4$–P, mg/L | 4.0 | 1.6 |
| TN, mg/L | 46 | 9 |
| $NH_4$–N, mg/l | 30 | 7 |
| $NO_3$–N, mg/L | 0.062 | 0.031 |
| $NO_2$–N, mg/L | 0.040 | 0.099 |
| pH | 6.8 | 0.5 |
| EC, mS/cm | 0.90 | 0.17 |

Table 2
Physical and chemical parameters of water in all nine test beds of pilot evapotranspirative willow system throughout the experiment (mean ± SD; $N$ = 32)

| | V 093 | V 160 | V 052 |
|---|---|---|---|
| EC, mS/cm | 1.77 ± 1.79 | 1.50 ± 1.20 | 1.11 ± 1.24 |
| pH | 7.1 ± 0.9 | 7.2 ± 0.4 | 7.1 ± 0.9 |
| DO, % | 30 ± 26 | 34 ± 30 | 29 ± 26 |
| T, °C | Mean 18 | Mean 18 | Mean 18 |
| | Minimum 9.2 | Minimum 8.7 | Minimum 9.1 |
| | Maximum 23 | Maximum 23 | Maximum 23 |

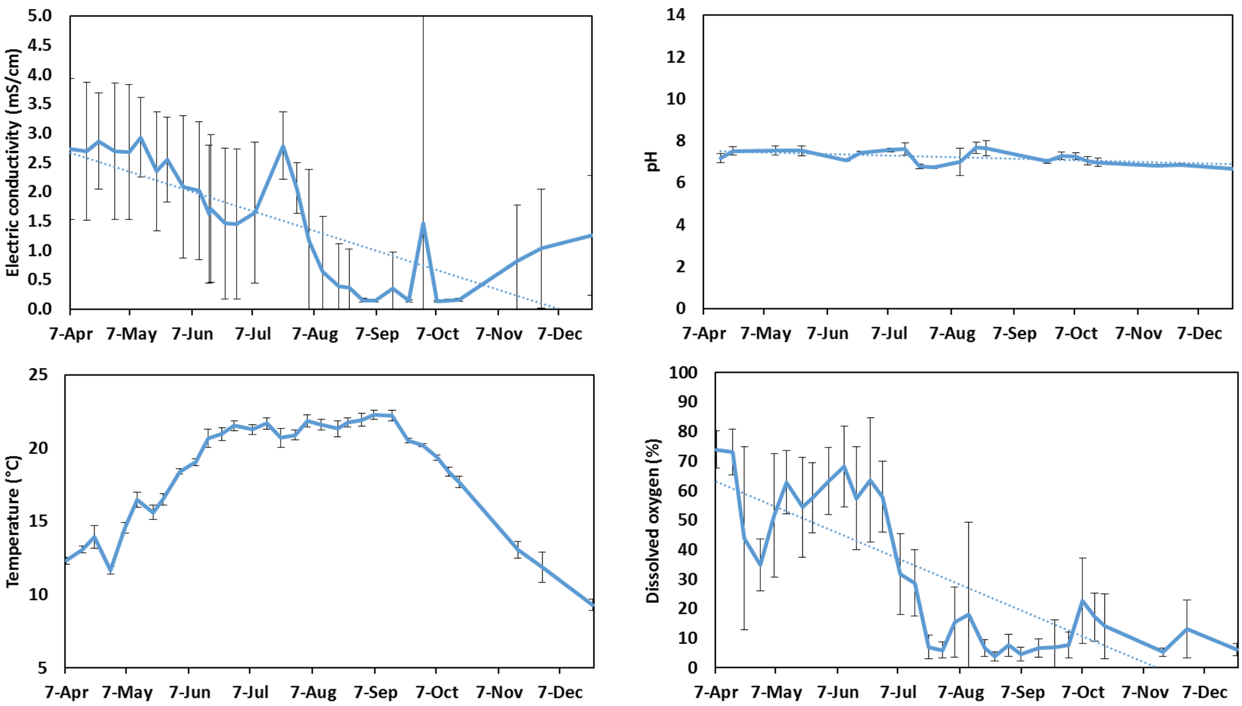


Fig. 2. Electric conductivity, pH, temperature and dissolved oxygen in all nine test beds of a pilot evapotranspirative willow system through vegetation season (mean ± SD; $N = 32$).

*3.2. Willow growth*

All willows planted in EWS and the control willows successfully sprouted. From February 2016 when they were cut back till mid-October, the willows in test beds have grown 2.58 ± 0.34, 2.57 ± 0.23 and 2.45 ± 0.21 m, for 'V 052', 'V 093', and 'V 160', respectively (Fig. 3). No significant differences in final height between the clones in test beds were observed; however, there were differences in final height between test and control willows. Specifically, at the end of vegetation season, the test willows outgrew the control willows for approximately 40 cm for all three clones. There were also differences in growth dynamics between the clones and the test and control trees. Due to a drought stress, the growth of control willows (fed only with rainwater) stopped at the end of June, beginning of July and late July for 'V 093', 'V 052', and 'V 160', respectively. The growth of test willows also continued after the test willows stopped growing; but the growth was slowed down significantly since early, mid-, and late August for 'V 160', 'V 093' and 'V 052', respectively.

From June to mid-July, the control willows of 'V 052' and 'V 093' were higher in comparison with the same clones in the test beds, showing a potentially inhibitory effect of wastewater or permanent flooding to the growth of these clones. In contrary, in the same period, the clone 'V 160' in the test beds was on average for 20 cm higher compared with the same clone in the control, showing better tolerance to the conditions in the EWS. A few weeks after the control willows stopped growing, there was a shift in growth also for 'V 093' and 'V 052' in which the willows in the test beds outgrew the control willows.

At the end of the vegetation season, the stem diameter of the willows in the test beds was 2.1 cm with no difference between the clones. The control willows of 'V 160' and 'V 052' had similar diameters (2.0 and 1.9 cm, respectively) as that of the test willows, while the diameter of the 'V 093' was smaller (1.6 cm); however, the difference was not statistically significant. For all three clones, the control willows at first showed better radial growth compared with the test willows; however, the radial growth of control willows stopped in mid-July for 'V 093' and 'V 052' and in mid-August for 'V 160', while the radial growth of the test trees continued. Comparing the shift between the control and test trees for vertical and radial growth, the shift in radial growth appeared later in the vegetation season; for 'V 093' and 'V 052', the stem diameter of test trees outgrew the control trees at the beginning of August, while for 'V 160' this was evident only from late September. In contrast to the height, results of 'V 160' for which the test trees outgrew the control trees during the first half of vegetation season, in the case of stem diameter, at that time the control trees of clone 'V 160' had wider stem diameter in comparison with the test trees (Fig. 4).

Regarding the number of shoots, 'V 160' and 'V 052' developed significantly more shoots in the test beds compared with the controls, while for clone 'V 093', the control trees developed significantly more shoots compared with the test willows (Fig. 5). Moreover, in the test beds, clone 'V 093' developed only 6 shoots on average, which was significantly less compared with 8 and 7.6 shoots developed in test beds for clones 'V 160' and 'V 052', respectively.

The willows in the pilot EWS produced 3.4 and 4.2 t DM ha$^{-1}$ a$^{-1}$ for 'V 160' and 'V 093' ('V 052'), respectively, while the control trees produced less biomass on average: 2.1, 3.1, and 3.6 t DM ha$^{-1}$ a$^{-1}$ for 'V 160', 'V 052', and 'V 093', respectively; however, the difference in biomass production

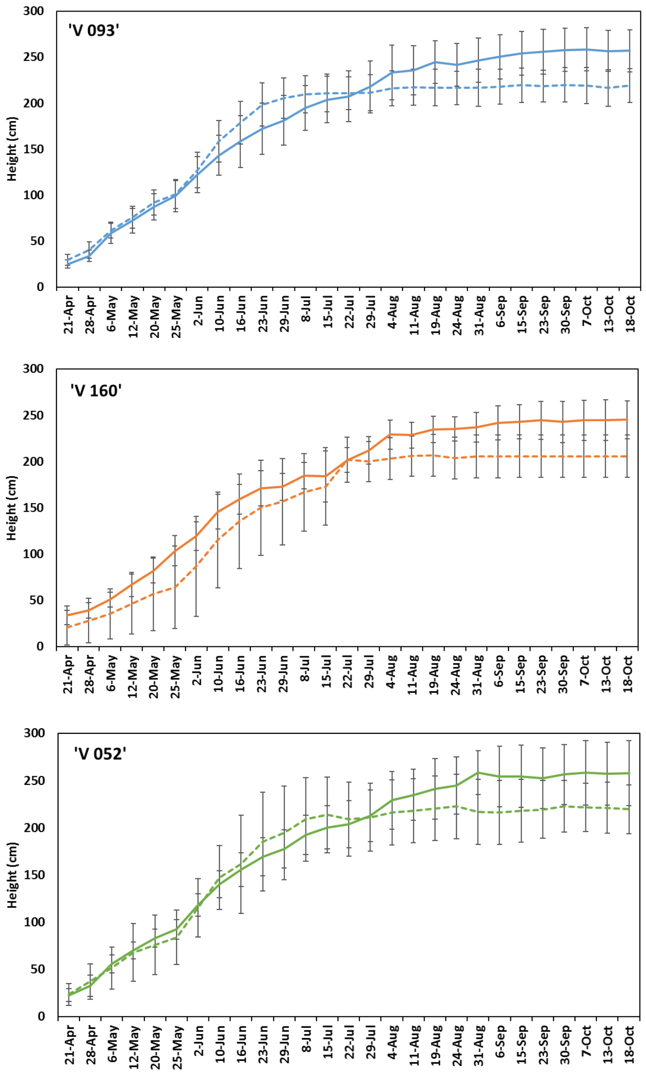


Fig. 3. Height of three willow clones 'V 093', 'V 160', and 'V 052' in pilot evapotranspirative willow system (solid line) and controls (dashed line) during first vegetation season. Means and standard deviations are given ($N$ = 9 and 6 for test and control willows, respectively).

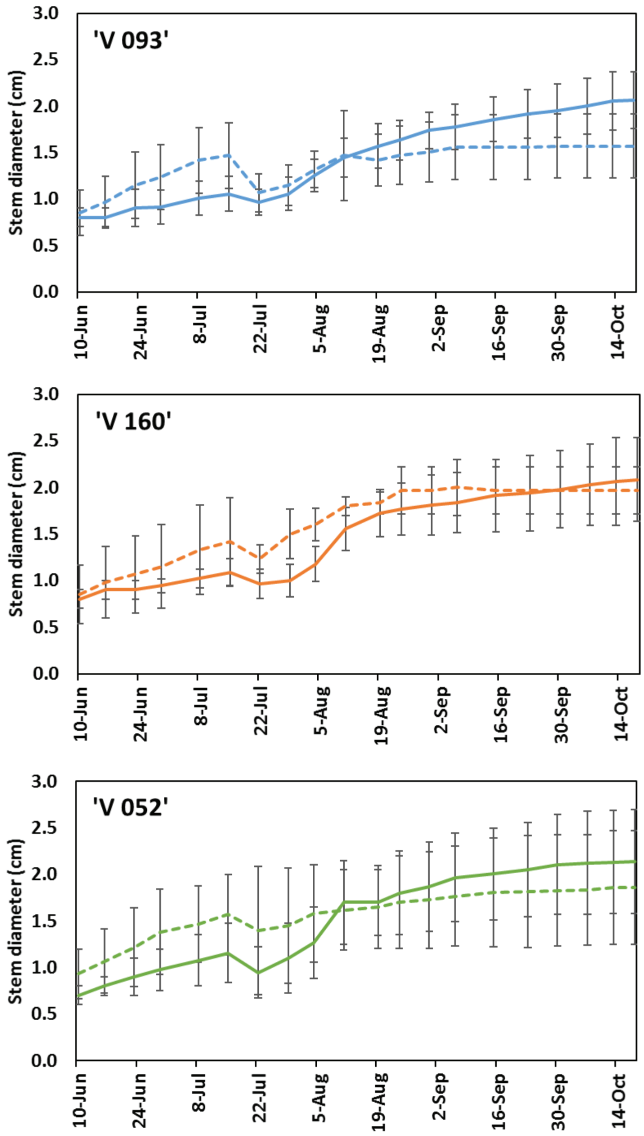


Fig. 4. Stem diameter of three willow clones 'V 093', 'V 160', and 'V 052' in pilot evapotranspirative willow system (solid line) and controls (dashed line) during first vegetation season. Means and standard deviations are given ($N$ = 9 and 6 for experimental and control willows, respectively).

between the test willows in the pilot EWS and control willows was statistically significant only for clone 'V 160'. There was no statistically significant difference in biomass yield between the clones in the pilot EWS. Despite this, the statistical analyses have shown that 'V 093' shoots have significantly higher DM compared with 'V 052' and 'V 160' indicating denser wood; however, due to its lower number of shoots, 'V 093' did not have higher biomass yield.

## 4. Discussion

The physical and chemical parameters of wastewater in all nine test beds were similar, resulting in equal growing conditions for all clones in the EWS. EC and DO in test beds were decreasing throughout the vegetation season (March–October), which can be explained by the degradation of dissolved organic matter and uptake of nutrients by plants. The water use did not differ significantly between the clones, which is in line with Guidi et al. [9] who found out that transpiration is subject to the development stage, plant nutritional status, and climate characteristics rather than to the willow species.

Regarding willow growth, the preliminary results have shown that irrigating willows with surplus wastewater has a positive effect on biomass production, stem height and diameter and in the case of 'V 160' and 'V 052' also on the number of the shoots. This is in accordance with Guidi et al. [9] who reported higher biomass production of wastewater irrigated willows that had increased stem diameter and height compared with non-irrigated willows. Numerous other studies have shown that the composition of wastewater matches to the nutrient requirements of willows;

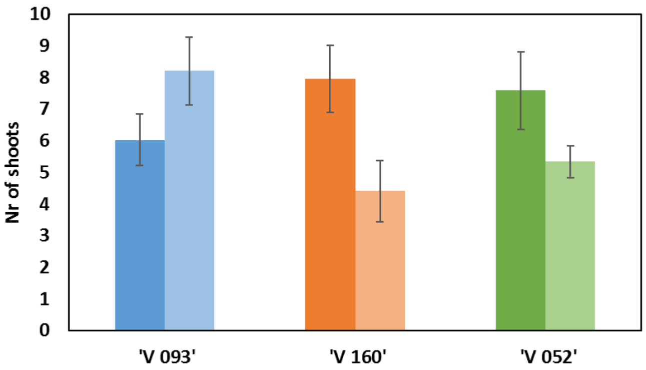


Fig. 5. Number of shoots for three willow clones 'V 093', 'V 160', and 'V 052' in the pilot evapotranspirative willow system (dark blue, orange and green) and control willows (light blue, orange and green). Means and standard deviations are given ($N$ = 9 and 6 for test and control willows, respectively).

moreover, wastewater can act as a good fertilizer [1,4,14]. Willow yield increase due to irrigation with wastewater can be as high as 30%–100% in a wastewater-fed system compared with commercial rainfed willow cultivation [4]. According to this, Curneen and Gill [2] report the highest biomass and evapotranspiration in willow cultivars receiving septic tank effluent compared with the systems fed with secondary treated effluent and rainwater, which justifies the use of EWS for domestic wastewater use for biomass production.

Better willow growth in test beds compared with the controls can also be the result of higher water availability in the beds. Willows have high water demand; therefore, control willows had lower growth due to lower water availability (note that the controls were only fed with rain). This is in accordance with Börjesson and Berndes [4] who pointed out that not only nutrients increase the biomass yield but also water availability. Moreover, the water availability may explain the shift in growth in mid-July when the willows in test beds outgrew the control willows for approximately 40 cm till the end of vegetation season. During summer, there was always plenty of water in test beds, while the control willows were subdued to drought stress, which resulted in the termination of first the vertical and later the radial growth. The most drought-sensitive clone was 'V 093', followed by 'V 052'. For them, the vertical and radial growth stopped 1 month earlier compared with 'V 160', which can result from genetic differences between the clones. The drought stress may also be typical for willows in the sub-Mediterranean climate; therefore, further studies of willows in EWS in sub-Mediterranean regions should consider the irrigation of control willows. In contrast to drought stress of control willows, permanent flooding can have negative effects on the growth of terrestrial plants, even on willows which are proven to be resistant to high soil water content: Guidi and Labrecque [19] have shown that permanent flooding had negative effects on most growth parameters in willows except the number of shoots per plant and root biomass; however, in this study, no such negative effects were observed.

In the first vegetation season, the willows in EWS produced 3.4–4.2 t DM ha$^{-1}$ a$^{-1}$ with no statistically significant difference between three different clones. Comparing test and control willows only indigenous clone 'V 160' in test beds produced significantly more biomass compared with control willows, while for the two hybrids, the difference was not statistically significant. Compared with other studies on *S. alba* clones, the annual biomass production in pilot EWS is in the same range as reported by Adegbidi et al. [8] (3.0–6.5 t DM ha$^{-1}$ a$^{-1}$) for older willow stands but much lower as reported by Guidi et al. [9] (64 t DM ha$^{-1}$ a$^{-1}$) for the first vegetation season. However, it is expected that the biomass yield in EWS will increase substantially in the second vegetation season with possible difference between the clones according to Kajba and Andrić [20] who reported the mean biomass production of non-fertilized willow stands in the first and second successive 2-year rotation cycles at age 2/3 (2-year-old stems with 3-year-old root system) and 2/5 (2-year-old stems with 5-year-old root system) 18.5 and 23.5 t DM ha$^{-1}$ a$^{-1}$ for 'V 093' and 10.4 and 19.3 t DM ha$^{-1}$ a$^{-1}$ for 'V 052'. This is likewise in line with the results of this study which indicate that 'V 093' shoots have significantly higher DM compared with 'V 052' and 'V 160' indicating denser wood. Denser wood, produced from 'V 093', can also result in potentially higher energy production and higher quality of wood chip or pellets.

In this study, there were few differences proven between the tested clones during the first vegetation season. The differences are expected to be more indicative in the following years, since significant differences between willow clones are also reported by other studies [10,20].

## 5. Conclusions

This paper compares the adaptability and biomass production of two selected *S. alba* hybrids with indigenous *S. alba* in pilot EWS during the first vegetation season. The pilot EWS had stimulating effect on willow growth since the willows in the test beds outgrew the control willows for approximately 40 cm until the end of vegetation season for all three tested clones. At the end of vegetation season, there were no significant differences between three tested willow clones neither in growth nor in biomass production; however, there were differences in growth dynamics. The indigenous clone showed better tolerance of the conditions in EWS at the beginning of the vegetation season compared with selected hybrids. The indigenous clone was also less sensitive to drought but had somewhat lower biomass production. The selection of appropriate willow clone turned out to be an important design parameter in EWS planning. In the following vegetation season, it is expected that the differences between the clones will increase, as well as biomass production and evapotranspiration. At the end of the second vegetation season, the willows will be harvested and analysed for nutrient contents, which will enable nutrient balance calculation.

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