Article

Novel Approaches to Optimise Early Growth in Willow Crops

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Abstract: Willow is a fast growing, high yielding biomass crop that can help reduce reliance on fossil fuels. However, long establishment times to get to profitability and sustainable yield may deter interest in planting the crop. A number of different approaches were investigated to optimise and accelerate early growth. These approaches were water immersion, plastic application, altering stem orientation at planting, altering coppicing timings and applying growth hormone. Glasshouse and field trials were used to test the different approaches. In this work, planting material was soaked for a varying number of days and plastic was applied or not applied in field trials. In the planting orientation approach, stems were planted diagonally or vertically with half of the planting material above the ground level or horizontally below ground level. Additionally, willow crops were coppiced at different times throughout their first growing season and a growth hormone trial was also incorporated in this work. Water soaking, plastic application, coppicing during the growing season or hormone application did not improve early growth or yield. However, early growth and yield were increased by manipulating the planting orientation of willow stems. Planting orientation treatments in which part of the stem was left above the ground increased early growth and yield significantly compared to the control without requiring extra inputs at planting. The beneficial effects of coppicing can be achieved by manipulating the planting procedure so that the first year's growth is not disregarded.

Keywords: willow; early growth; coppicing; stem orientation; pre-soaking

1. Introduction

Ireland imports 89% of fuel for energy which is mainly made up of fossil fuels [1]. There is an increasing need to find alternative sources of energy for today's growing society [2]. At the same time, there is a concerted effort by policy makers to develop indigenous sources of energy, mainly driven by European Union Directive 2009/28/EC, which outlines the requirement for 16% of Irelands energy to come from renewable sources by 2020 [3]. Ireland has one of the best tree growing climates in Europe yet still lags behind Europe for forestry cover [4]. Irish forestry cover is currently at 11% [5], while the European average is approximately 40% [6]. Half of Irelands forests are less than 25 years old [7], and thus, the time required to mobilise biomass resources from Ireland's limited current and future forestry may be too long and unfeasible to yield significant biomass supply in the short term [8]. Biomass is used for renewable energy generation at present in Ireland but the huge potential offered by bioenergy is relatively untapped, with an over reliance on imported fossil fuels [9]. Cost efficient allocation of biomass should get more attention to allow the sector to prosper and become an economically profitable farming enterprise [10].

Willow (*Salix*) is an excellent source of biomass that can be mobilised in a short period of time for use as an energy source. Improving cost effectiveness of willow crops for biomass depends strongly on achieving good yields [11,12] and establishing a strong, profitable crop as soon as possible. Willow is currently used as a source of biomass to offset some use of peat for energy production in Ireland [13]; however, factors such as poor establishment, high initial planting costs and a three year wait until the point of first income have delayed its uptake and attractiveness to landowners. Slow initial growth, particularly in the year after establishment with the first commercial harvest three years after planting of cuttings, means that the breakeven point occurs after seven years for a reasonably yielding crop [8]. This is a substantial deterrent for any prospective grower because of the initial cost and long wait for a return on investment, and therefore, any attempts to ensure significant early growth and yield assurance will make the crop more attractive.

Pre-soaking of willow planting material has been reported to increase root, shoot and leaf biomass as well as overall survival [14–18]. Edwards and Kissock [19] found that pre-soaking willow increased growth by improving weight, length and number of primordial roots. Tilley and Hoag [17,20] found that pre-soaking willow for 14 days, in a riparian restoration project, before spring planting on river banks improved establishment. Additionally, Schaff et al. [14] found 10 days soaking pre planting improved growth, biomass and survival rate of black willow in river bank restoration projects. Pre-soaking of willow stems prior to planting is currently practised by commercial operators in Ireland and may have stemmed from a practical approach to avoid desiccation before planting. Plastic treatments have commonly been used in maize crops in Ireland for many years to accelerate early growth [21] and more recently have been used successfully in bioenergy crops like *Miscanthus* [22,23]. Plastic applications are successful at accelerating early growth by increasing soil temperature and protecting seeds and planting material from frost [21]. Although numerous studies have been carried out on the effects and benefits of plastic treatments on C_4 crops such as *Miscanthus* and maize, no research has been carried out on the effect of the application of plastic on C_3 crops such as willow.

Current willow planting practise involves the use of a Step Planter, which inserts a 20 cm long cutting of stem vertically into the ground [8]. Minimal research has been carried out on planting orientation with the exception of horizontal (lay flat) planting [14,15]. Cao et al. [24] found that horizontal planting did not significantly increase biomass yield and McCracken [25] advised against it as considerable quantities of planting material are used in horizontal planting and could only be justified if significant savings could be found elsewhere. In many trials vertical planting with 20 cm cutting has been recommended over horizontal planting [16] with Edelfeldt et al. [26] recommending at least 20 cm cutting should be used. Minimal research has been carried out on other possible methods of planting stem cuttings such as leaving part of the stem exposed above ground. Observations from previous field work have noted that the more of the planting stem that is exposed to light and air the greater the inclination for new stems to form. Smith and Wareing [27] found gravity to have an effect on bud break and inclination of osier willow (*Salix viminalis*) resulted in stem propagation on the upper side of the stem.

Current common practice involves planting willow crops in May, allowing the crop to propagate and then in the following winter or early spring carrying out coppicing or cutting back of the first year's growth [8,28]. Coppicing removes apical dominance from the plant and redistributes growth to lateral buds [29] resulting in greater stem numbers. This willow biomass is not harvested and not only results in a year's delay to first commercial harvest but also the loss of the first years yield. Reported advantages of coppicing include increased stem numbers and stimulation of root growth [30]. There is little evidence and few publications to support the benefits of coppicing; in fact Finnan et al. [31] showed that omitting coppicing leads to an increase in biomass yield at first harvest without affecting yield at subsequent harvests. There are still potential benefits to a greater canopy development as a result of coppicing [32], including increased competitive ability with weeds [33]. Traditional coppicing usually occurs after leaf fall in the first growing season and before bud burst in the following growing season [8]. Little research has been conducted on altering the timing of the coppicing and removing

the apical dominance earlier in the first growing season to reduce complete loss of first years growth. Hytönen [34] found that coppicing during the growing season negatively affected growth of exotic willow varieties but did not hamper growth of local willow varieties. No research has been conducted on the effect of growth hormones on apical dominance in commercial willow crops. Cline [35] states that cytokinin originating in the roots and shoots is a key hormone in lateral bud growth. Alvim et al. [36] found a spike in cytokinin concentrations before bud burst in willow plants indicating that it was an important hormone in forming new stems.

The overall objective of this work was to optimise early growth of willow using a number of approaches that ranged from accelerating establishment through water immersion and the use of plastic to reducing the need to coppice by initiating more stem growth earlier on. These research hypotheses are that:

1. a. Pre-soaking of planting material will initiate root growth and accelerate the establishment of willow crop in the field. b. The application of plastic will accelerate the early growth of willow.
2. Stem orientation can affect early growth; exposing stem material above ground will promote buds to turn into stems instead of roots and result in a greater stem number after one year and remove the need to coppice.
3. a. Altering the time and method of coppicing will eliminate the need for a harvest after one year of growth. b. The application of the growth hormone cytokinin will override the auxin hormones dominance in exerting apical dominance and result in greater stem numbers and yield than coppicing.

2. Materials and Methods

2.1. Experimental Design and Site

All field trials were carried out at different sites in a close radius of Oak Park agricultural research centre in Carlow, Ireland. Glasshouse experiments were also carried out at the research centre as a means of conducting initial investigations of some approaches prior to field trials.

Three approaches were investigated in order to improve early growth and yield of willow crops. All approaches for improving growth were applied in the time around planting or shortly afterwards. A description of these approaches and their constituent treatments is displayed below in Table 1.

Table 1. Description of the treatments in the three approaches.

Treatment Number	Description
Approach 1—Pre-Soaking and Plastic Application	
1	No soaking of planting material, planted straight from cold store with/without plastic.
2	Soaked for 2 days pre planting with/without plastic.
3	Soaked for 4 days pre planting with/without plastic.
4	Soaked for 6 days pre planting with/without plastic.
5	Soaked for 8 days pre planting with/without plastic. N.B. Plastic was applied over planted willow cuttings on the same day as planting.
Approach 2—Planting Orientation	
1	Traditional planting of 20 cm stem planted perpendicular to the ground surface but below the surface.
2	10 cm of planting stem is exposed above surface of ground while 10 cm is buried.
3	10 cm of planting material is above surface and 10 cm below surface and stem is planted diagonally.
4	20 cm stem is planted vertically in the ground between 5 and 10 cm below the surface of the ground.

Table 1. *Cont.*

Treatment Number	Description
	Approach 3—Coppicing Timing
1	Stems planted and not coppiced.
2	Stems planted and coppiced after one year's growth (Traditional coppicing).
3	Stems planted and coppiced after 30 cm of growth (approximately 6 weeks after planting).
4	Stems planted and coppiced after 60 cm of growth
5	Stems pre-soaked in a cytokinin solution for 48 h pre planting.
6	Stems planted and coppiced at 20 cm high after approximately 6 weeks growth.

Genus *Salix*, cultivar Tora was used at all trials and was sourced from SalixEnergi, Krinova Science Park, Stridsvagnsvägen 14, Kristianstad, Sweden. The sites for all trials were prepared by spraying with an herbicide (Glyphosate 360 g active ingredient, dose 4 L ha^{-1}) before a suitable seedbed was prepared. The trials were hand planted in each year following standard spacing in double rows of 70 cm apart separated by 150 cm with the distance between each cutting of 60 cm. There were four double rows; each plot was guarded by a double row on both sides and two plants at top and bottom of plots. After sowing, the sites were sprayed with pre emergence herbicide pendimethalin (Stomp Aqua, 455 g active ingredient). All sites were also sprayed with an insecticide chlorpyrifos (Clynch, 480 g active ingredient). Weed control was carried out throughout the growing season by interrow spraying and hand weeding.

2.1.1. Pre-Soaking and Plastic Application

Glasshouse trials were conducted in 2013 and again in 2014 to investigate the potential benefits of soaking willow cuttings in water prior to planting. Cuttings (20 cm in length) were stored in a cold room (<4 °C) before being placed in a bath of tap water at ambient room temperature to a depth of 10 cm for 0, 2, 4, 6 or 8 days prior to planting (Table 1). Cuttings for all treatments were planted in 20 L pots filled with general, sterilised compost arranged in ten blocks with the five treatments arranged randomly in each block. Cuttings from all treatments were planted on the same day (Table 2). At harvest, plants were divided into stems and leaves before being weighed and samples taken for dry matter analysis.

Table 2. Planting, coppicing and harvesting dates.

Approach	Location	Planted	Coppiced	Harvested
1 (Plastic application and pre-soaking)	Glasshouse	20th Mar 2013		30th Sept 2013
	Field	27th May 2013		4th Feb 2014/10th Dec 2015
2 (Planting Orientation)	Glasshouse	20th Jun 2014		10th Oct 2014
	Field	2nd Jun 2014/7th May 2015		15th Dec 2015/8th Feb 2017
3 (Coppicing Timing)	Field	19th May 2014/7th May 2015	Treatment 3 and 6–14th July 2014/7th July 2015. Treatment 4–11th August 2014/4th Aug 2015.	15th Dec 2015/8th Feb 2017

Field trials involved a split plot, randomised complete block design with four replicates incorporating the five treatments with and without plastic. Water immersion was the main plot factor and plastic application was the split plot factor. The treatments involved pre-soaking the planting material for various lengths (as outlined in Table 1 above) with and without plastic. The plastic was sourced from Samco Agricultural Manufacturing Ltd., Adare, Limerick, Ireland. 20 cm stems of

planting material were soaked in water for two, four, six and eight days before planting, while the no soak treatment was stored in the cold store at −4 °C. The trials were then planted by hand on two different soil types within the research centre in each of two separate years. Each plot within the blocks had twenty plants that the treatments were applied to. For the treatments to which plastic was applied, the plastic was applied over the planted double rows of willow cuttings by a Samco single bed plastic applicator mounted behind a tractor on the same day as planting. Planting and harvesting dates are provided in Table 2. The trials were harvested (coppiced) after one year's growth and then at their first rotation two years later. The first harvest was carried out by a clippers and the subsequent harvest was carried out by a chain saw. In both harvests the weight of the samples was measured in the field on a weight cell mounted scales on a flatbed van and a random sample was chipped in the field and collected for dry matter analysis.

2.1.2. Planting Orientation

A glasshouse trial to investigate this approach was conducted during the 2014 growing season. Orientation treatments are described in Table 1 and Figure 1. The treatments included traditional method of planting with full 20 cm stem below ground (1), 10 cm above and 10 cm below the ground surface (2), 10 cm above and below ground surface but planted diagonally (3), layflat planting with the 20 cm stem planted horizontally 5 cm below the surface of the ground (4). Cuttings were planted on 20th June 2014 in 20 L pots filled with general, sterilised compost arranged in ten different blocks with the four treatments randomly arranged in each block. The glasshouse trials were watered daily. The pots were harvested on the 2 of October 2014 and a number of parameters were measured. A dry matter sample was taken and dried in an oven for 24 h to assess the dry matter content.
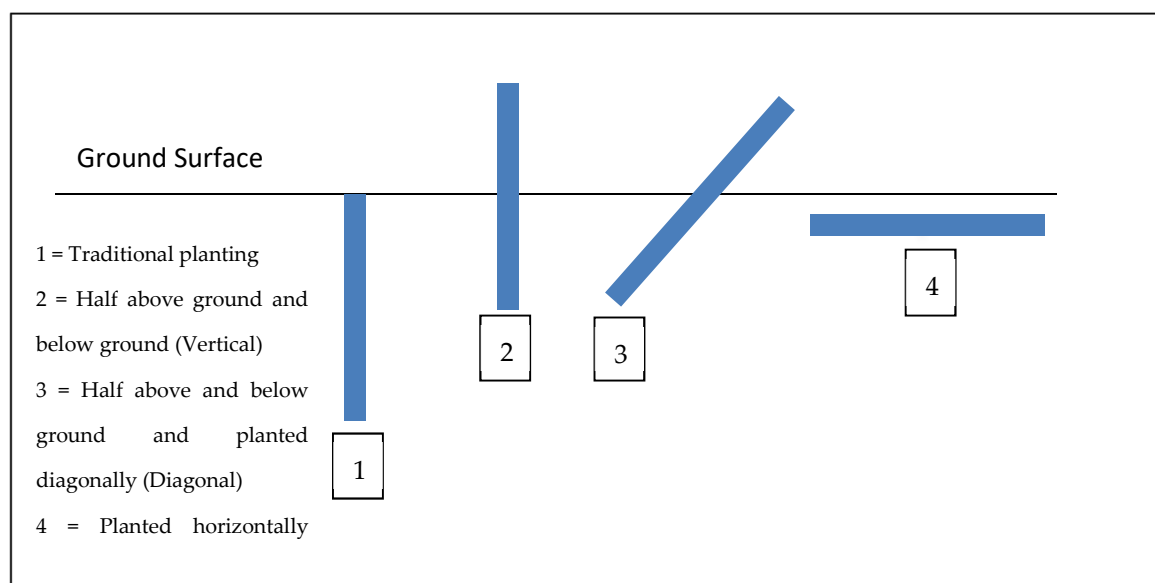

Figure 1. Treatment orientations below and above ground level.

The field trials involved a randomised complete block design with four replicates in total incorporating the four treatments described above. Field trials were planted in two separate years (2014 and 2015) on a surface water gley soil which had previously been in permanent grassland. The treatments used in the field trials were identical to those used in the glasshouse trial (Table 1). Planting and harvesting dates are provided in Table 2. Electrified rabbit fencing was erected around each trial to prevent ingress of rabbits and hares.

Measurements were conducted on 20 centre plants within each plot. These 20 plants were guarded by a double row on both sides and a set of plants at the top and bottom of each plot. Leaf area index was also measured using a Sunscan Canopy Analysis System (Delta-T Devices, 128 Low Road, Cambridge,

CB25 0EJ, United Kingdom). The yield was harvested at the end of two growing seasons by cutting down these 20 plants after leaf fall. The weight of these samples was measured in the field and a random sample was chipped and collected for dry matter analysis.

2.1.3. Coppicing Timing

The field trials involved a randomised complete block design with four replicates in total incorporating six treatments. The treatments are described above in Table 1. The trials were planted in 2014 and 2015 on two different soil types in each year. The trials were planted on a surface water gley in 2014 and 2015; the second trial in 2014 was planted on an acid brown earth and in 2015 on a grey brown podzolic. The treatments were applied to the crop throughout the first growing season with hand clippers (dates given in Table 2 below). Treatment 5 was pre-soaked in 6-Benzylaminopurine (Cytokinin), sourced from Sigma Aldrich, Arklow, Ireland, applied before-planting. Planting, coppicing and harvesting dates are provided in Table 2 below.

Measurements were conducted on 20 centre plants within each plot. Stem height was measured monthly while stem numbers were measured at the beginning and end of the growing season. Leaf area index was also measured to quantify the canopy development. The yield was measured destructively by cutting down these 20 plants after leaf fall after two growing seasons. The weight of these samples was measured in the field and a random sample were chipped and collected for dry matter analysis.

All trials had an emergence and survival rate of greater than 90% in both field trials and glasshouse trials, except for the trials under plastic that had a high emergence rate but of which the survival rate was 60%.

2.2. Weather Data

Meteorological data was collected from a synoptic weather station located within the research centre at Oak Park. Weather and growing conditions varied substantially in each growing season as displayed in Figures 2 and 3. Sites were situated in close proximity of each other in each year so meteorological conditions did not have an effect on growth at the different sites used within each growing season. However, growing condition varied between years. 2013 was a dry summer with low rainfall. 2014 had high rainfall and high soil temperatures in May and June. 2015 had high rainfall in May but less in June with lower soil temperatures.
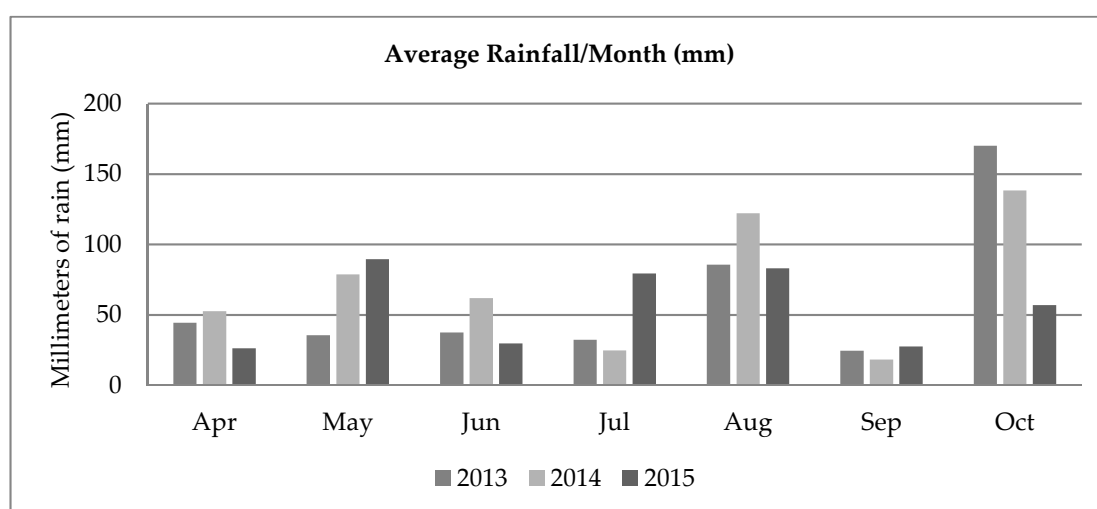

Figure 2. Average rainfall in the three trial years from April to October.
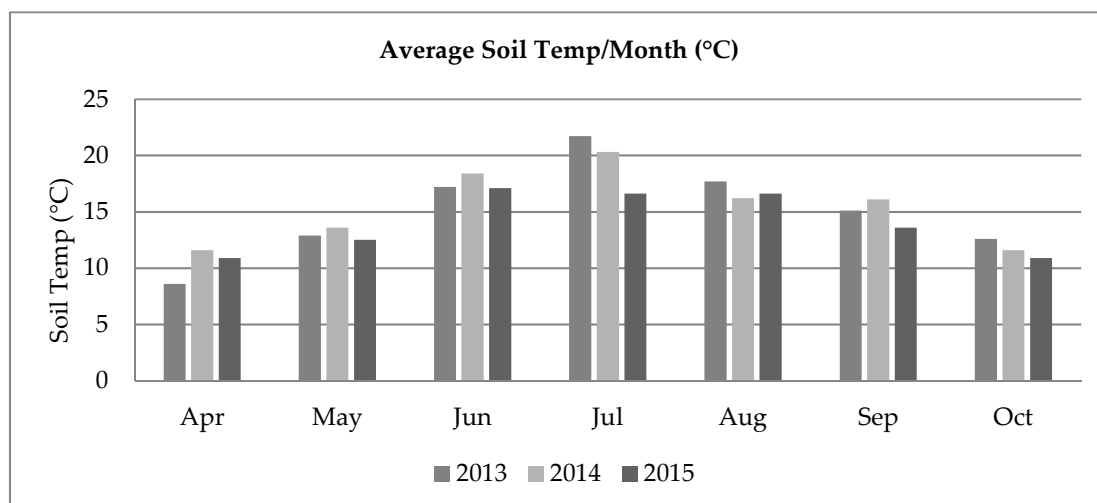
Figure 3. Average soil temperatures per month for the three trial years from April to October.

2.3. Data Analysis

Data from the glasshouse and field work was analysed with PROC GLIMMIX for analysis of variance in SAS (SAS/STAT V.9.4.2012. SAS Inc. Cary, NC, USA). Pairwise comparisons of treatments were conducted using Tukey's test.

2.4. Glasshouse Trials

The pre-soaking glasshouse trial had one factor, soaking. Similarly, the orientation trials conducted in the glasshouse had just one factor, orientation.

2.5. Field Trials

The field trials on pre-soaking and plastic application had two factors, pre-soaking and plastic. Year was not a factor as only one trial is presented.

The planting orientation field trials were conducted over two years, the factors were orientation treatment and year. Additionally, the interaction between year and orientation was studied.

The coppicing timing trial was conducted on two separate sites in each of two separate years. The sites were different between the two years; thus, each year was analysed separately. For each year, site and coppicing timing treatment were the factors included in the analysis; the interaction between these two factors was also studied.

No transformations of data were required and all statistical tests were performed at a significance level of 0.05.

3. Results

3.1. Approach 1—Presoaking and Plastic Application

3.1.1. Glasshouse Results

The initial glasshouse work showed promising results for soaking times with a significant increase in biomass yield with a greater soaking time. These trials were carried out in 2013 (Table 3) and 2014 (data not shown) with very similar results in both years. There was a significant difference in yields between the water soak treatments ($p = 0.03$). Treatment 5 (8 days soaking) yielded significantly higher than the control treatment with a 35% increase in yield. However, this effect did not translate to the field. Four field trials with water soaking and plastic treatments were planted on different sites over two years and since, the same results were found for all trials only one set of data is presented.

Table 3. Results from pre-soaking and plastic application from initial glasshouse investigation and results from field trials planted in 2013 and harvested after one growing season and then after two years growth (three-year-old roots). All weights are expressed on a dry matter basis. Pairwise differences between treatments ($p = 0.05$) were compared using Tukey's test. Numbers followed by the same letter are not statistically different.

Treatment	Glasshouse (g/Pot)	Yield at Coppice 2013 (Mg ha^{-1} a^{-1})	Yield at First Rotation 2015 (Mg ha^{-1} a^{-1})
1 (No presoaking)	21.0 B	0.7 A	17.4 A
2 (2 days presoaking)	26.7 AB	0.6 A	16.8 A
3 (4 days presoaking)	26.3 AB	0.7 A	17.2 A
4 (6 days presoaking)	26.1 AB	0.7 A	19.2 A
5 (8 days presoaking)	32.6 A	0.7 A	15.8 A
Plastic	–	0.6 B	15.5
No Plastic	–	0.8 A	19.1
p-values			
Water Treatment	0.03	0.90	0.30
Plastic	–	0.02	<0.01
Interaction	–	0.82	0.68

3.1.2. Field Results

In field trials, there was no significant effect of soaking treatment on yields after one year's growth (2013) or after a further two years growth (2015). Although initial growth of the willow underneath the plastic was vigorous, many shoots were not able to break through the plastic. Consequently, many plants died which resulted in a significant ($p = 0.02$) 30% reduction in biomass yield in 2013 and a 19% reduction in yield over no plastic in the 2015 harvest ($p < 0.01$). The yield at first rotation harvest was reduced from 19.1 Mg ha^{-1} a^{-1} down to 15.5 Mg ha^{-1} a^{-1} as a result of plastic application. This was a direct result of the plastic delaying willow growth. There was no significant interaction between water treatment and plastic.

3.2. Approach 2–Planting Orientation

3.2.1. Glasshouse

Planting willow cuttings vertically with the upper half of the stem above ground in the glasshouse resulted in a higher yield by enabling a greater number of stems to form and a higher leaf area to develop. Planting orientation had a significant effect on stem yield, leaf yield and stem numbers ($p < 0.01$) as displayed in Table 4 above. Highest stem and leaf yield were obtained from treatment 2 (planted vertically with the upper half of the stem exposed) and the lowest yields were obtained from treatment 4 where cuttings were planted horizontally beneath the surface of the soil. Stem and leaf yields from treatment 2 were significantly greater than those of treatment 4, whereas stem and leaf yields from treatments 1 and 3 did not differ significantly from treatment 4. The treatments in which the upper half of stems were exposed above the surface of the soil, treatments 2 and 3, resulted in a 43% and 39% increase in stem numbers over treatment 1 without the need to coppice. The root yield did not show a significant difference ($p = 0.09$) for treatment; treatment had a significant effect on aboveground biomass but not below ground biomass.

Table 4. Glasshouse planting orientation trial 2014. Stem yield represents above ground dry matter biomass measured in weight per total stems per pot, leaf yield is the dry matter weight of the leaves, root yield represents the average dry matter yield per pot and the stem numbers the average stems present per pot.

Treatment	Stem Yield (g)	Leaf Yield (g)	Root (g)	Stem Numbers/Plant
1 (Trad)	25.3 AB	15.5 AB	9.6 A	1.7 A
2 (Vert)	31.3 A	19.0 A	11.6 A	3.0 B
3 (Diag)	24.3 AB	17.4 AB	9.9 A	2.8 B
4 (Flat)	17.3 B	11.7 B	8.9 A	1.4 A
p-value	<0.01	<0.01	0.09	<0.01

Treatment descriptions are given in Table 1. Pairwise differences between treatments ($p = 0.05$) were compared using Tukey's test. Numbers followed by the same letter are not statistically different.

3.2.2. Field

In field experiments, willow stems which were planted vertically or diagonally produced higher yields as a result of significantly higher stem numbers per plant (results displayed in Table 5). Planting orientation had a significant effect on stem yield ($p = 0.04$), treatment 3 (diag) yielding significantly more (1.7 Mg ha^{-1} a^{-1}) than the layflat treatment, treatment 4.

Table 5. Stem yield (represented as dry matter tonnes per hectare measured after two growing seasons), Leaf Area Index (LAI), stem height (height of the tallest stem in each plant) and stem numbers for two trials planted in 2014 and in 2015.

Treatment	Stem Yield (Mg ha^{-1} a^{-1})	LAI	Height after One Growing Season (cm)	Height after Two Growing Seasons (cm)	Stem Numbers /Plant
1 (Trad)	7.4 AB	1.0 AB	131 A	415 A	2.2 B
2 (Vert)	8.1 AB	1.1 AB	128 A	408 A	3.7 A
3 (Diag)	8.8 A	1.4 A	129 A	408 A	3.5 A
4 (Flat)	6.4 B	0.7 B	131 A	403 A	1.9 B
2014	6.89 B	1.0 A	135 A	401 A	2.6 B
2015	8.5 A	1.1 A	125 A	416 A	3.1 A
			p-values		
Treatment	0.04	0.04	0.97	0.99	<0.01
Year	<0.01	0.32	0.13	0.89	<0.01
Interaction	0.89	0.29	0.55	0.99	0.03

Pairwise differences between treatments ($p = 0.05$) were compared using Tukey's test. Numbers followed by the same letter are not statistically different.

The different orientation treatments also had a significant effect on the canopy development, measured by LAI (Leaf Area Index) ($p = 0.04$). Treatment 3 had a significantly higher leaf area index than treatment 4 (1.4 versus 0.7). The treatments had no significant effects on willow height after either one growing season or two and there was no significant difference of year on height ($p = 0.97$ and $p = 0.99$).

Stem numbers per plant were significantly higher for treatments 2 and 3 (3.7, 3.5 stems per plant) compared to treatments 1 and 4 (2.2, 1.9 stems per plant) ($p < 0.01$) in both years. Treatment 2 and 3 had an average of 39% more stems over treatment 1 and a 47% increase in stem numbers over treatment 4.

There was a significant increase in yield and stem numbers in 2015 over 2014, but no significant differences for height and LAI. There were no significant interactions between year and planting

orientation treatment for yield, LAI and plant height. However, there was a significant interaction between year and orientation treatment for stem numbers because the difference between treatments 2 and 3 between years was greater than treatment 1 and 4 between years.

3.3. Approach 3–Coppicing Timing

3.3.1. 2014 Results

The several investigated treatments, displayed in Table 6, had a significant effect on stem yield ($p < 0.01$); there was no significant difference between sites ($p = 0.6$), but there was a significant interaction between treatment and site ($p = 0.03$) because yields of treatments 4, 5 and 6 were highest on site 2, whereas treatments yields of 1, 2 and 3 were highest on site 1. Treatment 1 (not coppiced) and treatment 5 (Cytokinin application and not coppiced) yielded significantly higher than all other treatments. The coppiced treatments 2 (traditional coppicing) and 6 (coppiced during growing season at 20 cm high) yielded significantly higher than coppicing fully during the growing season (treatment 3 and 4). Coppicing when the plants had reached 60 cm, treatment 4, reduced yield to 10% of the control.

Table 6. Stem yield (represented as dry matter tonnes per hectare measured after two growing seasons).

Treatment	Stem Yield (Mg ha^{-1} a^{-1})	LAI	Height after One Growing Season (cm)	Height after Two Growing Season (cm)	Stem Numbers/Plant
1 (No Coppice)	14.8 A	2.5 AB	171 A	532 A	2.2 C
2 (Trad Coppice)	8.8 B	1.9 BC	172 A	347 C	3.7 B
3 (Coppice after 30 cm)	5.1 C	1.1 DE	52 C	319 C	3.9 B
4 (Coppice after 60 cm)	1.4 D	0.6 E	9 D	61 D	5 A
5 (Cytokinin)	14.6 A	2.7 A	186 A	518 AB	2.1 C
6 (Coppice at 20 cm)	10.5 B	1.7 CD	103 B	465 B	2.3 C
Site 1 (Gley)	9.0 A	1.6 A	115 A	373 A	2.9 B
Site 2 (Brown Earth)	9.4 A	1.8 A	116 A	374 A	3.6 A
p-values					
Site	0.6	0.1	0.95	0.9	<0.01
Treatment	<0.01	<0.01	<0.01	<0.01	<0.01
Interaction	0.03	0.07	0.16	0.7	<0.01

Leaf Area Index, stem height (height of the tallest stem in each plant) and stem numbers displayed above for trials planted on two different sites but within close proximity of Oak Park Research Centre. Pairwise differences between treatments ($p = 0.05$) were compared using Tukey's test. Numbers followed by the same letter are not statistically different.

Treatment had a significant effect on LAI ($p < 0.01$) while there was no significant effect of site ($p = 0.1$). Treatments 1, 2 and 5 had a significantly higher LAI than the treatments that were coppiced during the growing season (3, 4 and 6). The treatment with the lowest LAI (O.6) was treatment 4 (coppiced after 60 cm) whereas the treatment with the highest LAI (2.7) was treatment 5 (cytokinin treatment). Treatment had a significant effect on height after one and two growing seasons ($p < 0.01$) with treatment 1 (non-coppiced) measuring significantly higher than all other treatments. Coppicing reduced height considerably; coppiced treatments were still considerably shorter than the non-coppiced treatments at harvest. There was no effect of site on height ($p = 0.9$) and there were no site and treatment interactions for height after one growing season ($p = 0.16$) or after two growing seasons ($p = 0.7$).

Treatment and site had a significant effect on stem numbers ($p < 0.01$). Treatment 4 had a significantly higher number of stems than all other treatments while treatments 2 and 3 also having significantly higher stem numbers than treatments 1, 5 and 6. Treatment 5 (cytokinin) had the lowest stem numbers but this did not negatively affect yield. There was a site and treatment interaction ($p = 0.01$) as there was a large difference in treatment 3 and 4 between sites but not for the remaining treatments.

3.3.2. 2015 Results

Treatment and site had a significant effect ($p < 0.01$) on yield as displayed in Table 7, results were similar to the experiments carried out in the previous year. Treatment 1 (no coppicing) yielded the highest (10.9 Mg ha^{-1} a^{-1}) and yielded significantly higher than the coppiced treatments. Similar to 2014, the treatment with the lowest yield was the treatment that was coppiced at 60 cm; the yield of this treatment was less than 10% of the yield of the control. Site 1 yielded significantly higher than site 2 and there was a significant site by treatment interaction ($p = 0.02$) as there was a greater difference between yields for treatment 1, 2 and 3 while treatments 4, 5 and 6 behaved similarly on both sites.

Table 7. Stem yield (represented as dry matter tonnes per hectare measured two growing seasons), Leaf Area Index.

Treatment	Stem Yield (Mg ha^{-1} a^{-1})	LAI	Height after One Growing Season (cm)	Height after Two Growing Season (cm)	Stem Numbers/Plant
1 (No Coppice)	10.9 A	2.1 A	257 A	392 A	2.1 BC
2 (Trad Coppice)	5.3 BC	1.3 ABC	123 C	324 BC	3.5 A
3 (Coppice after 30 cm)	3.6 CD	0.7 CD	121 C	257 C	2.6 ABC
4 (Coppice after 60 cm)	0.8 D	0.4 D	81 C	185 D	2.3 BC
5 (Cytokinin)	8.0 AB	1.9 AB	197 B	351 AB	1.9 C
6 (Coppice at 20 cm)	3.1 CD	1.0 BCD	116 C	272 C	2.9 AB
Site 1 (Gley)	7.1 A	1.3 A	153 A	359 A	2.3 B
Site 2 (Podzolic)	3.5 B	1.1 A	146 A	234 B	2.8 A
p-values					
Treatment	<0.01	<0.01	<0.01	<0.01	<0.01
Site	<0.01	0.40	0.50	<0.01	0.01
Interaction	0.02	0.25	<0.01	0.05	0.10

Stem height (height of the tallest stem in each plant) and stem numbers for trials planted on two different sites but within close proximity of Oak Park Research Centre. Pairwise differences between treatments ($p = 0.05$) were compared using Tukey's test. Numbers followed by the same letter are not statistically different.

Treatment had a significant effect on LAI ($p < 0.01$) while site did not ($p = 0.4$). Treatment 1 had the greatest canopy development. The treatments that were coppiced during the growing season (3, 4 and 6) had significantly lower LAI compared to the non-coppiced treatment. Treatment had a significant effect on height after both one growing season and two ($p < 0.01$). Treatment 1 measured significantly higher than all other treatments after one and two growing seasons. Similar to the results obtained in 2014, coppicing reduced height considerably and coppiced treatments were still considerably shorter than the control at harvest. There was no significant difference between sites after one growing season ($p = 0.5$) but there was a significant difference after two growing seasons ($p < 0.01$). There was a significant interaction between site and treatment on both occasions as the height of treatment 1 and 3 were lower on the site 2 compared with site 1 whereas the heights of the other treatments were similar between sites.

Treatment had a significant effect on stem numbers ($p < 0.01$). There was also a significant difference between sites ($p = 0.01$). Treatment 2 (traditional coppicing) had a significantly higher number of stems than treatment 1, 4 and 5.

The control and the treatment which received cytokinin, both of which were not coppiced, yielded the highest in both years while also producing the lowest stem numbers after two years of uninterrupted growth. The later in the growing season that coppicing is practised, the greater the detrimental effect on yield as displayed by treatment 4 (coppicing after 60 cm of growth). These results demonstrate

that coppicing willow crops, particularly during the growing season, retards growth and substantially delays yield development.

4. Discussion

Pre-soaking of willow planting material has been shown to increase the water content of willow cuttings and promoted root initiation and development [37,38], but our work found that these benefits do not translate to higher yields in a field setting. Pre-soaking of willow planting material showed potential in the glasshouse with yields increasing with increasing soaking lengths but this did not translate effectively to the field. Yields in the field did not follow as clear of a trend and it could not be justified to continue with further trials. A number of factors may have contributed to this including the fact that the pre-soaked planting material may have developed adventitious roots that were subsequently damaged at planting. The practicality of planting pre-soaked material which had longer adventitious roots was an inhibitory factor for this trial. However, soaking of willow in the field for a number of days while planting or just before planting may be beneficial to commercial growers to prevent desiccation. Plastic applications have been successfully used to accelerate early growth of maize crops in Ireland for decades and in more recent years in miscanthus crops [20]. Maize and miscanthus are able to break through the plastic but the soft initial vegetative growth of willow was unable to break through even perforated plastic and subsequently many plants died. Potentially planting willow cuttings through the plastic in a traditional mulching fashion may yield a more favourable outcome.

Previous attempts at altering planting orientation in willow have involved looking at lay flat planting to reduce costs and improve yields [39] but not to accelerate early growth nor to remove the need for coppicing. This research (planting orientation) showed that the beneficial effects of coppicing can be achieved by manipulating the planting procedure without the need to lose the first year's growth. This approach did not significantly affect root biomass yield in glasshouse trials, even though in some cases there was a much smaller area underground for roots to form. This work hypothesised that burying willow planting material may hinder stem formation for two reasons; 1. only buds near the surface of the soil will turn into stems thus reducing potential stem numbers; and 2. light exposure may play a part in stem formation. Sennerby-Forsse and Zsuffa [40] looked at factors that dictated root development and found that exposure to a humid environment was a main factor. There does not appear to be a definitive answer as to whether nodes will turn into shoots or roots but this research has shown that the more of the planting material that is exposed to air the more stems that form. The diagonally planted stems yielded slightly higher in the field trials than the vertically planted but not in the glasshouse. A more in-depth study of these two methods may be warranted but it is very apparent that they both achieved significant increases in stem numbers over traditional and horizontal (layflat) planting. The current approach for planting of commercial willow [8] may need to be altered to accommodate this new method of planting as there may be difficulties with rolling seed beds and applying herbicides but with such significant increases in stem numbers and yield this justifies further investigation.

Approach 3 using a coppicing timing approach to remove apical dominance at different stages in the growing season and chemical manipulation of the growth using cytokinin showed that the control treatment (i.e., no coppicing) produced the highest yields. However, the cytokinin did not have the expected effect of increasing stems numbers over treatment 1, the control treatment; in fact, it had an inhibitory effect on stem numbers in some years although not statistically significant. Cytokinin has been identified as peaking in willow plants just prior to bud burst [36], but there is still a lot of unknown factors about the hormonal interactions, mainly how cytokinin works in conjunction with auxins to allow/inhibit stems to form or roots to form. Ward et al. [41] have carried out some interesting work on trying to map the DNA pathways of these mechanisms but still have no definitive answers on the exact role of cytokinins on stem formation. Treatments 2 and 3 where complete coppicing was carried out during the first growing season managed to substantially increase stem numbers but

affected subsequent growth of the willow plants. It appears from this work and work carried out by Hytönen [34] that the later coppicing occurs in the growing season the more negative an effect on the yield. Treatment 6 where the plants were coppiced at 20 cm high thus removing the apical dominance but not all the stem biomass still had an effect on yield but did not significantly increase stem numbers. Although in 2014, the yield for this treatment was better than treatment 2, this did not follow through to the following year. From this work and previous studies [31], it appears there is no real requirement for coppicing to improve yield and if there is a need to coppice the best strategy is to coppice willow in dormancy [34].

Previous work on coppicing has found an average stem number of 2 stems per plant for uncoppiced experiments which is in line with our findings for the uncoppiced treatment in both the planting orientation and coppicing approaches. That same research found average stem numbers per plant to be approximately 3.3 after coppicing [17]. Thus, our research had a similar effect of traditional coppicing as stem numbers almost doubled compared to the no coppicing treatment, but without the need to cut back and disregard one year's growth of biomass. Additionally, the different orientation of the planting material improved yield over the control in both years.

Higher biomass yields in all experiments in 2014 compared to 2015 were associated with higher stem numbers per plant but also to taller stems. High rainfall in the earlier months of 2014 that preceded planting together with precipitation and high soil temperatures around the time of planting proved favourable for growth in 2014.

The hypothesis that pre-soaking of willow planting material, application of plastic, altering the method of coppicing and the use of a growth hormone would accelerate establishment and early growth of willow has been rejected. The hypothesis that altering planting orientation increases stem numbers without losing a year's growth and producing a good yield from two years growth can be accepted. The good canopy development allowed the willow to compete well with weeds in its first growing season. Although coppicing after one year's growth may still be required in some commercial cases, if sufficient, weed control is not achieved early in the crops first year. Traditional coppicing may initially result in an abundance of stems but self-thinning rates in some willow species can be high [40,42] resulting in wasted energy for the plant. The planting orientation method in this research allows an optimum numbers of stems to form for good yield and canopy development without the wasted energy of self-thinning. The yield of the non-coppiced willow crops in Finnan et al. [31] after three years of growth were 9.3 Mg/ha, while the yields in this work after only two years were similar, thus highlighting the advantage of the increased stem numbers benefit to early establishment and yield. Further work on scaling these trials from a plot scale to a bigger field scale and investigating machinery adaptations to facilitate the new planting method would be of great benefit and firmly establish the benefits of altering planting orientation in a commercial setting.

5. Conclusions

Applying plastic, pre-soaking of planting material and coppicing during growing season and application of growth hormones does not appear to offer any benefit to accelerating early growth of willow crops. Although soaking of planting material did not result in yield improvements it may still be beneficial in drier climates if delayed planting is envisaged. Additionally, there may be scope for further investigation regards alternative methods of applying plastics to the crop. Planting the willow through the plastic would potentially facilitate easier emergence of the crop.

Altering the planting orientation has shown immense potential in this research to improve early growth and improve returns on the crop. The most significant implication of this research is that the beneficial effects of coppicing can be achieved by manipulating the planting procedure so that the first year's growth does not have to be disregarded. This has huge potential for commercial growers of willow crops, as they can achieve a quicker return on investment and eliminate the expense of coppicing. This approach needs further investigation regards machinery adaptations and research on its suitability in drier climates.

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