# Influence of Pre-emergence Cutting Characteristics on Early Willow Establishment

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Cover: Cuttings planted in 2008 in an outdoor enclosure. Cuttings on the diagonal (with white labels) originate from the apex of the rod. (photo: N-E. Nordh)

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#### Abstract

In willow cultivation, successful establishment is crucial for the development of a willow crop. The overall objective of this study was to assess the effects of preemergence cutting characteristics on the performance of willow during early establishment. In the experiments described in this thesis, cuttings of different size, position on the original rod, quality (i.e. planting damages and storage effects), orientation (i.e. vertical or horizontal planting), and clone were planted in the field or in boxes in an outdoor enclosure. Effects of weed competition and nitrogen fertilization were tested in a bucket experiment, and long-term effects of cutting characteristics on development and growth were evaluated in a field experiment harvested twice between 2008 and 2015.

Cutting characteristics had a significant influence on the early establishment of willow. Cutting size had the most apparent influence, with performance generally increasing with increased size. There was a tendency for this effect to level off beyond a certain size. Cuttings sprouted earlier if derived from the apex, and the majority of the shoots on horizontally planted cuttings originated from the apical part. Cutting damage caused by storage or machine planting on compacted soil resulted in decreased performance and increased variation. Cuttings planted on compacted soil had higher probability of being damaged or landing on the soil surface instead of in the soil. Vertically planted cuttings were generally preferable to horizontally planted cuttings, especially when considering the amount of planting material needed. If planted horizontally, the depth should not exceed 5 cm. Weed competition resulted in a considerable decrease in performance if weeds sprouted before the willow had reached sufficient size. Nitrogen fertilization was likely to be of more use to the weeds than to the willow. In the long-term experiment, stool weight increased with cutting weight and early plant size at both harvests, indicating that the initial size hierarchy was maintained during the entire experiment. The performance responses in the experiments varied depending on clone.

*Keywords:* competition, cutting damage, cutting position, cutting size, nitrogen, planting depth, planting orientation, Salix, short rotation forestry, weeds

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# Dedication

To my family

They say a little knowledge is a dangerous thing, but it's not one half so bad as a lot of ignorance.

Terry Pratchett

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# List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Verwijst T., Lundkvist A., Edelfeldt S., Forkman J. & Nordh N-E. (2012). Effects of clone and cutting traits on shoot emergence and early growth of willow. *Biomass & Bioenergy*, 37: 257–64.
- II Edelfeldt S., Verwijst T., Lundkvist A. & Forkman J. (2013). Effects of mechanical planting on establishment and early growth of willow. *Biomass* & *Bioenergy*, 55: 34–242.
- III Edelfeldt S., Lundkvist A., Forkman J. & Verwijst T. (2014). Effects of Cutting Length, Orientation and Planting Depth on Early Willow Shoot Establishment. *Bioenergy Research*, 8 (2): 796-806, DOI: 10.1007/s12155-014-9560-3.
- IV Edelfeldt S., Lundkvist A., Forkman J. & Verwijst T. Establishment and early growth of willow at different levels of weed competition and nitrogen. (Manuscript).

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The contributions of Stina Edelfeldt to the papers included in this thesis were as follows:

- I Evaluated the data and wrote part of the manuscript.
- II Planned the study in collaboration with the co-authors, performed part of the experimental work, evaluated the data, and wrote the main part of the manuscript.
- III Planned the study in collaboration with the co-authors, performed part of the experimental work, evaluated the data, and wrote the main part of the manuscript.
- IV Planned the study in collaboration with the co-authors, performed the experimental work, evaluated the data, and wrote the main part of the manuscript.

# 1 Willow in Sweden

### 1.1 Renewable energy

Climate change is one of the major environmental problems we face today, and reduction in greenhouse gases needs to be taken into consideration when selecting future energy sources. Renewable energy is a sustainable and carbon dioxide-neutral source of heat and power. According to the EU's renewable energy directive, by 2020 20% of all energy consumption should be from renewable resources. The Swedish government has increased this policy target for Sweden to 50%. Furthermore, by 2020 greenhouse gas emissions in Sweden should be reduced by 40% and at least 10% renewable energy should be used in the transport sector. By 2050, the target for Sweden is to be an emissions-neutral country (Regeringskansliet, 2014).

To reach these targets, dedicated bioenergy crops are being planted to replace the dependency on fossil fuels such as oil. Willow (*Salix* sp) grown in short rotation coppice on arable land is one of these bioenergy crops and the one most commonly used in Sweden.

### 1.2 Willow as an energy crop

Willow is very suitable for use as an energy crop. To begin with, the input:output energy exchange is high at between 10–20:1 (Börjesson & Tufvesson, 2011). Willow is a fast-growing species, a property that is essential for a biomass-producing crop. Furthermore, it is easily propagated by using clones and cuttings (normally unrooted) that are derived from one-year-old shoots. Hybridization and development of new clones in willow is fairly easily accomplished, which facilitates breeding. Compared to many other specialized bioenergy crops, willow has a long growth period, which reduces the need for weed control and re-planting compared to annual and short-term perennial

alternatives. Weed control is normally needed only in the establishment year (Hollsten *et al.*, 2012) because after that time the willow will have grown large enough to tolerate and suppress weeds. Willow also regrows from the harvested stump resulting in a number of harvests from the same planting.

Willow has environmental and other alternative uses. It can be used as a vegetation filter at treatment plants to further reduce nitrogen and phosphorous in wastewater (Perttu & Kowalik, 1997; Börjesson & Berndes, 2006), for phytoremediation of heavy metals in contaminated soils (Dimitriou *et al.*, 2006), and for cleaning polluted drainage water from agricultural land (Elowson, 1999). These practices can be used in parallel with biomass production making the plantation a multi-purpose system. These environmental benefits might be an economic necessity for the further development and deployment of commercial willow cultivation (Volk *et al.*, 2004). Other uses of willow could be as wind, sound, and snow protection or to increase biodiversity on a landscape level.

#### 1.3 Willow cultivation in Sweden

Willow short rotation coppice is a perennial cropping system with an expected life span of about 20–30 years. In Sweden it is mainly grown on arable land with about 13,000 cuttings planted per hectare (Hollsten *et al.*, 2012). Attempts to grow willow on less fertile lands have resulted in yields that are too low to be economically viable. In 2013 there were about 10,300 ha of short rotation coppice willow being cultivated in Sweden (Jordbruksverket, 2015).

Preparation of a willow plantation starts in autumn by applying a broadspectrum systemic herbicide like Roundup (glyphosate) followed by ploughing a few weeks later. Planting takes place in spring, with the field harrowed as short a time before planting as possible. Commercial willow planting in Sweden is commonly performed by specialized planting machines (Step Planter or Woodpecker 601) (Fig. 1) using one-year-old shoots (rods) of about 2 m in length that are pushed vertically into the soil and cut at regular intervals a few centimetres above the soil surface to produce about 20 cm long cuttings (Verwijst *et al.*, 2013). This provides the cutting with good soil contact and minimizes the risk of drying out (Hollsten *et al.*, 2012). This practice has decreased establishment costs for short rotation willow coppice substantially during the initial phase of commercialization in Sweden (Nordh, 2005).

Alternative planting methods to the normal vertical planting are being tried. Billets are cuttings chopped into short lengths that are planted by dropping them into the bottom of a shallow trench (McCracken *et al.*, 2010; Gro & Culshaw, 2001). These could potentially be planted with a sugar cane planter (Gro & Culshaw, 2001). Another alternative is using a lay-flat system in which longer cuttings or rods are placed horizontally into prepared furrows of different depths (Gro & Culshaw, 2001, Lowthe-Thomas *et al.*, 2010). None of these alternative methods are currently used commercially in Sweden.

Chemical weed control in the form of a suitable soil-applied herbicide like Bacara (diflufenikan + flurtamon) should be applied as soon as possible after planting and before the cuttings have started to sprout (Hollsten *et al.*, 2012). Later during the establishment year, mechanized measures might be required to keep the weeds under control (Hollsten *et al.*, 2012). Weed control after the establishment year is normally only necessary if the establishment was not successful.

Fertilization might be required to achieve a sufficiently large crop yield. It is usually nitrogen that needs to be added (Hollsten *et al.*, 2012). This can be applied in the form of commercial fertilizer or treated sewage sludge from a local waste treatment plant. The latter is commonly applied to plantations in Sweden (Hollsten *et al.*, 2012). Commercial mineral fertilizers can also be applied, but the use of these fertilizers can be quite expensive in relation to the expected increase in yield, and the effect varies with willow clone and site. In a study by Aronsson *et al.* (2014), a positive net effect of moderate fertilizing was found for modern-bred willow varieties, but not for older varieties. Furthermore, the response to fertilizing varied considerably between sites. It is not recommended to use fertilization during the establishment year because this might increase weed growth rather than willow growth (Balasus *et al.*, 2012).

An earlier common practice was to cut back the first year's growth in order to facilitate fertilization and additional weeding during the second growth season (Verwijst *et al.*, 2013; Albertsson *et al.*, 2014a). However, because several studies have indicated that this practice has no or negative results on yield (Albertsson *et al.*, 2014b; Verwijst & Nordh, 2010; Verwijst & Volk, 2002), cutting back in Sweden has declined and is rarely used in current commercial practice.

The willow plantation is harvested every 3–4 years depending on the biomass production rate, which in turn largely depends on climate and soil as well as on the type of clone. The harvesting is usually done in winter or early spring when the ground is still frozen using a harvester that immediately cuts the willow shoots into wooden chips (direct-chip harvesting). There are alternative harvesting methods available, including one that cuts entire shoots and one that cuts and bundles the shoots into bales (Baky *et al.*, 2010). The harvested material is mainly driven to a district heating plant and burned for

heat. There are some trials for using willow to produce gas, but so far this has not been put into commercial use.

After harvesting, new willow shoots regrow from the remaining stump. If necessary, weed control is applied in early summer. This most often happens after the first rotation if the establishment was not successful.

The expected lifespan of a plantation is 20–30 years. In early summer after the last harvest, the plantation is sprayed with the herbicides Roundup (glyphosate) and MCPA (4-chloro-2-methylphenol) that kill the stools. In summer, the stumps are cut using a mulcher that cuts shoots and stumps above ground. The soil is then harrowed before sowing. The first ploughing is performed 2–3 years after the termination of the plantation.



Figure 1. Planting willow with a Woodpecker 601. Photo: Nils-Erik Nordh.

# 2 Willow planting

#### 2.1 Establishment

Various willow cultivation guides and manuals stress that a successful early establishment in commercial willow plantations is of major importance to the further development of the stand (Hollsten *et al.*, 2012; Caslin *et al.*, 2010; Gustafsson *et al.*, 2007) Establishment is considered the most important element in achieving a long-term high yield in the stand (Hollsten *et al.*, 2012), and a failed establishment often results in a failed plantation.

A good establishment can be characterized by large shoots that have higher survival expectancy and higher expected biomass production capacity (Rosenqvist, 1997), a large number of shoots that cover the ground quickly thereby reducing weeds by competition, and low variability in shoot size per unit area, which reduces the risk of developing size hierarchies and gaps within the stand.

If weeding procedures have been successfully performed during the establishment, there is initially little competition in willow stands. However, as the plantation approaches canopy closure, which implies a leaf area index (LAI) above six (Lindroth *et al.*, 1994; Merilo *et al.*, 2006) and rarely happens during the establishment year (Verwijst *et al.*, 2013), the plants eventually start to compete with each other and self-thinning processes begin to take effect. This intraspecific competition in willow plantations has consequences that need to be addressed to ensure an even and successful crop. Self-thinning reduces the number of shoots per stool (Willebrand & Verwijst, 1993) and in theory creates an even canopy. However, if early variations in plant size develop at establishment, either caused by small-scale differences in the environment or by differences in cutting quality, they will likely enlarge over time and lead to size hierarchies, mortality, and gaps in the stand (Nordh, 2005; Verwijst, 1996a). These size hierarchies are preserved in the remaining stumps

and in below-ground roots during consecutive harvests resulting in considerable production losses (Verwijst, 1996a, Verwijst, 1996b). Likewise, an initial establishment that results in high survival and vigorous growth is positively related to yield levels during consecutive harvests (Verwijst *et al.*, 2010).

Weed control is essential during the establishment year (Hollsten *et al.*, 2012; Caslin et al., 2010; Gustafsson et al., 2007). If weeds are not managed, the risk of plantation failure increases considerably. Helby et al. (2006) reported in a survey that 29% of the interviewed farmers stated that the primary reason for termination or reduction of plantations was failed growth due to weeds, and 14% stated weeds as the second-most important reason. Because planting density in willow plantations is relatively low, about 1.3 plants m<sup>-2</sup>, the risk of weed infestation is very high. Willow is sensitive to competition during its establishment and is considered to be a poor competitor (Balasus et al., 2012; Albertsson et al., 2014a, Albertsson et al., 2014b; Labrecque et al., 1994; Clav & Dixon, 1995; Sage, 1999). Weeds have been found to reduce willow growth by 46% to 96% (Clay & Dixon, 1997; Balasus et al., 2012; Albertsson et al., 2014b) and survival by 2.7%-37.4% (Albertsson et al., 2014b) in the establishment year. These effects, like the size hierarchies, are preserved through consecutive harvests. In a trial by Clay and Dixon (1997), there were still 40%–70% yield reductions after three years of regrowth despite plots being kept weed free after the cutback the first year.

If the establishment during the first year has been successful, further weed control measures normally do not need to be taken during the rest of the plantations' life cycle (Sage, 1999), not even directly after harvest (Gustafsson *et al.*, 2007). Willow grows fast, and has by this time reached sufficient canopy closure to tolerate and suppress weeds. In addition, the rapid development of an extensive root system further suppresses weed infestation (Gustafsson *et al.*, 2007).

Fertilization of the willow plantation is not necessary during the establishment year. Because willow is regarded as an efficient nitrogen user (Shield *et al.*, 2008), the nutrients stored in the cuttings and in the soil are usually adequate for establishment (Caslin *et al.*, 2010). Fertilization might instead give weeds an advantage by increasing their competitive ability and relative growth rate. Balasus *et al.* (2012) noted in their two-year study that while fertilization did not significantly affect the willow yield, weed biomass growth was increased by 46% in the first year. This suggests that weeds were more responsive to early fertilization than willow.

Establishment constitutes an important part of the total costs of commercial willow cultivation. In a report by Hauk *et al.* (2014), establishment, along with

land rent and harvesting and chipping, were found to be the largest contributors to the overall costs in short rotation coppice cultivation. In Sweden, establishment was estimated to be about 15% of the total production cost of a willow stand (Rosenqvist *et al.*, 2013).

One reason for the expensive establishment costs is the amount of planting material needed along with the specialized machinery used for planting. Heaton *et al.* (1999) estimated that planting material accounted for 47% of the establishment costs in the upland of Wales. To gain an economically viable cultivation, an establishment method should be chosen that uses as little planting material as possible while still resulting in successful establishment and a viable crop.

#### 2.2 Pre-emergence variation

The importance of pre-emergence traits has been noted in several agricultural crops. The size of the seed, propagule, or cutting and the variation in size might be determinants for the vitality and survival of the growing plant and consequently the developing crop. Benjamin and Hardwick (1986) presented a quantitative framework for analysing the physiological sources of variability in plant stands. They suggested that the yield and size of a plant can be predicted from plant size at the start of its growth in addition to growth rate and duration of growth. In addition, they distinguished between pre- and post-seedling emergence as two physiological periods in which weight accumulation occurs. Their framework has been used in several studies on plant competition, especially to explain the development of size hierarchies (Park *et al.*, 2003).

The impact of planting material size and size variation has been shown in several species. For example, Souza *et al.* (2014) showed that smaller seeds of *Copaifera langsdorffii* Desf. (*Fabaceae*) had a higher germination percentage and faster germination and that they allocated more resources to roots compared to larger seeds, while larger seeds resulted in more vigorous (larger) seedlings. Njoku *et al.* (2010) found that the longest (4-node) cutting of sweet potato was superior to smaller sizes in terms of establishment and yield. Studies on other tree species also show cutting size dependencies. Ahmad *et al.* (2014) compared three sizes (15, 20, and 25 cm) of olive cuttings and found that the 25 cm cuttings had the maximum number of shoots and roots per plant, the maximum shoot length, and the minimum root length (lower shoot:root ratio). OuYang *et al.* (2015) found that cutting length and diameter of Norway spruce (*Picea abies* (L.) Karst.) significantly affected rooting efficiency with cuttings 0.3–0.4 cm in diameter and 9–12 cm in length having the highest efficiency.

#### 2.3 Cutting pre-emergence characteristics and planting methods

Commercial willow is most commonly planted using cuttings of about 20 cm in length with a minimum diameter of 8–9 mm (Dawson, 2007) that are pushed vertically into the soil with a few cm protruding from the ground. This practice provides the cutting with good soil contact thereby minimizing the risk of drying out (Gustafsson *et al.*, 2007) as well as providing early exposure to direct sunlight enabling early photosynthesis. The planted cuttings have an initial reserve of nutrients that is mobilized during sprouting (Brereton *et al.*, 2013) and is an important part of the willow plant's nutrient requirements during the early establishment period. The quality and characteristics of these cuttings therefore have a large impact on the survival and viability of the crop, particularly during the establishment phase.

Willow growth and survival are known to generally increase with cutting length and diameter (Burgess *et al.*, 1990; Rossi, 1999; Shield *et al.*, 2008; Verwijst *et al.* 2012; Friedrich *et al.*, 2014). These positive effects of cutting size are generally attributed to the size of the carbohydrate pool available for allocation to roots and shoots (Carpenter *et al.*, 2008). Larger cuttings, in particular longer ones, might also be better able to withstand soil desiccation (Gage & Cooper, 2004).

These effects might only persist up to a certain size. Burgess *et al.* (1990) noted that use of *Salix alba* cuttings greater than 1.3 to 1.9 cm in diameter and 22.9 cm in length did not result in any significant increase in growth, and Rossi (1999) concluded that the differences in cutting length that are relevant for establishment in practice are between lengths of 10 cm and 20 cm. Vigl and Rewald (2014) found that 40 cm cuttings produced more total biomass than 20 cm cuttings producing about 66% more fine roots than 20 cm cuttings. Leaf, stem, and coarse root biomass production did not differ between lengths. This also affected the shoot:root allocation with 40 cm cuttings having more roots compared to shoots than 20 cm cuttings and possibly gaining a future rooting and nutrient uptake advantage.

The position on the shoot from which the cutting originated might have importance for the plant size. Flowering usually occurs in the apical part of the stems, and the number of buds is usually more abundant there compared to more basal parts. The timing of the bud burst and leaf area development is an important determinant of early plant size development (Weih, 2009).

Soil factors are also known to affect early willow performance in terms of survival and early growth (Tahvanainen & Rytkönen, 1999; Stolarski *et al.*, 2009; Alriksson, 1997; Schaff *et al.*, 2003). This is normally attributed to the soil's water-holding capacity and nutrient supply. Furthermore, compacted soil

might result in mechanical impedance of root growth and reduced uptake of water and nutrients (Alakukku & Elonen, 1995; Nambiar & Sands, 1992). Machines used for planting and harvesting might exert pressure on the soil and cause compaction, which is mainly a problem in the establishment phase (Watts *et al.*, 2005). Negative effects have been found during the first year when the root system is shallow and the roots are young and more susceptible to compaction damage (Souch *et al.*, 2004). Once the root system is well established, these effects have been shown to be relatively small in willow (Souch *et al.*, 2004, Kuzovkina, 2004; Wyatt, 1998). Compaction might also cause problems at planting. If the preparation of the soil is not thorough, the soil might still be compacted, which increases the risk of causing direct damage to the cuttings when they are forcefully pushed into the ground by the planter.

Alternative planting methods to the traditional vertical planting have been considered, such as lay-flat planting and the use of billets. The orientation of the cuttings and planting depth, as well as cutting size, might have effects on establishment success.

In lay-flat planting, longer cuttings or rods are placed horizontally into prepared furrows of different depths (Gro & Culshaw, 2001; Lowthe-Thomas et al., 2010). The longer cuttings and the cover of soil might reduce evapotranspiration and the risk of dehydration. They are also not subjected to the same potential planting damage that can occur in the traditional mechanized vertical planting system and can cause a decrease in performance. Lowthe-Thomas et al. (2010) found that stem diameter, weight, and estimated vield were significantly larger for the lay-flat system after three growing seasons. In addition, the lay-flat planter was able to plant at a faster rate compared to the Step Planter and could use a wider range of material, which could reduce the planting costs. Gro & Culshaw (2001) found in their study that the lay-flat system produced more biomass during the first year and had a higher canopy than traditional planting. However, McCracken et al. (2010) found no differences between lay-flat rods or vertical cuttings, making the overall conclusions uncertain. A disadvantage with the lay-flat system that needs to be taken into account when calculating costs is that it can require as much as three times more planting material as the vertical planting system (McCracken et al., 2010).

Another alternative planting method would be to use billets, which are cuttings chopped into short lengths and planted by being dropped into a shallow trench (McCracken *et al.*, 2010; Gro & Culshaw, 2001). These could potentially be planted by a sugar cane planter, and this has the advantage of not requiring material of the same quality as for the traditionally planted cuttings.

The disadvantage with billets seems to be the survival and vitality of the crop. McCracken *et al.* (2010) noted that some of the planted areas failed, and growth per unit area was generally less from billets after two consecutive three-year harvest cycles.

A disadvantage with both the billet system and lay-flat planting is that they both require the shoot to penetrate a layer of soil before reaching the surface. This not only delays photosynthesis, but could also result in weeds gaining a competitive advantage by giving them the opportunity to sprout before the willow shoot.

When comparing horizontally and vertically planted cuttings of the same size, the effect might be smaller than when comparing lay-flat and billet systems with traditional planting. In two separate studies by Cao *et al.* (2010, 2012), no differences between stem, leaf, or fine root biomass were found between vertically and horizontally planted cuttings of 25 cm length.

Field storage before planting can have an impact on the quality of the cuttings and reduce survival and yield. Planting material is normally in the form of entire shoots, which are considered more resistant to desiccation compared to cuttings (Danfors *et al.*, 1997). Nevertheless, storage in the field might increase the risk of desiccation. Volk *et al.* (2004) showed in their study that a prolonged time of field storage after cold storage might lead to a decrease in survival and growth rate.

When considering cutting quality, it is necessary to consider possible differences between clones. Clone variation might be considerable, especially when comparing yield and resistance abilities (frost, pests, drought, etc.) between older and newer varieties. Differences in biomass production between a wide range of clones have been shown in numerous studies in a number of countries, including greenhouse experiments (Yang *et al.*, 2015), field trials (Larsen *et al.*, 2014; Serapiglia *et al.*, 2013; Mleczek *et al.*, 2010), and commercially growing plantations (Nord-Larsen *et al.*, 2015).

# 3 Overall objectives and specific hypotheses

The overall objective of this thesis was to assess the effects of different preemergence cutting characteristics on the performance of willow in the early establishment phase. Because the success of the establishment often determines the success of the plantation, it is crucial to understand the impact of these characteristics on the growth and survival of willow cuttings and on shoot development. The papers and the long-term field experiment included in this thesis cover a wide range of cutting characteristics and their effect on a number of performance attributes resulting in an overall understanding of the processes involved in the early development of a willow plantation crop.

Performance attributes considered in the specific hypotheses below include cutting survival, bud burst time (only Paper I), biomass production, leaf area (only Papers II–IV), stem-to-leaf ratio (only Paper IV), number of shoots, and maximum shoot height for each cutting.

## 3.1 Paper I

The aim of this study was to assess and quantify the effects of pre-emergence variation in willow cuttings on early performance of shoots. The specific hypotheses tested were that (1) thicker, longer, and heavier cuttings will show a better performance than thinner, shorter, and lighter cuttings, (2) cuttings from the basal part of the shoot will show better performance than cuttings closer to the apex, (3) the performance will be clone specific, and (4) the performance of cuttings will be worse with increased field storage time.

### 3.2 Paper II

The aim of this study was to assess and quantify the effects of machine planting on sprouting and the growth of shoots. The specific hypotheses were that (1) cuttings planted in compacted soil will be more damaged than cuttings planted in non-compacted soil, (2) damaged cuttings will have a lower performance than undamaged cuttings, (3) cuttings planted in compacted soil will have a lower performance than cuttings planted in non-compacted soil, which in turn will have a lower performance than the control, and (4) the relative performance will be dependent on clone and cutting size.

# 3.3 Paper III

The major purpose of the two experiments in this study was to assess and compare the early performance of horizontally planted cuttings of different lengths with the performance of vertically planted cuttings. The relative variation (coefficient of variation) in shoot height was also calculated to assess if cutting length and other factors might underlie a developing size hierarchy in a stand. In the first experiment, we also studied if shoot origin and subsequent performance was affected by apical dominance that is possibly present in the horizontally planted cuttings. In the second experiment we assessed planting depth as an additional factor.

## 3.4 Paper IV

The main objective of this study was to assess the competitive ability by evaluating the performance of willow at different levels of weed pressure and nitrogen levels in the early establishment phase using both a thin- and a broadleaved model weed. Furthermore, the effect of willow on weed performance in terms of biomass and shoot height was assessed.

# 3.5 Field experiment 2008–2015

The objective of this study was to validate the assumption that cutting characteristics underlie the development of an early size hierarchy, and that this early hierarchy may persist over several cutting cycles.

# 4 Materials and methods

### 4.1 Sites

All trials were conducted either in a fenced field (Paper III and Field experiment 2008–2015) or in boxes or pots standing in an outdoor net enclosure (Papers I–IV) located at Ultuna, close to Uppsala, in Sweden (59°48'N, 17°39'E). The soil in the field consisted of heavy clay and was ploughed prior to planting in all field experiments.

## 4.2 Cutting plant material and clones

Plant material for all trials consisted of one-year-old shoots (rods) of about 200 cm in length from a field plantation close to Uppsala harvested in the winter or early spring prior to planting. The rods were placed in a freezer at between  $-4^{\circ}$ C and  $-6^{\circ}$ C. Prior to planting, the rods were taken out of the freezer and a 5 cm long part of the base of each rod was removed to standardize the effects of drying, moulds, and other kinds of storage damage. Cuttings were then derived from the rods either by cutting them manually (Papers I, III, and IV) or with a Wood Pecker 601 (Paper II).

Five different commercial willow clones were used in the trials. Gudrun (*Salix dasyclados*), Jorr (*Salix viminalis*), Olof (*Salix viminalis* × (*Salix schwerinii* × *Salix viminalis*)), Sven (*Salix viminalis* × (*Salix schwerinii* × *Salix viminalis*)), and Tora (*Salix schwerinii* × *Salix viminalis*) were used for the experiments described in Papers I and II and the Field experiment 2008–2015, and Tora was used for Papers III and IV (Table 1).

	Paper I	Paper II	Paper III	Paper IV	Experiment 2008–2015
Cutting characteristics	Length, diameter, position	Weight, damage	Length	Diameter	Weight
Clones	Gudrun, Jorr, Olof, Sven, Tora	Gudrun, Jorr, Olof, Sven, Tora	Tora	Tora	Gudrun, Jorr, Olof, Sven, Tora
Experiment type	Outdoor box experiment	Outdoor box experiment	Field experiment, outdoor box experiment	Outdoor bucket experiment	Field experiment
Planting method	Vertical	Vertical with different soil compaction levels	Horizontal at different depths, vertical	Vertical at different nitrogen levels, weed intensities, and weed types	Vertical
Experiment time	Six weeks	Eight weeks	Seven weeks	Nine to ten weeks	Seven growing seasons

Table 1. Summary of the experiments described in Papers I–IV and Field experiment 2008–2015.

### 4.3 Experimental designs

The experiments described in Papers I (Fig. 2) and II (Fig. 3) were both performed in  $80 \times 80 \times 20$  cm boxes in the net enclosure. Each box was assigned one of five different clones and one of three different cutting lengths (Paper I) or planting regimes (Paper II). Cutting position on the long shoot from which the cutting was derived was also noted. The cuttings were placed in an  $8 \times 8$  pattern in the boxes. The boxes were then arranged randomly in the net enclosure.

Both experiments described in Paper III were randomized complete-block designs. In the field experiment, eight blocks with five treatments (cutting length and orientation) were used (Fig. 4). Each block was divided into six rows with each treatment planted in one (50, 100, and 200 cm vertically planted cuttings) or two (25 cm vertically and horizontally planted cuttings) rows. In the box experiment, seven blocks with twelve treatments (three cutting lengths and four planting depths/orientation levels) were used. Six cuttings originating from the same rod were evenly spaced in each  $35 \times 25 \times 21.5$  cm box.

The experiment described in Paper IV (Figs. 5 and 6) was a completely randomized design using buckets with 30 treatments (willow and weed presence, two model weeds, four planting times for model weeds, and two nitrogen levels). Five cuttings originating from the same rod were evenly spaced in each bucket.

In the Field experiment 2008–2015, cuttings of 20 cm length were used. The experiment was conducted in an arrangement of 32  $(4 \times 8)$  plot squares with  $8 \times 8$  cuttings in each square using 20 cm spaces between cuttings and 40 cm spaces between each square (Fig. 7). Each square was randomly assigned either to be a monoculture or a mixture of two different clones using two replicates for every possible clone combination in the entire experiment. In mixtures, cuttings of different clones were placed in a checkered pattern alternating the two different clones. Edge effects were taken into account by assigning the four rows closest to the edge to separate edge groups and putting all the remaining cuttings into a fifth group (Edge 1-4 and No edge). Two square plots in the arrangement were fillers added to make the design as similar between squares as possible regarding edge effects. These were not included in the analysis. The cuttings were planted by pushing them manually into the ground until about 5 cm protruded from the ground. During the first year, the experiment was weeded regularly during the entire growing season. There was no weeding during the following years.



*Figure 2.* Cuttings planted in 2008 (Paper I). Cuttings on the diagonal (with white labels) originate from the apex of the rod. Photo: Nils-Erik Nordh.



*Figure 3*. Cuttings planted in 2009 (Paper II). Cuttings on the diagonal (upper left corner to lower right corner) originate from the apex of the rod. Photo: Nils-Erik Nordh.



Figure 4. Cuttings planted in eight plots in 2010 (Paper III). Photo: Stina Edelfeldt.



Figure 5. Cuttings planted in pots in 2011 (Paper IV). Photo: Stina Edelfeldt.



Figure 6. Harvest time 2011 (Paper IV). Photo: Stina Edelfeldt.



Figure 7. Cuttings planted in 2008 for the Field experiment 2008–2015. Photo: Nils-Erik Nordh.

#### 4.4 Measurements

All experiments described in the papers were conducted between six and ten weeks during the growing season (May–August) (Table 1). The Field experiment 2008–2015 was harvested at the beginning of 2013 (i.e. after the growing season of 2012) and in the winter of 2014–2015 (i.e. after the growing season of 2014). In addition, non-destructive measurements were taken after the growing season each year.

The cutting characteristics assessed in the trials were cutting length (Papers I and III), cutting diameter (Papers I and IV), cutting weight (Paper II and Field experiment 2008–2015), cutting position on original shoot from which the cutting was derived (Paper I), storage time of shoots in the field before planting (Paper I), planting damage on the cutting (Paper II), cutting landing on or in the ground at planting (Paper II), planting orientation (i.e. vertical or horizontal planting) (Paper III), and planting depth (Paper III) (Table 1). In Papers I and II, different clones were tested to determine if the effects of the characteristics were assessed both in an environment where competition between plants was assumed to be negligible (Papers I–III) and in an environment where model weeds were densely planted with the willow (Paper IV). Furthermore, in Paper IV, the effects of two different nitrogen concentrations were assessed.

The following performance measurements were taken in all trials for each cutting: survival, shoot biomass as dry weight (Papers I–IV) or fresh weight (Field experiment 2008–2015), height of the highest shoot, and number of shoots. Furthermore, leaf area was measured for the experiment described in Papers II–IV, bud burst for Paper I, planting damage on cutting for Paper II, shoot emergence and distance to cutting base for Paper III, and leaf-to-stem ratio for Paper IV.

All dry weights were measured after drying the plant material at 105°C for 24–48 h depending on the analysis being performed.

#### 4.5 Statistical analyses

In all papers, linear models with fixed effects of treatments were fitted using the SAS procedure MIXED. Allometric relations between leaf area and shoot length were determined using the NLIN procedure (Papers III and IV). The GLIMMIX procedure was used to analyse bud burst as an ordered multinomial variable (Paper I). Independence between the number of damaged and normal cuttings found in and on the soil was tested using the FREQ procedure (Paper II). Probability graphs for orientation of cutting (in or on the soil) and for major cutting damage were made using the GENMOD procedure with binomial distributions. Factors and possible interactions between factors were tested in all analyses and removed from the model if not significant. Random effects, if any, were accounted for.

In the Field experiment 2008–2015, effects on biomass production at harvest regardless of clone mixture type were analysed using clone, edge group, cutting fresh weight, and shoot height in 2008 as explanatory variables and stool as the observational unit. A linear model including random effects of clones within plot squares was fitted using the MIXED procedure in SAS 9.3 (Cary, 2011). Because biomass production showed increasing residual dispersal with increasing values, a logarithmic transformation was used.

All statistical analyses were performed in SAS 9.1-9.3 at a significance level of 0.05.

# 5 Results and discussion

#### 5.1 Effects of cutting size on performance

Cutting size had a considerable effect on the majority of the examined performance attributes. Increases in length (Papers I and III) or diameter (Papers I and IV) led to a higher biomass production and a larger leaf area, with the exception of cuttings at a planting depth of 17 cm. The effect was the same for weight (Paper II) with one exception (clone Olof as a control for biomass production). For maximum shoot height and number of shoots, there were a few exceptions for some combinations of treatment factors, but in the majority of the treatments an increase in size attribute led to an increase in height or number. Cutting diameter was not found to be significant to stem-to-leaf ratio (Paper IV). The probability of early bud burst decreased with diameter (at a given cutting position on original rod) but did not depend on cutting length, and this indicated that the amount of stored resources per cutting did not influence the onset of shoot emergence from buds.

In the studies of Burgess *et al.* (1990) and Rossi (1999), an increase in biomass production with cutting length showed a tendency to decrease and become insignificant at higher lengths. This effect might have been present in our study in Paper I because the difference between lengths often was larger between 12 cm and 18 cm cuttings than between 18 cm and 24 cm cuttings. However, in the horizontally planted cuttings (Paper III) this effect was only evident in maximum shoot height with no difference between 100 cm and 200 cm horizontally planted cuttings. This was probably a result related to planting orientation as well as length, with shoots emerging from the soil at several locations instead of just one. In vertically planted cuttings of smaller size (7–21 cm), cutting length did not show any pattern of decreasing importance because the relative size of the difference between cutting lengths varied between performance attributes.

Despite the continued increase in performance with cutting length, there might still be reason to limit the size of the cuttings. Cutting material is a considerable cost in the establishment of a new plantation (Heaton *et al.*, 1999), and an increase in cutting length needs to be followed by a similar increase in production for the increase in length to be economically viable. When comparing performance per length unit of cutting material (2 m rod), longer horizontally planted cuttings produced similar or less biomass and fewer shoots than shorter cuttings (Paper III). This could lead to considerable costs if using the lay-flat system because this planting method might use up to three times as much material as conventional planting (McCracken *et al.*, 2010). In smaller cuttings, there was no difference between 21 cm and 14 cm long cuttings when comparing performance per length unit. The 7 cm cutting still produced less, which was probably due to the overall considerably lower performance of the very small cuttings.

In addition to the direct effect on performance attributes, cutting size might also have other effects. When planting on compacted soil (Paper II), the probability of cuttings landing on the soil surface instead of being put into it, and the probability of major damage to the cuttings, decreased with increasing diameter, especially for cuttings with a diameter below about 11 mm.

Survival was very high in our experiments, probably due to the short experimental periods and regular irrigation. However, in the box experiment of Paper III, where very small cutting sizes were used, there were considerable effects of cutting size on survival. Of the vertically planted cuttings, 45% of the 7 cm cuttings died compared to only 5% of the 14 cm cuttings and 0% of the 21 cm cuttings. For the 7 cm cuttings, this might have been a desiccation effect because the relative evaporation was probably very high in the small cuttings due to the high surface-to-volume ratio. The larger cuttings not only had larger nutrient reserves, but they also reached further down into the ground and probably had a larger root system making them less susceptible to desiccation. Larger root systems on longer cuttings were observed by Vigl & Rewald (2014) and could be the reason that longer cuttings have a relatively higher ability to withstand soil desiccation (Gage & Cooper, 2004). Another reason for the decreased performance in small cuttings could be the presence of only one or two nodes. In addition to the low survival, the 7 cm cuttings had significantly lower performance compared to longer cuttings, suggesting that there is a limit in length below which cuttings should not be used.

# 5.2 Effects of position on cutting performance

The relative position of the cutting on the long shoot (rod) from which the cutting is derived and the relative position on the cutting itself was of importance for performance. At an average diameter, cuttings originating from a position closer to the apex of the rod had higher biomass production and higher number of shoots for all clones except Gudrun (Paper I). This effect was likely related to phenology differences because cuttings from a position relatively closer to the apex had an earlier bud burst (Fig. 2). However, the effect of position was relatively small compared to the effects of length and diameter. Nevertheless, cutting position on the rod might be considered when planting so as to avoid size hierarchy effects.



*Figure 8*. Horizontally planted 25, 50, 100, and 200 cm cuttings with shoots (only half of the 200 cm cutting shown) (Paper III). The apex of the cuttings is to the right. Photo: Stina Edelfeldt.

When long cuttings are planted horizontally, the emergence position of the shoots is of importance, especially with regard to achieving an even establishment. In the field experiment described in Paper III, the majority of the shoots sprouted close to the apex of the cutting regardless of cutting length (Fig. 8). Performance attributes were considerably higher close to the apex,

especially biomass production and number of shoots. Apical dominance was present in the cuttings even if fragmented, and the part of the cutting closest to the tip on the original rod acted as a new tip. This could be of major importance in lay-flat planting where the apical dominance, especially in longer fragments, might cause gaps that lead to size hierarchies or give weeds an opportunity to gain a foothold in the stand.

## 5.3 Effects of quality on cutting performance

The quality of the cutting material, i.e. the level of damage and decreased vitality due to external influence after harvesting the rods, had an impact on the performance of the cuttings. Field storage of rods had a negative effect on cutting biomass production (Paper I). Cuttings made from rods stored outside for two weeks had a 21% reduction in biomass productivity and had a higher coefficient of variation than cuttings from rods that had not been stored. There was no effect on the number of shoots or maximum shoot height. Apparently, there was quality loss despite the planting material being stored as long rods that should be more resistant to desiccation (Danfors *et al.*, 1997). The desiccation process might have led to a loss of primary shoots that sprouted before planting causing resource loss and forcing secondary buds to develop and resulting in a delay in biomass accumulation.

Damages from the machine planting procedure had an evident effect on cutting performance (Paper II). Biomass production was 26%–73% lower in cuttings with major damages compared to cuttings without or with very minor damages depending on clone. For maximum shoot height and leaf area, the majority of the clones showed less production in damaged cuttings. The effect on the number of shoots was less, and only the clone Olof showed a difference between damaged and normal cuttings. Furthermore, variation was generally considerably higher in the damaged cuttings for all performance attributes, although this effect might to some extent be influenced by the lower number of damaged cuttings was probably due to damaged buds, desiccation effects, and direct damages on the cuttings causing stored carbohydrates and other nutrients to become unavailable to the cutting.

Damages on cuttings differed depending on soil compaction level and cutting weight (Paper II). The probability of cuttings landing on the soil surface and the probability of major damages were dependent on soil compaction level. No cuttings landed on the surface of non-compacted soil, in comparison with 6%–29% of the cuttings on compacted soil (depending on clone). Major damages were found in 0%–6% of the cuttings planted in non-compacted soil,

while 6%–31% of the cuttings planted in compacted soil were damaged (depending on clone). Cuttings planted in compacted soil produced less biomass and had a smaller leaf area than the control (manually cut) for all clones except Gudrun, and they produced less biomass than cuttings from non-compacted soil for Olof and Jorr. Tora and Jorr produced a higher leaf area in non-compacted soil compared to compacted soil. Differences between soil compaction levels generally decreased with the weight of the cuttings and became insignificant at higher weights for some comparisons. These differences between cutting planting procedures can be attributed to damages on cuttings, especially because there was a tendency for the effects to decrease with weight. As noted in section 5.1, damages on cuttings decreased with diameter.

Reduction in cutting quality, in combination with a higher variation in performance, could cause considerable variation in the field in addition to a general decrease in early production, leading to facilitated weed infestation, gaps, and size hierarchies in the stand (Nordh, 2005; Verwijst, 1996a).

### 5.4 Effects of planting orientation and depth on cutting performance

Lay-flat planting using long willow rods and planting using small billets are alternatives to traditional vertical planting with 20 cm cuttings. Not considering possible differences in cutting quality and spacing caused by the actual machine planting, these planting methods basically differ from traditional planting in three aspects: cutting length, planting orientation, and planting depth. Effects of cutting length have already been discussed in section 5.1.

Shoots from vertically planted cuttings in the field experiment that used longer cuttings (simulating lay-flat planting) sprouted before any on the horizontally planted cuttings reached the surface (Paper III). The performance (biomass production, number of shoots, and maximum shoot height) of individual 25 cm cuttings planted in the field was closer to the 50 cm horizontally planted cuttings than the 25 cm horizontally planted cuttings. When analysing performance per length unit (2 m rod) in the field experiment, vertically planted cuttings produced more biomass and shoots than horizontally planted cuttings having the highest performance. In some cases the performance was similar between vertically planted cuttings and cuttings planted at 5 cm depth. There was a similar pattern for biomass production and leaf area when analysing per length unit (2 m rod). For number of shoots, only

cuttings from the planting depth of 17 cm showed a significantly lower performance compared to the other treatments. Furthermore, cuttings planted at 17 cm depth had an overall lower survival than the other treatments, with the exception of 7 cm vertically planted cuttings, suggesting that 17 cm was deeper than the optimal planting depth for willow.

The prolonged time for shoot emergence was clearly a disadvantage that the horizontally planted cuttings could not overcome during the experimental period. Cuttings planted at a greater depth were more likely to run out of carbohydrates before they could be replenished by photosynthesis once the shoot reached the surface. This effect should be especially evident in smaller cuttings with more limited reserves. It is possible that the reduced risk of desiccation might have an effect later in the development of the stand, but for the early establishment, desiccation effects were probably only present in the small 7 cm cuttings.

The combined effects of depth and length were considerable. Cuttings of 7 cm planted at 17 cm depth produced 1/50 of the biomass of vertically planted 21 cm cuttings and 1/30 of the biomass of 21 cm cuttings planted at 5 cm depth. When comparing cuttings of the same size, the horizontally planted cuttings produced less biomass, suggesting that the positive effect of lay-flat planting lies in the larger cuttings. Similarly, the reduced performance and survival of smaller cuttings suggest shortcomings with the billet system, which is in accordance with the study by McCracken *et al.* (2010).

In general, vertically planted cuttings seemed to have better performance than horizontally planted cuttings, and if planted horizontally, a shallow depth of about 5 cm is to be preferred.

# 5.5 Effects of weed competition and nitrogen fertilization on cutting performance

Competition had a significant effect on early willow development. Willow with weeds planted 15 days after the willow produced less biomass (27%–35%), smaller leaf area (17%–21%), and had a lower maximum shoot height (10%–14%) compared to willow monocultures and treatments in which weeds were planted later among the willow (Paper IV). However, when weeds were planted 26 or 30 days after the willow, there was no effect on willow performance compared to willow planted in monoculture. By this time, at the very high willow planting density of the experiment, the willow had grown large enough and gained enough canopy closure to withstand and suppress weed competition. The willow LAI estimated around this time suggests that there was only minor competition for light, which shows that a full canopy

closure might not be needed for willow to gain a sufficient competitive advantage. This supports the practice of not weeding after the first establishment year (Gustafsson *et al.*, 2007) even if canopy closure is rarely reached during this time (Verwijst *et al.*, 2013). Despite being sensitive to weed competition (Balasus *et al.*, 2012; Albertsson *et al.*, 2014a, Albertsson *et al.*, 2014b; Labrecque *et al.*, 1994; Clay & Dixon, 1995; Sage, 1999), willow had a large effect on weed growth, reducing weed biomass by 57%–95% depending on weed planting time and weed type.

Weed type seems to be less important for early willow competition because there were no differences between the model weeds white mustard and spring barley in any performance analysis (Paper IV). Because all plants are dependent on light, and because both model weeds were fast-growing annuals, the supressing effect might have been the same regardless of growth pattern. Furthermore, there might still be an indirect effect of weed type because willow had a larger impact on biomass and height production in white mustard, which might lead to possible future differences in model weed competitive ability.

There was probably some minor competition for nutrients from weeds in treatments with weeds planted after 15 days because the weeds in this treatment showed a small positive response to nitrogen fertilization while willow did not (Paper IV). Reversed conditions were present in treatments with weeds planted 26 and 30 days after the willow, where willow showed a small but positive response to fertilization while weeds did not. The lack of response from weeds might have been influenced by the shorter development period and, consequently, less biomass accumulation and nutrient requirement of the later-planted weeds. Regardless of the level of weed competition, the effect of fertilization was minor, which is probably due to the stored reserves in the cutting. These results are in accordance with Sage (1999), who concluded that competition during the first year was mainly for light rather than nutrients, and with Fircks *et al.* (2001) who found little differences in leaf area and dry weight of *Salix dasyclados* between nutrient regimes during the first two months.

## 5.6 Long-term effects of cutting characteristics

The influence of cutting characteristics and early advantages in size had a longterm impact. In the Field experiment 2008–2015, biomass production increased significantly with cutting weight at both harvests (Figs. 9 and 10). Furthermore, the height of the cutting shoots at the end of 2008 had a significant influence on both harvests even when the effect of cutting diameter



*Figure 9.* Stool (aboveground cutting shoots) fresh weight at harvest after growing season 2012. Different letters indicate significant differences between treatments. 'Edge 1-4' show effects on the first four rows closest to the edge of the plantation and 'Not edge' show effects on the remaining cuttings.

was taken into account. This effect varied depending on how close the cuttings were to the edge of the plantation. These results suggest that a size hierarchy dependent on both initial cuttings size and initial plant size after the first growing season had developed in the canopy and that the effects of early competition persisted over the two harvests. In addition, the high competition in the very dense canopy (21.5 cuttings m<sup>-2</sup>) resulted in a total of 182 (9%) dead stools in 2013, which increased to 773 (38%) in 2014, further suggesting an increase in competition effects over multiple harvests.

Edge effects (Zavitkovski, 1981) were also present in the Field experiment 2008–2015. Cuttings closer to the edge showed better performance than cuttings further into the canopy, especially the row of cuttings at the very edge of the stand. Because this effect generally increased between harvests, it is



*Figure 10.* Stool (aboveground cutting shoots) fresh weight at harvest after growing season 2014. Different letters indicate significant differences between treatments. 'Edge 1-4' show effects on the first four rows closest to the edge of the plantation and 'Not edge' show effects on the remaining cuttings.

likely that the advantage of growing close to the edge will increase with time causing a considerable difference between stools at the edge and those within the canopy at consecutive harvests.

There was generally higher variation in biomass production per stool at harvest 2014 than at harvest 2013. This is probably because the self-thinning processes (Willebrand & Verwijst, 1993) had not yet reduced the number of shoots per stool. At the 2013 harvest, there were only one or two shoots on the living stools, while about 50% of the stools had more than 2 shoots at harvest 2014 (up to 17 shoots).

The above findings validate the assumption that cutting characteristics underlie the development of an early size hierarchy, and that this early hierarchy may persist over several cutting cycles, as previously shown in other studies (Nordh, 2005; Verwijst, 1996a; Verwijst, 1996b).

### 5.7 Effects on cutting performance depending on clone

There were effects on performance depending on clone. In the experiment described in Paper I, there were differences between clones for biomass production, number of shoots per cutting, maximum shoot height, and bud burst development. In contrast with all other clones, Gudrun did not show any dependency on cutting position on the original rod and showed no differences in the number of shoots for different length cuttings. Only maximum shoot height for Olof increased significantly with cutting position. Jorr and Tora started their bud burst earlier than Gudrun and Sven, which in turn started earlier than Olof.

The probability of cuttings landing on the soil surface instead of being put into it or being damaged when planted in compacted soil varied between clones (Paper II). For example, a high percentage of Jorr cuttings (29%) landed on compacted soil in comparison with only 6% of Tora cuttings. Olof had a very high number of cuttings showing major damage (31%), while only 8% of Gudrun cuttings were damaged. There were also differences between clones in their dependency on diameter for the probability of landing on soil or being damaged. Olof in particular showed a different response to the probability of landing on soil, with the probability decreasing linearly with diameter instead of exponentially like the other clones.

The effect of cutting damage on performance differed between clones in terms of biomass production, leaf area, maximum shoot height, and number of shoots (Paper II). In contrast with the other clones, there were no differences between normal and damaged cuttings for Jorr and Sven in leaf area, for Gudrun in maximum shoot height, or for Olof in number of shoots. Furthermore, clone response differed between planting procedures. Gudrun, for example, showed no difference between planting procedures in terms of biomass production.

Long-term performance differed between clones (Figs. 9 and 10). Although the effect of diameter was similar for all of the clones, biomass production per stool and edge effects were not. Tora and Gudrun generally produced the largest amount of biomass and showed the largest edge effect, while 'Edge 3' in Jorr surprisingly produced less biomass than edge rows closer to the edge.

# 6 Conclusions

Cutting characteristics had a large influence on the early establishment of willow. Cutting size in terms of length, diameter, and weight had the most apparent influence and were significant in all studies for almost all performance attributes and most treatments. Performance generally increased with cutting size. There was a tendency of this effect to decrease beyond a certain size, even if this tendency was not present for the horizontally planted cuttings except for maximum shoot height. Large cuttings were less susceptible to damage and landing on soil when planted in compacted soil. Survival was low in very small cuttings (7 cm), which was probably due to desiccation effects or having only a few viable nodes.

The main effect of position on the original rod from which the cuttings were obtained was in emergence of shoots. Cuttings sprouted earlier if derived from the apex, and the majority of the shoots on horizontally planted cuttings were from the apex. However, in traditionally planted cuttings, this effect was countered by the increase in performance with diameter, which decreased towards the apex. Commercially, the largest impact of position would probably be in lay-flat planting in which the increased emergence at the apex could cause gaps in the stand.

Damages to the cutting material by storage or machine planting on compacted soil resulted in a decrease in performance. Furthermore, variation in performance was higher in the damaged cuttings. The level of soil compaction contributed significantly to the damages, and cuttings planted on compacted soil had a higher probability of being damaged or landing on soil instead of being placed into it. These effects decreased with diameter, further emphasizing the advantages of larger cuttings. If planting in compacted soil, cuttings of a minimum 10–11 mm in diameter should be used. However, planting in compacted soil in general is not recommended.

In this study, vertically planted cuttings were generally preferable to horizontally planted cuttings, especially when considering planting material costs. If planted horizontally, the depth should not be greater than 5 cm. The effects increased considerably when combining small cuttings (7 cm) with large depths (17 cm), producing only 1/50 of the shoot biomass of large (21 cm) vertically planted cuttings.

Early competition against weeds was of major importance to early willow development. If weeds sprouted before the willow had reached sufficient size, which at the given high planting density of this study was when weeds were planted 15 days after the willow, there was a considerable effect on performance. Weeds planted later (after 26–30 days) had little effect on willow. Nitrogen fertilization was of lesser importance and seemed to be more beneficial to the model weeds than to the willow. All in all, this study confirmed the importance of weeding during the establishment year and indicated that fertilization should not be applied during this time.

The long-term effect of cutting characteristics and early plant size was validated in a field experiment between 2008 and 2015 during which two harvests were performed. Stool shoot weight increased with cutting weight and early plant size for both harvests indicating that the initial size hierarchy was maintained during the entire experiment.

Finally, differences were found in performance between clones. Some clones responded only slightly to treatments that had a considerable effect on others. To be able to draw reliable and general conclusions on the effect on a treatment for willow cultivation, tests should be performed on a variety of clones.

# References

- Ahmad, S., Bostan, N., Munir, M., Rabi, F. & Rehman, J. (2014). Effect of Different Sizes of Olive Cutting on Growth Capacity. *Pure and Applied Biology*, 3(3), pp. 92-94.
- Alakukku, L. & Elonen, P. (1995). Long-term effects of a single compaction by heavy field traffic on yield and nitrogen uptake of annual crops. *Soil and Tillage Research*, 36(3-4), pp. 141-152.
- Albertsson, J., Verwijst, T., Hansson, D., Bertholdsson, N.O. & Åhman, I. (2014a) Effects of competition between short-rotation willow and weeds on performance of different clones and associated weed flora during the first harvest cycle. *Biomass and Bioenergy*, 70, pp. 364-372.
- Albertsson, J., Hansson, D., Bertholdsson, N.O. & Åhman, I. (2014b) Site related set-back by weeds on the establishment of 12 biomass willow clones. *Weed Research*, 54, pp. 398-407.
- Alriksson, B. (1997). Influence of site factors on Salix growth with emphasis on nitrogen response under different soil conditions. Doctoral thesis. Department of Short Rotation Forestry, Swedish University of Agricultural Sciences, Uppsala, Sweden. Acta Universitatis Agriculturae Sueciae - Silvestria No 46.
- Aronsson, P., Rosenqvist, H. & Dimitriou, I. (2014). Impact of nitrogen fertilization to short-rotation willow coppice plantations grown in Sweden on yield and economy. *Bioenergy Research*, 7(3), pp. 993-1001.
- Baky, A., Forsberg, M., Rosenqvist, H., Jonsson, N. & Sundberg, M. (2010). Skördeteknik och logistik för bättre lönsamhet från små odlingar av Salix. *Rapport 1143, Värmeforsk*, Stockholm.

- Balasus, A., Bischoff, W.A., Schwarz, A., Scholz, V. & Kern, J. (2012). Nitrogen fluxes during the initial stage of willows and poplars in short-rotation coppices. *Journal of plant nutrition and soil science*, 175, pp. 729-738.
- Benjamin, L.R. & Hardwick, R.C. (1986). Sources of variation and measures of variability in even-aged stands of plants. *Annals of Botany*, 58(6), pp. 757-778.
- Brereton N., Pitre F, Shield I., Hanley S., Ray M., Murphy R. & Karp, A. (2014) Insights into nitrogen allocation and recycling from nitrogen elemental analysis and 15N isotope labelling in 14 genotypes of willow. *Tree Physiology*, 34(11), pp. 1252-1262.
- Burgess, D., Hendrickson, O.Q. & Roy, L. (1990). The importance of initial cutting size for improving the growth performance of *Salix alba* L. *Scandinavian Journal of Forest Research*, 5(1-4), pp. 215-224.
- Börjesson, P. & Berndes, G. (2006). The Prospects for Willow Plantations for Wastewater Treatment in Sweden. *Biomass and Bioenergy*, 30, pp. 428-438.
- Börjesson, P. & Tufvesson, L.M. (2011). Agricultural crop-based biofuels resource efficiency and environmental performance including direct land use changes. *Journal of Cleaner Production*, 19(2–3), pp. 108-120.
- Cao, Y., Lehto, T., Repo, T., Silvennoinen, R. & Pelkonen, P. (2010). Effects of planting orientation and density of willows on biomass production and nutrient leaching. *New Forests*, 41, pp. 361-377.
- Cao, Y., Lehto, T., Piirainen, S., Kukkonen, J.V.K. & Pelkonen, P. (2012). Effects of planting orientation and density on the soil solution chemistry and growth of willow cuttings. *Biomass and Bioenergy*, 46, pp. 165-173.
- Carpenter, L.T., Pezeshki, S.R. & Shields, F.D. (2008). Responses of nonstructural carbohydrates to shoot removal and soil moisture treatments in *Salix nigra*. *Trees*, 22(5), pp. 737-748.
- Cary, N.C. (2011). SAS Institute Inc SAS/STAT® 9.3 User's Guide: The MIXED Procedure. SAS Institute, Inc.
- Caslin, B., Finnan, J. & McCracken, A. (2010). Short Rotation Coppice Willow - Best Practice Guidelines. Teagasc, Crops Research Centre, Oak Park, Carlow, Ireland. Available from: <u>http://www.seai.ie/</u><u>Renewables/Bioenergy/Willow\_Best\_Practice\_Guide\_2010.pdf</u> [2015-02-15].
- Clay, D. & Dixon, F. (1995). Vegetation management in the establishment of poplar and willow short-rotation coppice. *Brighton crop protection*

conference: weeds. Proceedings of an international conference, Brighton, UK, pp. 979-984.

- Clay, D. & Dixon, F. (1997). Effect of ground-cover vegetation on the growth of poplar and willow short-rotation coppice. *Aspects of Applied Biology*, 49, pp. 53-60.
- Danfors, B., Ledin, S., & Rosenqvist, H. (1997). *Short Rotation Willow Coppice - Growers Manual.* Swedish Institute of Agricultural Engineering. 40 p.
- Dawson, W.M. (2007). *Short rotation coppice willow best practice guidelines*. Belfast: Omagh College, 50 p.
- Dimitriou, I., Eriksson, J., Adler, A., Aronsson, P. & Verwijst, T. (2006). Fate of heavy metals after application of sewage sludge and wood-ash mixtures to short-rotation willow coppice. *Environmental Pollution*, 142(1), pp. 160-169.
- Elowson, S. (1999). Willow as a Vegetation Filter for Cleaning of Polluted Drainage Water from Agricultural Land. *Biomass and Bioenergy*, 16, pp. 281-290.
- Fircks, Y., Ericsson, T. & Sennerby-Forsse, L. (2001), Seasonal variation of macronutrients in leaves, stems and roots of *Salix dasyclados* Wimm. grown at two nutrient levels. *Biomass and Bioenergy*, 21, pp. 321-334.
- Friedrich, V. & Boris, R. (2014). Size matters? The diverging influence of cutting length on growth and allometry of two Salicaceae clones. *Biomass and Bioenergy*, 60, pp. 130-136.
- Gage, E.A. & Cooper, D.J. (2004). Controls on willow cutting survival in a Montane Riparian area. *Journal of Range Management*, 57(6), pp. 597-600.
- Gro, V. & Culshaw, D. (2001). How is life if you try to live from developing SRC in Denmark? Experiences, results and recommendations. In: *Proceedings from the first meeting of IEA- Bioenergy Task 30*, Denmark, 86, pp. 29-34.
- Gustafsson, J., Larsson, S. & Nordh, N. (2007). Manual för salixodlare. Available from: <u>http://www.bioenergiportalen.se/attachments/42/406.</u> <u>pdf</u> [2015-02-15].
- Hauk, S., Knoke, T., & Wittkopf, S. (2014). Economic evaluation of short rotation coppice systems for energy from biomass - A review. *Renew Sustainable Energy Reviews*, 29, pp. 435-48.
- Heaton, R.J., Randerson, P.F. & Slater, F.M. (1999). The economics of growing short rotation coppice in the uplands of mid-Wales and an economic comparison with sheep production. *Biomass and Bioenergy*, 17, pp. 59-71.

- Helby, P., Rosenqvist, H. & Roos, A. (2006). Retreat from Salix Swedish experience with energy crops in the 1990s. *Biomass and Bioenergy*, 30, pp. 422-427.
- Hollsten, R., Arkelöv, O. & Ingelman, G. (2012). Handbok för Salixodlare. Jordbruksverket (Swedish Board of Agriculture), Jönköping, Sweden. URL Available from: <u>http://www2.jordbruksverket.se/webdav/files/</u> <u>SJV/trycksaker/ Pdf\_ovrigt/ovr250.pdf</u> [2015-02-15].
- Jordbruksverket (2015). Jordbruket i siffror. Odling av Salix 2005- 2013. Available from: https://jordbruketisiffror.wordpress.com/2014/02/26/ odling-av-salix-2005-2013/ [2015-04-22].
- Kuzovkina, Y.A., Knee, M. & Quigley, M.F. (2004). Effects of soil compaction and flooding on the growth of 12 willow (*Salix L.*) species. *Journal of Environmental Horticulture*, 22(3), pp. 155-160.
- Labrecque, M., Teodorescu, T.I., Babeux, P., Cogliastro, A. & Daigle, S. (1994). Impact of herbaceous competition and drainage conditions on the early productivity of willows under short-rotation intensive culture. *Canadian Journal of Forest Research*, 24, pp. 493-501.
- Larsen, S.U., Jørgensen, U. & Lærke, P.E. (2014). Willow Yield Is Highly Dependant on Clone and Site. *Bioenergy Research*, 7, pp. 1280-1292.
- Lindroth, A., Verwijst, T. & Halldin, S. (1994). Water-use efficiency of willow: Variation with season, humidity and biomass allocation. *Journal of Hydrology*, 156(1–4), pp. 1–19.
- Lowthe-Thomas, S.C., Slater, F.M. & Randerson, P.F. (2010). Reducing the establishment costs of short rotation willow coppice (SRC) a trial of a novel layflat planting system at an upland site in mid-Wales. *Biomass and Bioenergy*, 34, pp. 677–686.
- McCracken, A.R., Moore, J.P., Walsh, L.R.E. & Lynch, M. (2010). Effect of planting vertical/horizontal willow (*Salix* spp.) cuttings on establishment and yield. *Biomass and Bioenergy*, 34, pp. 1764-1769.
- Merilo, E., Heinsoo, K., Kull, O. & Koppel, A. (2006). Above-ground production of two willow species in relation to radiation interception and light use efficiency. *Proceedings of the Estonian Academy of Sciences, Biology and Ecology*, 55(4), 341–354.
- Mleczek, M., Rutkowski, P., Rissmanna, I., Kaczmarekc, Z., Golinski, P., Szentner, K., Strazynska, K. & Stachowiak, A. (2010). Biomass productivity and phytoremediation potential of Salix alba and Salix viminalis. *Biomass and Bioenergy*, 34, pp. 1410-1418.
- Nambiar, E.K.S. & Sands, R. (1992). Effects of compaction and simulated root channels in the subsoil on root development, water uptake and growth of radiata pine. *Tree Physiology*, 10(3), pp. 297-306.

- Njoku, J.C., Muoneke, C.O. & Okocha, P.I. (2010). Effect of propagule size and storage period on establishment and yield of sweetpotato in a tropical ultisol. *Journal of Sustainable Agriculture and the Environment*, 12(1), pp. 43-56.
- Nordh, N.E. (2005). Long term changes in stand structure and biomass production in short rotation willow coppice. Doctoral thesis. Swedish University of Agricultural Sciences. 26 p. Acta Universitatis Agriculturae Sueciae No. 120.
- Nord-Larsen, T., Sevel, L. & Raulund-Rasmussen, K. (2015). Commercially Grown Short Rotation Coppice Willow in Denmark: Biomass Production and Factors Affecting Production. *Bioenergy Research*, 8, pp. 325-339.
- OuYang F., Wang J. & Li Y. (2015). Effects of cutting size and exogenous hormone treatment on rooting of shoot cuttings in Norway spruce [*Picea abies* (L.) Karst.]. *New Forests* (2015), 46, pp. 91–105.
- Park, S.E., Benjamin, L.R. & Watkinson, A.R. (2003). The theory and application of plant competition models: an agronomic perspective. *Annals of Botany*, 92(6), pp. 741-748.
- Perttu, K.L. & Kowalik, P.J. (1997). Salix Vegetation Filters for Purification of Water and Soils. *Biomass and Bioenergy*, 12, pp. 9-19.
- Regeringskansliet (2015). *Climate policy*. [online] (2004-04-28) Updated 2014-09-17. Available from http://www.government.se/sb/d/5745 [2015-03-31]
- Rosenqvist, H. (1997). *Salixodling kalkylmetoder och lönsamhet*. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala, 241 p. Acta Universitatis Agriculturae Sueciae Silvestria No 24.
- Rosenqvist, H., Berndes, G. & Börjesson, P. (2013). The prospects of cost reductions in willow production in Sweden. *Biomass and Bioenergy*, 48, pp. 139-147.
- Rossi, P. (1999). Length of cuttings in establishment and production of shortrotation plantations of *Salix* 'Aquatica'. *New Forests*, 18(2), pp. 161-177.
- Sage, R. (1999). Weed competition in willow coppice crops: the cause and extent of yield losses. *Weed Research*, 39, pp. 399-411.
- Schaff, S.D., Pezeshki, S.R. & Shields, Jr F.D. (2003). Effects of soil conditions on survival and growth of black willow cuttings. *Environmental Management*, 31(6), pp. 748-763.
- Serapiglia, M.J., Humiston, M.C., Xu, H., Hogsett, D.A., Orduna, R.M., Stipanovic, A.J. & Smart, L.B. (2013). Enzymatic saccharification of

shrub willow genotypes with differing biomass composition for biofuel production. *Frontiers in Plant Science*, 4(57), pp.1-8.

- Shield, I.F., Macalpine, W. & Karp, A. (2008). The effect of the size of the cuttings planted on the subsequent performance of three contrasting willow cultivars for short rotation coppice. *Aspects of Applied Biology*, 90, pp. 225-31.
- Souch, C.A., Martin, P.J., Stephens, W. & Spoor, G. (2004). Effects of soil compaction and mechanical damage at harvest on growth and biomass production of short rotation coppice willow. Plant and Soil, 263(1), pp. 173-182.
- Souza, M. & Fagundes, M. (2014) Seed Size as Key Factor in Germination and Seedling Development of *Copaifera langsdorffii* (Fabaceae). *American Journal of Plant Sciences*, 5, pp. 2566-2573.
- Stolarski, M., Szczukowski, S., Tworkowski, J. & Bieniek, A. (2009). Productivity of willow coppice Salix spp. under contrasting soil conditions. *Electronic Journal of Polish Agricultural Universities*, 12(1), pp. 1-10.
- Tahvanainen, L. & Rytkönen, V.M. (1999). Biomass production of Salix viminalis in southern Finland and the effect of soil properties and climate conditions on its production and survival. *Biomass and Bioenergy*, 16(2), pp. 103-117.
- Verwijst, T. (1996a). Cyclic and progressive changes in short-rotation willow coppice systems. *Biomass and Bioenergy*, 11, pp. 161-165.
- Verwijst, T. (1996b). Stool mortality and development of a competitive hierarchy in a *Salix viminalis* coppice system. *Biomass and Bioenergy*, 10, pp. 245-250.
- Verwijst, T. & Nordh, N.E. (2010). Effekter av skottnedklippning efter etableringsåret på produktionen under första och andra omdrevet i salixodlingar. Grödor från åker till energiproduktion. *Rapport 1136, Värmeforsk Service AB (Thermal Engineering Research Association)*, Stockholm, Sweden. 33 p. Available from: <u>http://www.varmeforsk.se/</u> <u>rapporter?action=show&id=2543</u> [2015-03-30].
- Verwijst, T., Nordh, N.E. & Lundkvist, A. (2010). Effects of cutting characteristics on sprouting and early growth of willow. *Report No* 1144, Värmeforsk Service AB, Stockholm, 24 p.
- Verwijst, T., Lundkvist, A., Edelfeldt, S. & Albertsson, J. (2013). Development of Sustainable Willow Short Rotation Forestry in Northern Europe. In: *Matovic MD (ed) Biomass Now - Sustainable Growth and Use. InTech.* DOI: 10.5772/55072.

- Vigl, F. & Rewald, B. (2014). Size matters? The diverging influence of cutting length on growth and allometry of two *Salicaceae* clones. *Biomass and Bioenergy*, 60, pp. 130-136.
- Volk, T. (2002). Alternative methods of site preparations and coppice management during the establishment of short-rotation woody crops. Doctoral thesis. Syracuse, NY: State University of New York, College of Environmental Science and Forestry.
- Volk, T., Ballard, B., Robinson, D.J. & Abrahamson, L.P. (2004). Effect of cutting storage conditions during planting operations on the survival and biomass production of four willow (*Salix* L.) clones. *New Forests*, 28(1), pp. 63-78.
- Volk, T.A., Verwijst, T., Tharakan, P.J., Abrahamson, L.P. & White, E.H. (2004). Growing Fuel: A Sustainability Assessment of Willow Biomass Crops. *Frontiers in Ecology and the Environment*, 2, pp. 411–418.
- Watts, C.W., Clark, L.J., Chamen, W.C.T. & Whitmore, A.P. (2005). Adverse effects of simulated harvesting of short-rotation willow and poplar coppice on vertical pressures and rut depths. *Soil and Tillage Research*, 84(2), pp. 192-199.
- Weih, M. (2009). Genetic and environmental variation in spring and autumn phenology of biomass willows (Salix spp.): effects on shoot growth and nitrogen economy. *Tree Physiology*, 29(12), pp. 1479-1490.
- Willebrand, E. & Verwijst, T. (1993). Population Dynamics of Willow Coppice Systems and their Implications for Management of Short rotation Forests. *The forestry chronicle*, 69, pp. 699-704.
- Wyatt, G. (1998). Short rotation coppice harvesting: ground damage and yield effects. *Technical Note No 21/98. Dumfries: Forestry Commission, Technical Development Branch*, 13 p.
- Yang, W., Zhu, Z., Zhao, F., Ding, Z., Rafiq, M.T., Wang, Y., Zhang, X. & Yang, X. (2015). Variations in growth, nitrogen accumulation and nitrogen use efficiency among 18 willow clones under two nitrogen regimes. *Agroforestry Systems*, 89, pp. 67-79.
- Zavitkovski, J. (1981). Small plots with unplanted border can distort data in biomass production studies. *Canadian Journal of Forest Research*, 11, pp. 9-12.

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