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Methods of Poplar Propagation and Establishment on Forest and Agricultural Land in Sweden

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Contents

Abstract

Currently, in Sweden, the interest in growing poplars is increasing. But there is still scarce of knowledge about the appropriate species and clones that should be used for the specific conditions of Sweden. The disposal of information to farmers is also limited, which can difficult the establishment of poplar plantations. Most poplar plantations in Sweden are located on agricultural lands. A special attention should be given to the establishment phase. It is necessary to consider the specific conditions of Sweden: hard winter (extremes temperatures, late frost, and soil frost), high densities of wildlife (moose, deer, and voles) and weed competition that can cause problems for the establishment.

This paper has two parts: the first part treats different methods that can be used in order to get a good regeneration and successfull establishment of poplars and the second part aims to compare the growth and root development of cuttings pretreated with water, Allgrow and naphthalene acetic acid.

Normally, pre-soaking cuttings into growth regulators or hormones stimulates rooting and increases plant survival. But in this case, the results achieved in the greenhouse do not support the hypothesis regarding the positive effects of soaking cuttings in NAA (naphthalene acetic acid).

Introduction

Since the 1940s many foresters in the World have been interested in commercial poplar plantations, but the production goals have been changing during the last few decades between producing pulpwood, sawlogs or biofuels. Genetic improvements of plant material have contributed to farther expand poplars in the world. In 1947, the International Poplar Commission (IPC) was established in order to *"promote the cultivation, conservation and utilization of members of the family Salicaceae, which includes poplars and willows"* (FAO, http://www.fao.org/forestry/ipc/en/).

The World Oil Crisis in 1973 gave as a reaction an increase of the interest for the utilization of biomass for energetic purposes, emerging as a need of replacing fossil fuels by new energy sources (Dimitriou I. & Aronsson P., http://www.fao.org/docrep/008/a0026e/a0026e11.htm). In this sense, forest plantations and plantations of fast growing species such as poplars could be important sources of raw material for sustainable energy production.

At the end of eighteenth century poplars from North America were introduced in Europe. Spontaneous hybrids between American and European species appeared to have superior growth compared to their parents. These hybrids were also used in the first industrial plantations established in Italy providing timber for mechanical pulpwood and plywood (FAO, 1979).

Currently, in many European countries there is a trend of land conversion using non-food crops for bioenergy purposes. Poplar plantations are becoming more popular because of the great production potential. Most poplar plantations are grown in short rotations. This enables maximum use of production potential on fertile arable land and provides additional income to farmers in relatively short intervals compared to traditional forestry.

Short Rotation Forestry (SRF) has been defined many times. An often used definition by Hansen (1991) says that short rotation forestry is *"a silvicultural practice where fast-growing species such as poplars and willows are grown with an intensive management and are harvested in short cycles between 2-10 years"*.

The use of willow in short rotations for the purpose of energy production in Sweden has started during the 1980s. Poplars have been planted mostly in the beginning of 1990s as a result of Governments subsidies to farmers for conversion of agricultural land from food to energy crops. Short rotation willow coppice (SRWC) is usually intensively managed as other agricultural crops using intensive cultural practices, whereas poplar plantations are intensively managed only in the establishment phase. The rotation period depends on climate and location, and therefore it will be shorter if the conditions are more favourable. In Sweden, willows are grown as coppice with a rotation period between 3 and 5 years, whereas poplars are grown as forest with a rotation between 15 and 30 years.

Introducing SRF on agricultural land introduces significant aesthetic change to the landscape that it is usually not appreciated by the general public. On the other hand, SRF may have positive effects on biodiversity and the environment. For example, some authors found an increase of faunal diversity after introducing SRWC (Göranson & Boel, 1989; Twedt *et al.*, 1999; Bergström, 2001; Berg, 2002). Similarly, willow and poplar plantations have been found to increase floristic diversity in Sweden (Weih, 2004).

The interest in growing poplars in Sweden is presently increasing. However, the knowledge about suitable species and clones, and establishment and management procedures adapted to Swedish conditions is not available to farmers. There is also very little research on this topic. This could be a result of the fact that the traditional forestry in Sweden promoted conifers at the expense of deciduous species such as *Populus tremula* L, *Betula verrucosa* Ehrh. and *Alnus incana* Moench.

Presently, only clone OP42 is commercially used in Sweden. This is a hybrid between *Populus maximowiczii* Henry and *Populus trichocarpa* T. & G. Poplar plantations established with this clone can reach a high survival (more than 90% after one season) and a mean annual production of 10 tones ha^{-1} yr^{-1} (Karačić, A., 2005). The primary goal of these poplar plantations is biomass production for energy purposes (heat and power), but at the same time poplars are a source of carbon sequestration and thus contribute to the greenhouse gas mitigation.

Other potential uses of poplar plantations in Sweden are as a source of fibre for pulp and paper industry, particle boards and other products such as adhesives and organic chemicals. Poplars can also be used in soil conservation and stabilization, phytoremediation and as shelterbelts.

The main aim of this paper is to present issues related to poplar establishment and plant propagation relating them to the specific conditions of Sweden. Also a pilot experiment on rooting poplar cutting is conducted in order to approach the topic of commercial plant production.

Natural and plantation forest of poplars

The total area of natural poplar forest is approximately about 80 million hectares. The largest poplar forests occur in Canada (28.3 million ha), the Russian Federation (21.9 million ha) and the U.S.A. (17.7 million ha), contributing with 97% of the total area of natural poplar forests. The main use of the natural poplar forests in these countries is wood production (International Poplar Commission, http://www.fao.org/forestry/media/9497/1/0/).

The worldwide area of poplar plantations is about 6.7 million ha. The main uses of these poplar plantations are wood production (3.8 million ha) and environmental purposes (2.9 million ha). The total planted area in China is about 4.9 million ha, which means that China contributes with 73% of the global total. India, with 1 million ha, has the second largest area of planted poplars (International Poplar Commission, http://www.fao.org/forestry/media/9497/1/0/).

The worldwide area of willow plantations is about 176,000 ha, mainly for environmental purposes and wood production. China has the largest area of planted willows with 80,000 ha, followed by Argentina 46,000 ha, New Zeeland 20,100 ha and Sweden 15,100 ha, but the largest area of wood production plantations of willows is established in Argentina with 46,000 ha, followed by China with 21,000 ha and Sweden with 15,000 ha (International Poplar Commission, http://www.fao.org/forestry/media/9497/1/0/).

The taxonomy of *Populus*

The genus *Populus* contains a great variability in terms of resistance to frost, drought, wind, pests and diseases. There are about 30 species of poplars classified into six sections: Abaso, Turanga, Leucoides (swamp poplars), Aigeiros (black poplars and cottonwoods), Tacamahaca (balsam poplars) and Populus (aspens and white poplars, table 1, Eckenwalder, 1996).

In general, it can be said that hybrid poplars of the *Tacamahaca* and *Aigeiros* sections respond well to intensive silviculture and reach growth rates much higher than native poplars. In Boreal regions, for example, hybrid poplars achieved yields of 16-25 m^3 ha^{-1} yr^{-1} compared to 2 m^3 ha^{-1} yr^{-1} in natural stands of poplars (Jarvis, 1968). Species of the *Aigeiros* and *Tacamahaca* sections also are easy to propagate vegetatively, usually by cuttings.

Table 1. Taxonomic classification of genus Populus (Eckenwalder, 1996)

Section	Species	NA[a]	Notes and common name
Abaso	*P. mexicana* Wesm.	Yes	*Mexican poplar*
Turanga (Afro-Asian poplars)	*P. euphratica* Oliv. *P. ilicifolia* (Engler) Roul. *P. pruinosa* Schr.		Euphrates poplar Kenyan poplar
Leucoides (Swamp poplars)	*P.glauca* Haines *P. heterophylla* L. *P. lasiocarpa* Oliver	 Yes	Formerly *P. wilsonii* Swamp cottonwood
Aigeiros (Cottonwoods and Blackpoplars)	*P. deltoides* Marsh. *P.fremontii* S. Wats. *P. nigra* L.	Yes Yes	Eastern cottonwood; includes P. Sargentii and P. wislizenii Fremont cottonwood Black poplar
Tacamahaca	*P. angustifolia* James	Yes	Narrowleaf cottonwood

(Balsam poplars)	***P. balsamifera* L.**	**Yes**	**Balsam poplar**
	***P. ciliata* Royle**		**Himalayan poplar; heterotofore placed in section *Leucoides***
	***P. laurifolia* Ledeb.**		**Laurel poplar**
	***P. simonii* Carr.**		**Simon poplar**
	***P. suaveolens* Fisch.**		**Asian poplar; includes *P. cathayana, P. koreana* and *P. maximowiczii***
	***P. szechuanica* Schn.**		**Szechuan poplar**
	P. trichocarpa* Torr. & *Gràу	**Yes**	**Black cottonwood**
	***P. yunnanensis* Dode**		**Yunnan poplar**
Populus*[b] *(Aspens and white poplars)	***P. adenopoda* Maxim.**		**Chinese aspen**
	***P. alba* L.**		**White poplar**
	***P. gamblei* Haines**		**Himalayan aspen**
	***P. grandidentata* Mich.**	**Yes**	**Bigtooth aspen**
	***P. guzmanantlensis* Vazq. & Cuevas**	**Yes**	**Manantlán white poplar**
	***P. monticola* Brand.**	**Yes**	**Baja white poplar**
	***P. sieboldii* Miquel**		**Japanese aspen**
	***P.simaroa* Rzedo**	**Yes**	**Balsas white poplar**
	***P. tremula* L.**		
	***P. tremuloides* Mich.**	**Yes**	**Quaking (trembling) aspen**

[a] NA, native to North America
[b] Formerly section *Leuce*

Ecology of *Populus*

According to FAO (1958) all the poplars demand high light rates and are able to grow on great variety of soil types (table 2). Poplars usually require high soil moisture, and are often found close to the rivers, lakes or ponds. They are characterised by fast growth, high productivity and vegetative propagation. They are easy to hybridise through intra- and interspecific breeding. Poplars are wind-pollinated species with female and male flowers borne on individual trees. The great capacity to sprout new shoots from stamps or roots enables vegetative regeneration in the second rotation.

Site requirements and plantation management

The choice of a site suitable for the establishment of poplar plantation is of great importance for its future development. According to Baker & Broadfoot, (1979), "*best performance can be expected on soils that are well-aerated, have sufficient moisture and nutrients, are sufficiently deep (>1.0 meter to the water table), have medium texture (sand/loam) and have a soil pH in the 5.0 to 7.5 range*". From the economical point of view it is a good idea to choose a site close to a mill or energy producer to reduce the costs of management and transportation.

However, poplars are able to grow under many site conditions. It would be difficult to describe all of them and therefore it is better to describe some factors that they do not tolerate well. For example, saturated and waterlogged soils are not appropriate for growing poplars, because the oxygen content in such soils is low and even anaerobic conditions may occur. The symptoms of suffering from waterlogged conditions appear as yellowish-green leaves or decrease of growth. Eventually the trees can even die especially young trees in the spring season. Another characteristic of soils that greatly affects poplars is the soil texture that directly influences the site quality (table 2). Generally it can be said that coarser soils are more favourable for poplar growth than heavy soils with clay, clay loam, silty and clay loam textures. Usually, if the texture is fine, the drainage and aeration are poor, which sometimes causes difficulties in using heavy machinery for weed control and other management operations. Finally, saline conditions are usually not tolerated by poplars and should be avoided. In saline conditions, poplars are under drought stress, showing the symptoms similar to those described for saturated soils, with necrosis at the edge of leaves.

Table 2. Soil texture and drainage condition influencing site quality for poplar (Dickman & Stuart, 1983)

	Natural drainage class		
Dominant profile textures	**Well and moderately well drained**	**Somewhat poorly drained**	**Poorly and very poorly drained**
Fine clay (>60% clay)	**Fair**	**Fair**	**Poor**
Clay (40-60%)	**Fair**	**Fair**	**Poor**
Clay and silty clay loam	**Good**	**Poor**	**Poor**
Loam and silt loam	**Good-very good**	**Fair**	**Poor**
Loam and silt loam 25-50 cm over well-decomposed peat	**Good-very good**	**Poor**	**Poor**
Loam and silt loam marbled with well-decomposed peat	**Good-very good**	**Fair-good**	**Poor**
Sandy loam	**Very good**	**Fair-good**	**Poor**
Loamy sand	**Very good**	**Fair-good**	**Poor**
Sand		**Fair**	**Poor**
Sandy loam 35-100 cm over clay	**Very good**	**Fair**	**Poor**
Sandy loam 50-100 cm over loam-clay loam	**Very good**	**Fair**	**Poor**
Sandy loam 50-100 cm over sand	**Good**	**Very good**	**Poor**
Loamy sand 35-100 cm over clay	**Very good**	**Fair**	**Poor**
Sand-loamy sand 50-100 cm over loam-clay loam	**Very good**	**Very good**	**Poor**
Sand-loamy sand 100-150 cm over loam-clay	**Good**	**Very good**	**Poor**
Muck	**N/A**	**N/A**	**Poor-fair**

The quality of a site can be improved using different cultivation techniques. The most efficient is application of nutrients and irrigation water. Application of herbicides for weed control is important in the establishment phase, whereas additional improvements can be achieved by ditching, installing drain tile, subsoiling or a combination of these in order to increase soil aeration. It is also possible to improve yields by matching different poplar varieties to the available site qualities, equipment and the final product.

The productivity of poplar plantations depends on climate, water and nutrient supply, plant material, intensity and type of silvicultural system and the efficiency of weed, disease and pest control. In this sense, it can be said that the production potential of poplar plantations is higher in the regions with longer growing season; meaning that production is higher in temperate than in Boreal regions. The most effective improvement of yield is obtained by a combination of irrigation and addition of fertiliser. At a wider spacing a more intensive weed control is required during the establishment phase in order to secure a good plant survival.

Poplars in Sweden

The only native species of poplar in Sweden is aspen (*Populus tremula* L.). It appears almost in the whole country with the exception for the alpine regions of Sweden (Hulten, 1971).

At present there are approximately 500 ha of poplar plantations in Sweden established mainly at the beginning of 1990s. At this time the establishment of energy forest on agricultural land was supported by the Government as a part of general land conversion policy. Most poplar plantations were established in southern Sweden with clone OP42 using one meter long container plants, usually at 3m x 3m spacing and grown at rotations of 20 years (figure 1). The wood is used in pulp and paper industry or for energy purposes.

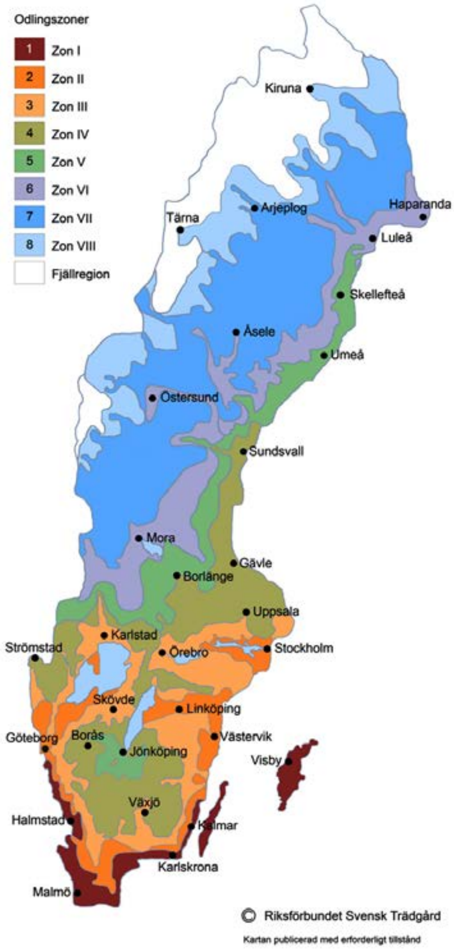

Figure 1. Sweden is usually divided into eight growing zones, depending on the length of the growing season, mean temperature and occurrence of early and late frosts. Poplars can be grown in zones I-V. The commercially sold clone OP42 is recommended for zones I and II, but it grows well in zone III as well.

Different methods of poplar propagation

There are two main methods of poplar propagation: sexual (by seeds) and asexual (by cuttings). Poplars have a very short seed life (generally less than two or three weeks) and small range of appropriate seedbed conditions for germination (Dickmann *et al*., 2001). Propagation by seeds is important for establishment of poplar populations in their natural habitats but is not widely used in poplar plantations. Asexual propagation refers to reproduction by vegetative parts of plants. Ramet is a vegetativelly reproduced copy of a plant which has the same genotype as the original parent tree. The original parent tree is known as ortet (Forest Genetic Glossary, http://www.esf.edu/for/maynard/GENE_GLOSSERY.html#-O-). Vegetative reproduction is easier, cheaper and requires less time than propagation by seeds.

This paper focuses on propagation by cuttings. Vegetative propagation is frequently used for dicotyledonous plants, but in some cases it can be used for some monocots, as well. This kind of propagation consists of extracting a portion of a stem, leaf or root from the parent plant in order to induce the cuttings to produce roots and shoots, and eventually a new independent plant. It is very important to choose cutting material from plants free from diseases, with known identity and moderately vigorous (Hudson *et al*., 1975).

Propagation by cuttings is the cheapest and easiest method of poplar propagation. Cuttings can easily be stored and transported on long distances. Poplars can be propagated by stem cuttings, obtaining a high number of trees genetically identical to their parents. This is the most common type of cutting used for commercial propagation of poplars and willows where portions of shoots containing terminal or lateral buds are planted in order to obtain a new independent plant from the bud, with its own root and shoot system. These cuttings are also called hardwood cuttings. Adventitious root initials in poplars and willows are formed during the stem development that usually remains dormant until cuttings are exposed to rooting conditions (Hudson *et al.*, 1975). This is possible because many cells are able to return to the meristematic condition, start cell division and produce new root or/and shoot systems. At the basal end of a cutting callus usually appears together with first roots.

Planting stock

There are mainly two different planting stock types depending on desired end product and available budget: unrooted stock and rooted stock (table 3). Rooted stock refers to plants derived from cuttings that are sold and planted as bare root or containerised plants.

Unrooted stock is divided into two groups: cuttings and sets. Unrooted dormant cuttings are cut from one year old stems and can vary in length from small sizes (2-3cm) to normal sizes from 15 cm to 1 m, Dickmann *et al*., 2001). One study carried out in North America shows that rooting of 10 cm cuttings was superior than rooting of 5 cm cuttings for four studied hybrid poplar clones. Consequently the use of 10 cm cuttings was recommended in order to maximise greenhouse rooting success of dormant cuttings. No additional treatment was recommended except soaking cutting in water for ca 48 hours. If the aim is to maximise root/shoot ratio DesRochers & Barb (2002) recommend the use of 5 cm cuttings with adding rooting hormones (Indole Butyric Acid, IBA) substances at low concentrations.

Table 3. Different planting stock types of poplars that can be used under various conditions (Dickmann et al., 2001)

	Unrooted stock		Rooted stock		
	Cuttings	Sets	Bare root Small	Sets	Container plants
Density stem ha^{-1}	>700	<400	>700	<400	>700
Planta-tion purpose	Fibre and solid wood	Solid wood	Fibre and solid wood	Solid wood	Research trials; new stoolbeds; extreme drought conditions at planting
Soil moisture conditions	Good	Excellent	Good	Good	Needs irrigation if planted in full leaf
Weed control	Excellent	Reasona-ble	Excel-lent	Rea-sonabl e	Excellent
Threat of browsers	High[a]-low	High	Low	High	Low
Time of planting	U.S. South:early winter U.S. Midwest, Pacific Northwest, and	Late winter to early spring	Late winter to late spring	Late winter to late spring	Late winter to late spring (irrigated)

	Canada: late winter to early spring	

[a]***With a high threat of browsers, deer fencing may be necessary.***

Zalesny *et al* (2003) at Iowa State University performed rooting experiments with 20 cm long cuttings of five genomic groups (BC = [*P. trichocarpa x P. deltoides*] *x P. deltoides*, D = *P. deltoides*, DM = *P. deltoides x P. maximowiczii*, DN = *P. deltoides x P. nigra*, NM = *P. nigra x P. maximowiczii*). These cuttings were cut in December and January of 2001 and 2002. The results show that the shoot position of dormant unrooted cuttings affects the future development of root system and early growth and development of a plant. In the cases of BC, D and DN genomic groups the cuttings from basal position of the shoot show almost two times better rooting than the cuttings from middle and apical position (in terms of number of rooted plants). On the other hand, cuttings from apical and middle positions exhibited similar rooting ability. This is explained by the higher content of carbohydrates in the basal position of hardwood cuttings (Fege & Brown, 1984). Nguyen *et al* (1990) have shown that the high levels of carbohydrates are associated with an increase of plant survival and root production. For DM and NM genomic groups the results exhibited no superiority in terms of rooting by cuttings from basal positions over middle or apical positions. For these two genomic groups all cutting positions were dependent on the year (2001 and 2002) and site conditions.

Unrooted dormant sets are produced from one or two year old stem material, but development of root is better from one year old dormant material. In this case the length of sets can vary from 1.5 m to 5 or 6 m (Dickmann *et al*., 2001).

Rooted stock can be planted in two different forms: bareroot cuttings and container plants (Dickmann *et al*., 2001). Rooted cuttings are produced by planting unrooted dormant cuttings, so that they can develop a viable root system. These rooted cuttings are also known as barbatelles. They can be planted outside as bare root plants.

The container plants are produced from seeds, small single-bud stem cuttings or root cuttings (for example in aspen). The container plants are usually dormant when they are planted, but after planting they break the dormancy and start to develop an adequate root system. For the Swedish latitude that means that the best moment to transplant the containerised plants are at the end of April in the Southern part of Sweden and May and June in the rest of the country.

Different systems of stock production

Most of the planting stock of poplars is produced in stoolbed nurseries (Dickmann *et al*., 2001). Stools may be formed from any stock type, but the most common are dormant cuttings. During the winter stools should be cut back annually to a height between 5 to 15 cm and new sprouts will be formed each year (Dickmann *et al*., 2001). If very long planting stock is needed, then the stools should cut back every second year in order to obtain a two–year-old sets. Planting stock in form of cuttings should be stored in coolers or freezers (for long-term storage between -2ºC to -4ºC and for short-term storage between 2ºC to 4ºC) until they are out-planted. The normal stool spacing is 0.3 x 0.3 m with a lifespan of a healthy stoolbed between 3 and 7 years (Dickmann *et al*., 2001).

Weed control is important for the successful establishment of a new stoolbed. The most effective weed control technique is the use of herbicides. Mulching can be used as an alternative method to control weeds, but it is not always recommended because it can cause soil acidification, decreasing the available nitrogen and creating a habitat suitable for rodents (Dickmann *et al*., 2001). Deficiencies of nutrients and water must be avoided in stoolbeds.

Deficiency of some nutrients can inhibit growth, but the excess of nutrients such as nitrogen can increase weed competition and promote formation of sylleptic branches. For this reason, fertilization and irrigation should be planned considering the specific local conditions in order to provide enough nutrients and water to maintain an even growth.

A special attention is required to avoid pests and diseases. In Sweden, some of them may appear such as leaf rusts (*Melampsora epitea* and *Melampsora medusae*), cottonwood clearwing borer (*Paranthrene dollii*), cottonwood twig borer (*Gypsonoma haimbachiana*) and aphids (Miller & LaGasa, 2001; Morris *et al*., http://arjournals.annualreviews.org/doi/pdf/10.1146/annurev.ento.50.071803.130431?cookieSet=1 ; Solomon, 1985; Royle & Hubbes, 1992). Combating pest and diseases is best done through a combination of chemical control and cultural practices, and the use of resistant varieties of hybrid poplars. High densities of stools promote foliage diseases. Some commercial insecticides has been used successfully in eastern cottonwood and hybrid poplar in the U.S. (Dickmann *et al*., 2001).

Another system of stock production consists of dormant material that is harvested from branches of young plantations. The branches that are near the top of the tree produce cuttings of appropriate diameters. Approximately 20-30 cuttings are produced by a 2-year-old tree (Dickmann *et al*., 2001).

Yet another system of plant production is the use of bareroot cuttings to establish poplar plantations. After one year of growth in the nursery, the bareroot plants are out-planted in the field with the root system intact. In winter or early spring (when the trees are dormant) the bareroot stock is lifted. Normally, large stock (several meters tall) can not be kept easily in store, which is why the transporting and planting is done immediately after lifting. It requires planting machinery such as a tractor with equipment for digging the hole for the plants. This system is labouring intensive and expensive and is normally not used for fibre plantations.

Container plants are grown from seeds, single-bud hardwood cuttings or root cuttings. The size of the container is very important for the development of root system. The containers should be designed to avoid root spinning and allow good aeration of the rooting medium. Dormant single-bud stem cuttings are used in breeding programs where the amount of available material is limited or for those varieties that are difficult to propagate. Each branch or sprout is divided into small cuttings with at least one bud per cutting and then rooted in containers. Once the fully rooted plants are obtained, they are out-planted with the soil that was in the container. It is also frequent to add rooting hormones or growth regulators, which is explained in the next section. This method is also expensive and labour intensive, but the advantage is that a small amount of original material can be multiplied into thousands of identical plants in an easy and quickly manner.

Container plants can also be produced from dormant root cuttings. In that case, root cuttings should be placed in containers in the greenhouse in the late winter until they develop a full root system. During the late summer the container plants are placed outside and in the next winter, dormant seedlings can be removed from the containers, packaged and kept in store in a freezer or cooler. The following spring these seedlings should be out-planted. This method is often used to propagate *Populus alba* (white poplar), *Populus tremula* (European aspen), *Populus tremuloides* (quaking aspen) and their hybrids (Hudson *et al*., 1975).

In the Prairie Region of Canada containerized seedlings are produced for reforestation (Dickmann *et al*., 2001). Normally, the seeds used come from open-pollinated trees. These seeds are sowed in containers during the late spring and normally the seedlings are out-planted in the autumn the same year.

Treating cuttings with hormones or growth regulators

The use of hormones or growth regulators (auxins, gibberellins, cytokinins...) can help to initiate adventitious roots on stems. In some cases, it is more effective to use a mixture of root-promoting substances than either component alone. However, it is important to use an adequate concentration for each component, otherwise the plant growth can be inhibited and, in some cases, it can even cause eventual death of the cuttings. When applied to promote rooting of dormant stem cuttings, indole butyric acid (IBA) and naphthalene acetic acid (NAA) have achieved better results in most of the cases compared to untreated cuttings (Hudson *et al.*, 1975).

There are different methods of application depending on whether the hormones or growth regulators are in powder or liquid condition. The most common procedure consists of dipping the basal part of the cutting into a dilute solution with hormones or growth regulators for 24 hours before the cuttings are inserted into the rooting medium. Dormant stem cuttings can also be treated with fungicides to prevent infections.

Different techniques of propagation by cuttings

Wounding is a technique that consists of stripping off the bark of the stem cutting in order to stimulate rooting. It can be made with a sharp knife or with a special tool designed for this task. The vertical cut must penetrate through the bark and into the wood and the length will depend on the size of the cutting. Wounded cuttings permit higher absorption of water and growth regulators from the soaking medium.

In some cases, it is advisable to provide an intermittent mist water spray over the cuttings to decrease loss of humidity by transpiration. Mist bed can be installed in the greenhouse (figure 2). Nozzles are set up over the mist beds to provide a fine mist over the whole bed. The application of water can be controlled by timer mechanism or by a thermostat that shuts off the mist flow at certain temperature.

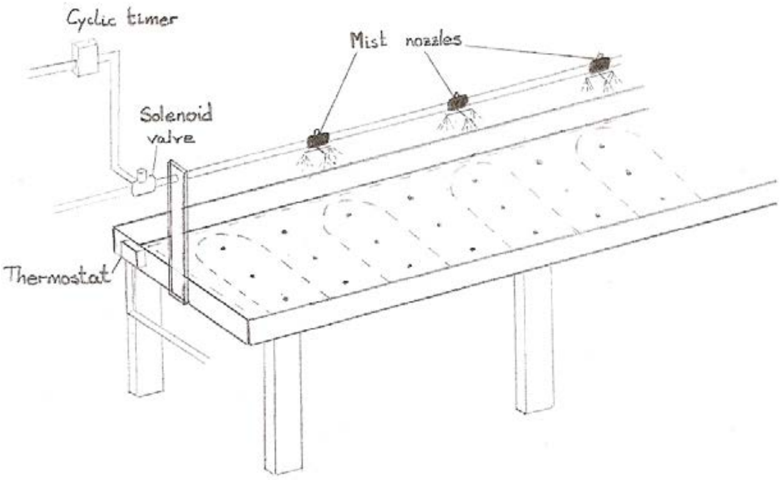

Figure 2. Design for a mist bed system. Drawing: Teresa Brage Tuñón.

Harvesting methods and processing of cuttings

Harvesting can be done by cutting of individual stems or by mass harvesting (cutting many stems at the same time). Individual stem harvesting requires qualified personnel that are able to identify which sprout is a good material and which is poor. The material with low quality, undersized or diseased will not be cut. This method is costly, and it is usually not mechanized. With mass harvesting it is possible to use equipment for cutting the sprouts, such as hand-held brushing saw or mower implemented on a tractor. This method enables high productivity with lower costs of harvesting.

Processing can be carried out individually or using assembly line techniques depending on whether it is for individual stems or for mass processing. Processing individual stems means that each stem is processed separately by the cutter, using a saw or power saw, pneumatic or hydraulic shears. There is a great advantage which is that cutters work independently, so, if some assembly line breakdown takes place it does not affect the whole processing. Other advantage is that cutter can better recognize the defects on cuttings. This method is usually used at small scale, for cutting of several varieties simultaneously. The main disadvantage is the high cost, especially if the procedure is applied at a large scale. At mass processing, this task is done by applying assembly line. One or two persons cut the sprouts with a set of mechanized cutting saws. Other group of workers sort the cuttings and finally, yet another group package the stock. Using assembly line speeds processing, but its main disadvantage is assembly line breakdown, for example when it is necessary to change the saws. This technique is useful at large scales when processing a single variety.

Packaging and storage

Once cutting stock have been harvested and processed, it should be packaged properly in plastic bags in order to avoid loss of humidity. Each bag should be sealed and labelled with the number or variety name, the quantity, packaging date and name of the nursery. These bags are kept in storage bins or cardboard boxes that should be stored at different temperatures depending on whether it is a short- (less than one month) or a long-term storage. For short-term storage the proper temperature is about 2ºC to 4ºC, whereas for long-term storage the adequate temperature is -2ºC to -4ºC (Dickmann *et al*., 2001). The boxes should be stacked to allow the free circulation of air. It is also possible to store them in a snowbank, but only if the temperatures are around 0°C. Sets that are up to 2 m long are covered using plastic sleeves, but the temperature conditions of storage are similar.

Quality control

Quality control is important for achievement of the expected results without mixing up varieties that can increase the costs of processing. Material should be free of pests and diseases, culled, properly identified and labelled. Individual stems processing enables good quality control.

Site preparation

Before planting, it is essential to prepare the site to ensure the success of poplar plantations. This is one of the most important steps for decreasing the mortality of seedlings. Furthermore, it reduces planting costs, damages by mechanical cultivation, improves internal aeration and drainage (by disruption of the impervious layers of the soil), and increases the efficiency of weed control and the accessibility of the site. There are different methods depending on the previous use and conditions on the site where the poplar plantations will be established, available equipment, costs, planting time and method, etc.

When the chosen site is an agricultural land or an open pasture, the task is relatively simple and it requires only a combination of conventional methods like chisel plowing, disking, mowing and subsoiling. An application of herbicides is optional. If the chosen site is a previous plantation or cutover natural stand, the task of site preparation might become very expensive and complicated. In that case, the task is complicated by logging debris, stumps, roots, heavy vegetation and even compactation caused by logging traffic. The methods used for the preparation of these sites are shearing, raking, ploughing, scarifying, harrowing, piling and burning.

Once the site is prepared, the rows are marked according to the selected spacing pattern. If fertigation technique is applied the task is more expensive and difficult, because it involves a heavy infrastructure including the replacement of the old irrigation system.

Planting

Before planting, it is important to make an inspection of cuttings for damages and signs of diseases. It is especially important that the buds are completely healthy. The spacing of plantations varies from 2 x 3 m to 4 x 4 m for the latitude of Sweden (55º - 70º N) and depends on the desired final product (Dickmann *et al.*, 2001). Planting is usually done manually, but it can also be mechanized. Depth of planting will vary with the cutting size, but it should be enough for the good contact between cutting and soil, which provides cutting with access to sufficient nutrients and water. Shallow planting should be avoided. The cuttings should be planted on cool, cloudy and unwind days, leaving about 5 cm free above the ground and with at least one vegetative bud above the ground. In Sweden, cuttings should be planted only in spring between beginning of May and the end of June. Planting the cuttings in July or later should be avoided because the risk of draught and because many clones will not be able to develop new shoots from cuttings so late in the growing season.

Weed competition

Plant survival and growth in newly established poplar plantations are strongly affected by weed competition. Thus, weed control is necessary to ensure the success of the plantation establishment as weeds are strong competitors for water, nutrients and light. The consequence of weed competition is decrease of growth and increase of mortality of planted poplars. It is particularly important to control weeds in the phase of plantation establishment. The strategies of control are different depending on the region, for example the annual rainfall pattern gives an idea about the amount of herbicides that will be leached, and the allowed herbicides vary in different countries. Herbicides should be applied the year before the plantation establishment and just before or after planting when the cuttings are still dormant. It has been reported that poplars are very sensitive to damages by herbicides (Buhler *et al.*, 1998; Netzer & Hansen, 1992, 1994; Netzer *et al.*, 1997; OMNR, 1991). There are also some herbicides that are tolerated by poplars. During the growing season many types of cultivators are used for mechanical weed control (steam shovel, discs, rototillers, cultivators), but the depth of cultivation should be maximum 5 cm to avoid damages on the roots of poplars. The slope, accessibility and even the type of soil will determine the kind of machinery that will be used.

Fertilization

The goal of fertilization is to maximize the growth of the plantation. It is essential to have a balance of nutrients in the soil. The most limiting element for poplar plantations is nitrogen (N). N-deficiency can result in reduced growth, sylleptic branches, etc. Excess of fertilizers, on the other hand, can result in increased weed competition or prolong the growth of poplars until late into growing season. A consequence of a prolonged growing season in Swedish climate may be poor frost hardiness, which results in frost damage on shoots during the winter. The most common method to determinate the existence of nutrient deficiencies is through the leaf analysis. Phosphorous can be limiting as well in those soils that are coarse-textured and well-drained. Due to Dickmann *et al.* (2001) the adequate ratios of nutrients for poplar plantations are 100 N : 48 K : 11 P : 7 Ca : 7 Mg. Other nutrients can be applied separately or together with N.

Fertigation can be used in order to improve the supply of nutrients. It consists of the application of nutrients in the irrigation water. This technique requires high initial costs and constant costs of maintenance.

Coppicing

Coppicing is a method of regeneration that works as an alternative to replanting. This regeneration method is suitable for poplars because of their great ability to build new sprouts from stumps or root collars. It is generally used in second rotation in eastern cottonwood plantations, but it should be avoided if the desired end products are veneer logs, sawlogs or pulp, because the quality of stems is poor. It is an interesting technique in economical terms, because the costs of establishment are low, which makes it an interesting alternative for non-industrial, private landowners. The disadvantage is that harvesting operation can be expensive. The main factors that affect coppice regeneration are the age of the stand and the time of harvesting. Harvesting should be started before an age of 10 years, during the dormant season, for Sweden approximately between October and April.

Suckering

Suckers arise from adventitious primordia, building a new plant with an independent root and shoot system. It is well-known that the task of removing apical dominance and increasing soil temperatures provoke the stimulation of flushing and expansion of adventitious primordia into suckers (Dickmann *et al*., 2001). According to DesRochers (2000), *"if the arising suckers do not have to spend energy on the development of a new root system, they are able to invest more energy into height and leaf area growth"*. The time required to complete suckering is approximately two years.

There are many factors that can negatively affect suckering such as: site conditions, age of the stand, plant competition, and presence of herbivores, *Armillaria* root rot infection, and soil disturbance. Soil compaction, for example can change its physical properties and damage the root system. The main limiting factor in the boreal forest is the low soil temperature, especially in those soils that have a high and thick organic content (Hogg & Lieffers, 1991). Some studies show that a possible threshold of 15ºC can be suggested for expecting a successful regeneration by suckers (Hungerford, 1988; Maini, 1967). Suckering has been observed in almost all of the riparian poplars, especially in the *Tacamahaca* section (Rood *et al*., 1994).

A comparison between seedling and sucker growth of aspens in North America shows that suckers grow 3-5 times faster the first decade of stand development (Dickmann *et al*., 2001).

While there have been suggestions that wounding of aspen roots can increase suckering, there have also been some general statements that wounding is detrimental to suckering on the long-term growth of the stands (Steneker, 1973; Navratil & Bella 1990).

Poplars plantation establishment in Sweden

The climate conditions of Sweden can convey difficulties for the establishment of poplar plantations. The most severe problems are related to the late frost during the periods of transition between dormant and growing seasons, which increases susceptibility to attacks by fungi and bacteria. To counteract this problem new material of poplar from North America has been studied for suitability to the Swedish climate conditions (Christersson, 1996). Other problems are related to browsing of the wild fauna such as moose, red deer and roe deer that feed on shoots and bark of young trees (FAO, 1979). This kind of damages is more severe in plantations established on a land surrounded by large forest areas. The most effective protection against browsing is by fencing the whole plantation, which increases the costs of establishment, especially for relatively small plantations. Other problems are mostly related to weeds.

Damages caused by wildlife

Moose (*Alces alces* Lin., figure 3) is widespread all over Sweden with a population of over 400,000 individuals (Naturetrek, 2006). The Swedish moose population is the densest moose population per square kilometre in the world. On average, an adult moose can reach around 1.8-2.1 m in height (Macdonald & Barrett, 2008), and is able to browse on several meters long shoots.

Figure 3. Moose. Photo: Teresa Brage Tuñón, Sweden.

With a population of around 1 million individuals the most common species of deer in Sweden is the roe deer (*Capreolus capreolus* Lin.) that usually appears in the south and middle of Sweden, (http://www.sverigeturism.se/smorgasbord/smorgasbord/natrecspo/nature/wildlife.html). Other common species mostly in southern Sweden are the red deer (*Cervus elaphus* Lin.) and the fallow deer (*Dama dama* Lin.).

Deer browse a variety of seedlings, especially when the forage is limited and their population density is high. Generally, browsing caused by deer occurs from the ground up to 1.85 meters (Hygnstrom *et al*., 2008). Browsing causes the development of multiple leaders, increases the susceptibility to frost damages, decreases the height of seedlings and increases enormously their mortality (NRCS, December 2007).

During the browsing activities terminal and lateral shoots are removed from saplings and seedlings. In many cases seedlings are entirely uprooted with broken tops and the whole plantation may have a ragged appearance. The moose peels the strips of the young bark off using its lower incisors leaving vertical toothmarks in the exposed sapwood (figures 4 and 5). During antler polishing in the late summer and autumn bark is pulled up from the trunk and branches of young trees. If the browsing activities are frequent the seedlings and saplings can acquire stunted and bushy shapes.

Figure 4. Bark of hybrid aspen striped off by moose.

Figure 5. Moose damage appears on almost all the trees of the stand. This stand was damaged at an age of 13 years, after the removal of fence. The extent of damages implies the necessity to maintain the fence during the whole first rotation period of hybrid aspen. Photo: Almir Karačić, SLU.

Open wounds favour development of pests and diseases and quickly decreases survival rate in a newly established poplar plantation. There are other mammals that can make the establishment of poplar plantations difficult, such as voles, mice, rabbits (*Oryctolagus cuniculus* Pollas) and hares (*Lepus caprensis* Lin., *Lepus timidus* Lin.). Voles (*Microtus* spp.) can be a problem because they feed on the lower parts of stems and roots of newly established poplars, decreasing heavily the survival rates.

Solving the problems of poplar establishment

According to Christersson (1996), frost damages in poplars plantations in Sweden could be avoided by using the crosses of *Populus trichocarpa* from higher latitudes in the USA and Canada. The use of hybrid poplars obtained in advanced breeding programs can improve yield considerably by providing the poplar growers with new clones of superior growth and resistance (or tolerance) to pests and diseases.

One of the best options to decrease the browsing damages is to reduce the density of the game. However, hunting is an important social activity in Sweden with approximately 300,000 registered hunters and the game management strategy is designed to maintain high density of moose, roe deer and red deer.

Fencing poplar plantations is the most efficient protection against animal browsing. This kind of protection has been practiced in some countries since 1939 (NRCS, December 2007). There are temporary and permanent fencing designs that can be applied depending on costs, type of game in the area and goals of the landowners. The recommended standard fence is the permanent one, high tensile, woven wire fence with a lifespan of more than 20 years (figure 6). This kind of fence is expensive, but the advantage is that the maintenance costs are small. It costs about $13-20 per meter excluding labor .The height of the fence should be at least 1.45 meters (Hygnstrom *et al*., 2008). This kind of fencing should be chosen when there are risks of damages by deer.

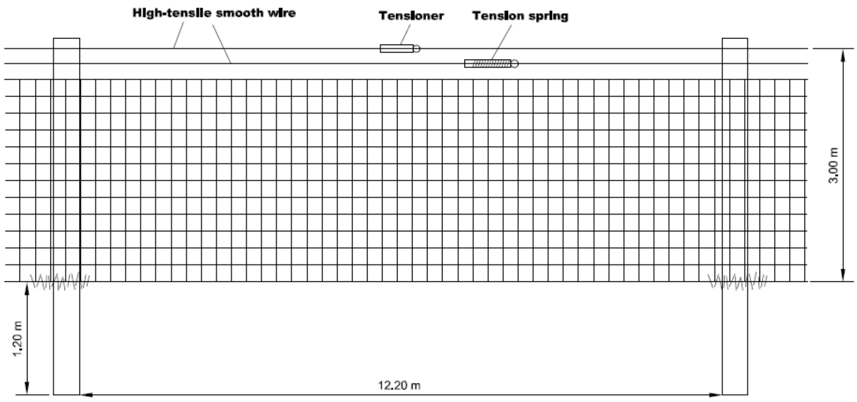

Figure 6.-Permanent woven wire fence charged with electricity (high tensile). Drawing: Teresa Brage Tuñón.

Also a less expensive ($6.6-13.1 per meter excluding labour) electric fence (figure 7) can be used. It normally consists of 6 or 7 strands of high tensile wire with a spacing of about 0.3 meters between the wires. The disadvantage of this kind of fencing is that frequent maintenance is required to ensure the lifespan of more than 20 years (Hygnstrom *et al.*, 2008). This kind of fence should be chosen when the pressure of deer is moderated.

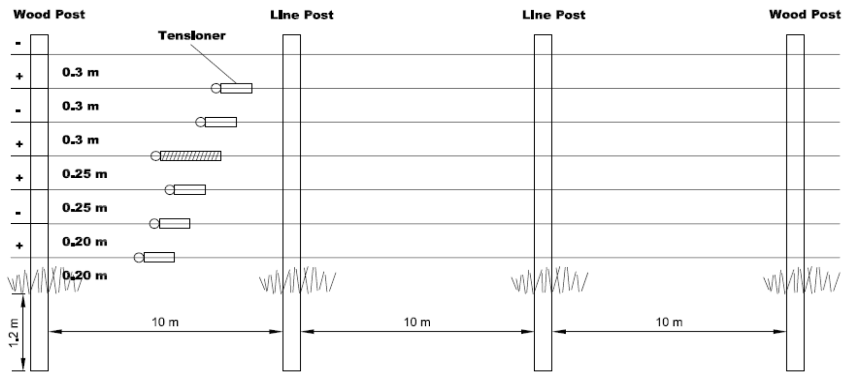

Figure 7. High tensile vertical fence. Design: Teresa Brage Tuñón

Among the temporary fences it is possible to find poly-tape and poly-twine fences that are portable electric fences (figure 8), which are useful for plantations that are larger than 17 ha, and in places under a moderated pressure of deer (Hygnstrom *et al*., 2008). In this case the lifespan is about 20 years, as well, but it should be removed from the field at the end of growing season and stored indoors during the winter period, what do it not so efficient. Baited fences with peanut butter (figure 9) attract a deer to contact the charged wire with its mouth and nose learning the animal to avoid fenced areas. The price of these fences can be $2-3 per meter for double or single-strands fences excluding the costs of labour (Hygnstrom *et al*., 2008). Higher prices can be reached if poly-materials with higher quality are used.

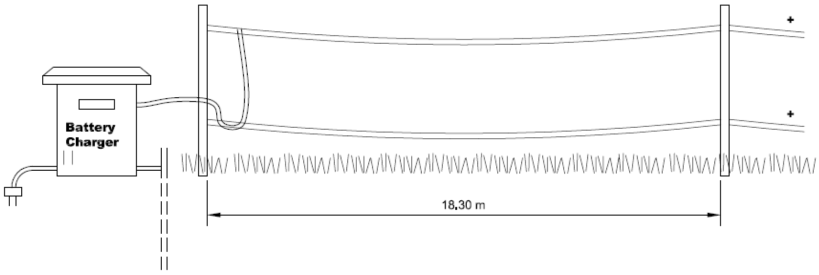

Figure 8. Temporary fence: Polytape fence. Drawing: Teresa Brage Tuñón.

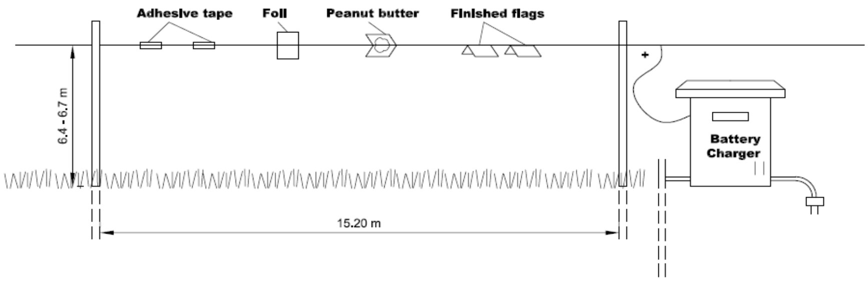

Figure 9. Temporary fence: Fence with peanut butter. Drawing: Teresa Brage Tuñón.

An option should be to construct fences without electricity large enough to protect plantation against moose. This option is much cheaper than electric fences and protects a plantation year around. Another advantage is that it also can be used against rabbits because it is fine-meshed in the lower part.

Individual plant protection with different types of covers (figure 10) is possible, but this option is more expensive and the cover usually cannot provide protection against moose. The most common individual protection is woven wire, chicken wire and plastic netting manufactured in degradable materials. There is a great variability of sizes, and the prices depending on supplier. As reference, it can be said that individual tubes and plastic cylinders vary in price between 2 to 5 $ per tube (Hygnstrom *et al*., 2008).

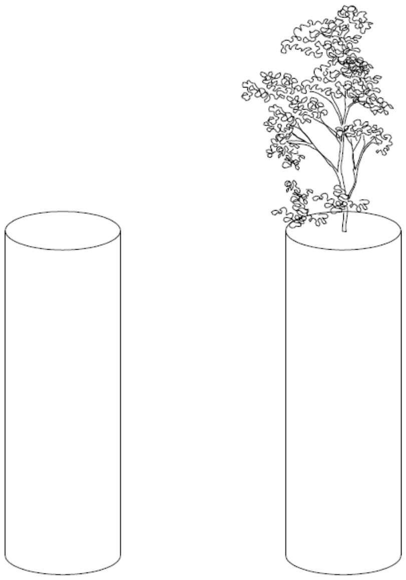

Figure 10. Plastic tube protection. Design: Teresa Brage Tuñón.

One option to decrease browsing damage is a selection of species and varieties that are less attractive to the browsing mammals. Palatability of plants is one of the breeding goals in some breeding programs.

Chemical repellents affecting the taste and smell (bitter tasting) can be used, provoking pain in receptors of deer. They are usually not used in a large scale, but are more appropriated for small areas (NRCS, December 2007). The efficiency of the repellents depends on the palatability of the protected plant compared to the availability or desirability of any alternative forage, the density of the browsers, the weather, and the amount and concentration of the repellent. Some studies show that the efficiency is higher on less palatable plants (NRCS, December 2007). These chemical products are effective only on the part of the plant that has been covered with them. The new emerging shoots that appear after the application are not protected. Weather greatly affects the efficiency of repellents. Both rain and snow reduce their efficiency.

Some researches have shown that deer is able to adapt quickly to frightening devices. It has been shown that shell crackers, propane cannons, deer guards and lasers are not effective against deer damages. However, visual and audio frightening devices are not typically used because of the public opinion. Dogs can be used to frighten deer from poplar plantations. The best pedigree of dog for Swedish conditions is the Husky, at least in the north of Sweden. First it is necessary to make an investment in fencing the zone and second to cover the costs of maintenance, veterinary, feeding and shelter for the dogs, approximately $650 per dog and year (Hygnstrom *et al*., 2008).

Any of these solutions is 100% effective according to researchers, but it can be said that a 50% reduction of browsing damages can be considered as successful (NRCS, December 2007). When protection has not been successful, replanting is required in order to establish poplar a plantation.

A pilot study: Pre-treatment of poplar cutting for better rooting

The success of poplar plantation establishment using cuttings depends on the ability of the cuttings to develop a root system. Many studies have been conducted to show how cuttings response to pre-treatment with growth regulators or hormones. According to Edward, Howard & David (1979), dipping cuttings into treatment with naphthalene acetic acid (NAA) and indole butyric acid (IBA) generally increases the percentage of rooted cuttings and the number of roots per cuttings. On the other hand, Petersen & Philippis (1976) concluded that certain hybrid poplar cuttings can improve rooting after pre-soaking them only in water.

Material and methods

This pilot study of rooting cuttings of two different poplar clones used in Sweden was done in the greenhouse at the Department of Energy and Technology, Swedish university of Agricultural Sciences in Uppsala. The goal of this experiment is to test the effect of NAA on rooting and plant development from single-bud cuttings grown in a commercial container system. For each clone three solutions were used: control (only water), Allgrow and the solution of naphthalene acetic acid (NAA). The expected result was that cuttings treated with the solution of NAA were going to develop the most vigorous rooting system.

First step was to prepare the three solutions. Second, preparing the cuttings and the substrate where they were planted. The cuttings where then soaked into prepared solutions (controll, Allgrow and NAA solution) for 42 hours. After planting the cuttings were planted in the substrate and fertilised with “Allgrow” and “Blomstra” during four weeks. Four plants per treatment and clone were harvested after four weeks and several parameters were measured in order to analyze plant growth.

The cuttings were planted at high density in two trays with 77 cells per tray according to the design showed in figure 11.

0	0	0	0	0	0	0	0	0	0	0
0	91	92	93	106	107	108	121	122	123	0
0	94	95	96	109	110	111	124	125	126	0
0	97	**98**	**99**	112	113	**114**	127	**128**	**129**	0
0	**100**	**101**	**102**	**115**	**116**	**117**	**130**	**131**	**132**	0
0	**103**	**104**	**105**	**118**	**119**	**120**	**133**	**134**	**135**	0
0	0	0	0	0	0	0	0	0	0	0

0	0	0	0	0	0	0	0	0	0	0
0	136	137	138	151	152	153	166	167	168	0
0	139	140	141	154	155	156	169	170	171	0
0	142	**143**	**144**	157	158	**159**	172	173	**174**	0
0	**145**	**146**	**147**	**160**	**161**	**162**	**175**	**176**	**177**	0
0	**148**	**149**	**150**	**163**	**164**	**165**	**178**	**179**	**180**	0
0	0	0	0	0	0	0	0	0	0	0

	SW+B
	SA+AI
	SNAA+B

Figure 10. Trial design for "Rooting poplar cuttings". SW - water solution (controll), SA - allgrow solution, SNAA - NAA solution. Bold numbers - clone OP42, Normal numbers - clone Bullstofta

Preparation of solutions

The first step was to prepare three different solutions where cuttings were being soaked before planting into cointainers:

1. Controll - pure water without sand or other components
2. Allgrow solution – commercial fertiliser "Allgrow" dissolved in water at at ratio 1:30 (table 4)
3. Naphthalene acetic acid (NAA) is a synthetic growth regulator called auxin that can be used to stimulate the development of the rooting system in poplar cuttings. The materials used for preparing this solution were:
    - 1-Naphthylacetic acid (PESTANA©, $C_{12}H_{10}0_2$) delivered as powder.
    - Dymethyl Sulfoxide (DMSO, $(CH_3)_2S0$). This is the medium used to dissolve NAA.
    - Test-tube
    - Pipette
    - Funnel

Table 4. Nutrient composition of "Allgrow"

N 660 mg/l	Zn 8.5 mg/l
P 184 mg/l	B 44 mg/l
K 722 mg/l	Pb 0.1 mg/l
Cu 3.7 mg/l	Hg 0.0002 mg/l
S 310 mg/l	Fe 5.6 mg/l
Cr 0.05 mg/l	Ca 15 mg/l
Ni 0.07 mg/l	V 0.89 mg/l
Na 27 mg/l	Mo 0.28 mg/l
Mn 17 mg/l	Mg 21 mg/l
Co 0.49 mg/l	Cd 0.01 mg/l

Usually, the NAA concentration of 0.5-1% is used for rooting poplar cuttings. I have used a concentration of 0.75% that is prepared as follows:

- A piece of aluminium foil is weighed without NAA to calibrate the scale.
- 0.05 g of NAA was weighed.
- The aluminium foil was removed making sure that all the powder of NAA has been poured into the test-tube.
- The NAA is dissolved in 50 ml of DMSO. All the powder is carefully removed from the walls of the aluminium foil.
- Stirring the mixture untill all the powder is dissolved in DMSO.
- This basic solution in DMSO is later used to make water solution of NAA by diluting 75 ml of basic solution in 999.25 ml of pure water with pH of 6.5.

Cuttings

Two hybrid poplar clones were used in this study: OP42 (*Populus maximowiczii* Henry x *Populus trichocarpa* T. & G), a commercially produced clone that is widely used southern Sweden and Bullsofta (*Populus trichocarpa* Hook x *Populus deltoides* Bartr.) bred to fit the climate conditions in southern Sweden.

Single-bud cuttings, at least 5 cm long were prepared, and soaked into the solutions for approximately 42 hours. The two clones were soaked in separate containers and with solution covering cuttings but not submerging the buds (figures 11 and 12).

Figure 11. Cutting immersed in three different solutions: water, Allgrow and NAA. Photo: Teresa Brage Tuñón

Figure 12. Detail of the cuttings absorbing the solutions. Photo: Teresa Brage Tuñón

Preparation of the growth substrate

There are three functions of the rooting medium:

1. To keep the cutting during the rooting period.
2. To provide moisture for the cutting.
3. To allow the entrance of air to the base of the cutting.

An optimal rooting medium must provide a good aeration and have a high water-holding capacity. The rooting medium is usually prepared of a mixture of peat moss with perlite, or a mixture of sand and peat moss. The pH of the substrate is usually neutral. The substrate used in this study was a mixture of 75% commercial soil with a pH 6.5 (peat), 15% perlite and 10% vermiculite. The mineral composition of the soil is given in table 5.

Table 5. Mineral composition of soil used for rooting poplar cuttings

Näringsinnehåll g/m^3	
N 180	Fe 6.0
P 110	Mn 3.5
K 195	Cu 2.5
Mg 260	Zn 1.5
S 100	B 0.6
Ca 2000	Mo 3.0
pH 5.5-6.5	

The cuttings were planted into 150 cm^3 containers with air strips on the side walls. The containers were placed into a tray system Flexi Frame 77 (BCC AB, Landskrona, Sweden). Each tray contained 77 cells 15 cm deep and with an opening on the top of 4x4 cm (figure 14 and 15).

Figure 13. Single cell from Flexi Frame 77 (BCC AB, Landskrona, Sweden) Photo: Teresa Brage Tuñón

Figure 14. Flexi Frame 77 (BCC AB, Landskrona, Sweden). Photo: Teresa Brage Tuñón

Planting the cuttings

As it was mentioned before, the cuttings were planted after soaking them for 42 hours leaving one bud above the substrate surface (figure 16 and 17). Air pockets in the substrate were eliminated permitting a good contact between cutting and the soil.

Figure 16. First leaves appearing the buds within a few days after the cuttings have been planted. Photo: Teresa Brage Tuñón

Figure 17. Cuttings planted following the trial for rooting poplar trial. Photo: Teresa Brage Tuñón

Fertilization

The plants were fertilised seven times with two different kinds of fertiliser. The cuttings pre-treated in NAA-solution or pure water were fertilised with balanced fertiliser "Blomstra" (table 6), whereas the cuttings pre-treated in Allgrow solution were fertilised with Allgrow (table 4). Each plant received 4.7 mg N in a 15 ml solution per each fertilisation occasion. Due to the high concentration of nitrogen solution a new, lower levels of N were applied after the three fertilisation occasions. Each plant continued to receive 1.9 mg of nitrogen per application. The total amount of nitrogen applied per plant was 13.3 mg.

Table 6. Nutrient content in 100 ml "Blomstra"

N 5.1 g	Fe 350 mg
P 1.0 g	Mn 20 mg
K 4.3 g	B 10 mg
S 0.4 g	Zn 3 mg
Ca 0.3 g	Cu 1.5 mg
Mg 0.4g	Mo 0.4 mg

Plant harvest

Four weeks after the start of the trial six and seven plants per treatment and clone (13 plants per treatment) were harvested. Plants were removed from containers and measured for relevant parameters.
The parameters measured are cutting length and diameter, number of roots per cutting, length of the longest root per cutting, number of leaves per cutting, leaf area (LA), shoot length, and the weight of cutting, root, leaves and shoot per each cutting (Annexe no. 1).

Each plant was removed from the container and immersed into water in order to remove as much substrate as possible. After the first immersion into water, the plant was moved to another container with clean water; where the roots were washed carefully (figure 18). The diameter and length of cutting, and root and shoot length were measured using digital calliper. Leaf area was measured using surface area meter (LICOR 3100, Lincoln, NE, figure 19). The weight of cuttings, roots, leaves and shoots was measured one week later after drying in an oven at 100 ºC (figure 20).

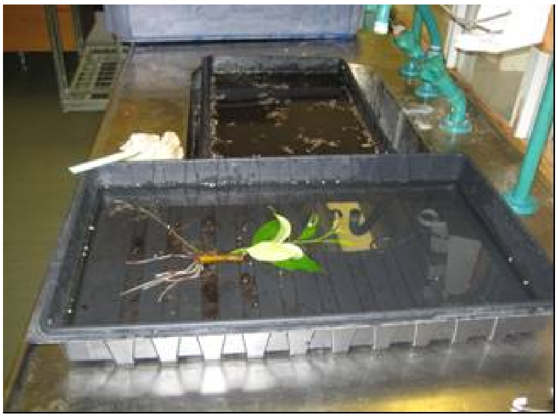

Figure 18. Plants immersed into the water. Photo: Teresa Brage Tuñón

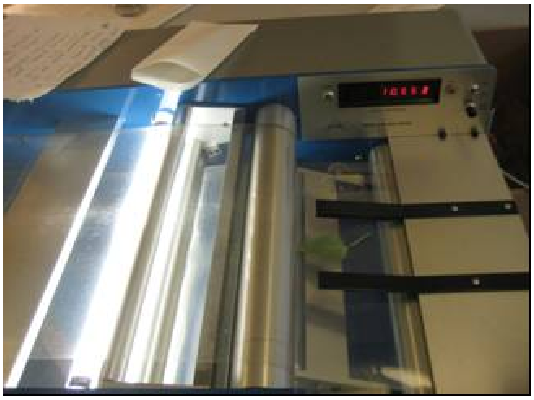

Figure 19. Surface area meter (LICOR 3100, Lincoln, NE). Photo: Teresa Brage Tuñón

Figure 20. Electronic scale used to measure the dry weight of cuttings, shoots, roots and leaves. Photo: Teresa Brage Tuñón

Statatistical analysis

The effects of treatments and treatment-clone interactions on length of the longest root, root weight, root/shoot ratio, leaf area and specific leaf area were analysed using covariance analysis with the length of cutting as a covariate. The assumptions are that the dependent variable is distributed according to a normal distribution and that variances are approximately equal.

The data was analysed using SYSTAT 12 software (2008). The study was design as a full factorial experiment. Comparisons of means were done using "Least Squares Means". A chosen significance level of P-value was < 0.05.

There are two factors or independent variables: treatments (NAA solution, Allgrow solution and pure water solution) and clones (OP42 and Bullstofta). On the other hand, several dependent variables were chosen: length of the longest root, root weight, cutting length, leaf area, specific leaf area and root/shoot ratio.

After analysing the data, it was discovered that the data did not comply with the normal distribution. Therefore, the data transformations were made using the best adjustment for each variable. Root length was transformed to square root length (figure 21). Root weight and cutting length were not transformed, because it was not necessary. Leaf area was transformed to logarithm of leaf area (figure 21). Root shoot ratio was transformed to logarithm to base 10 (figure 21). Specific leaf area (SLA, it is relationship between leaf area and leaf weight) and net assimilation rate (NAR, it is biomass assimilation per leaf area in a certain time period) were not transformed.

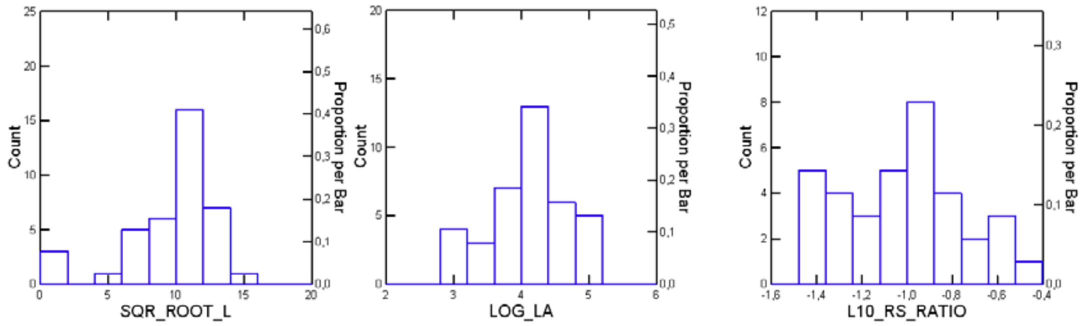

Figure 21. Distribution of dependent variables converted in order to comply with the normal distribution. Left up diagram represents the distribution of square root transformed to root length. Right up diagram represents the distribution of leaf area logarithm transformed to leave area. Down diagram represents the distribution of logarithm to base 10 of root shoot ratio transformed to root shoot ratio.

Results and discussion

The ANOVA results are shown in ANOVA tables for each dependent variable. The analysis of variance for square root length is shown in table 7. There were significant differences between treatments (figure 22, P-value = 0.003 < 0.05). The cuttings pre-treated with NAA had significantly shorter length of the longest root compared to the cuttings pre-treated with Allgrow. A negative effect of NAA on the length of longest root could have been caused by an inadequate concentration. Another option would be that combination of given concentration of NAA and a high concentration of applied “Blomstra” fertiliser caused damages on tissues in cuttings. There were no significant differences in length of the longest root between clones or the clone-treatment interactions. The length of cutting did not have a significant effect on this variable.

Table 7. ANOVA table for the length of the longest root

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	138,654	2	69,327	7,264	0,003
CLONES	10,040	1	10,040	1,052	0,313
TREATS*CLONES	3,456	2	1,728	0,181	0,835
CUTTING LENGTH	16,715	1	16,715	1,752	0,195
Error	305,388	32	9,543		

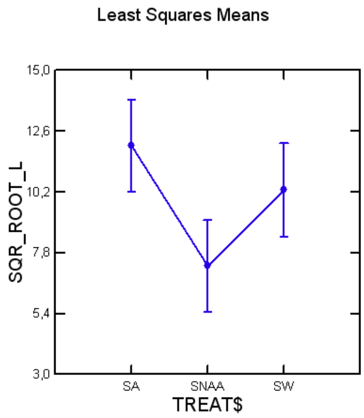

Figure 22. Least squares means for the dependent variable of the length of the longest root. The xlabel includes the three treatments for soaking the cuttings: allgrow solution (SA), solution of NAA (SNAA) and water solution (SW).

Results for root weight are shown in table 8. According to the obtained F-ratios and P-values there were no significant differences between treatments, clones, and clone-treatment interactions. It is though important to conclude that root biomass of clone OP42, but not of clone Bullstofta, was negatively affected by NAA pre-treatment (figure 23).

Table 8. ANOVA table for root weight

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	1 931,179	2	965,589	2,699	0,083
CLONES	70,655	1	70,655	0,198	0,660
TREATS*CLONES	1 299,037	2	649,518	1,816	0,179
CUTTING LENGTH	144,191	1	144,191	0,403	0,530
Error	11 447,471	32	357,733		

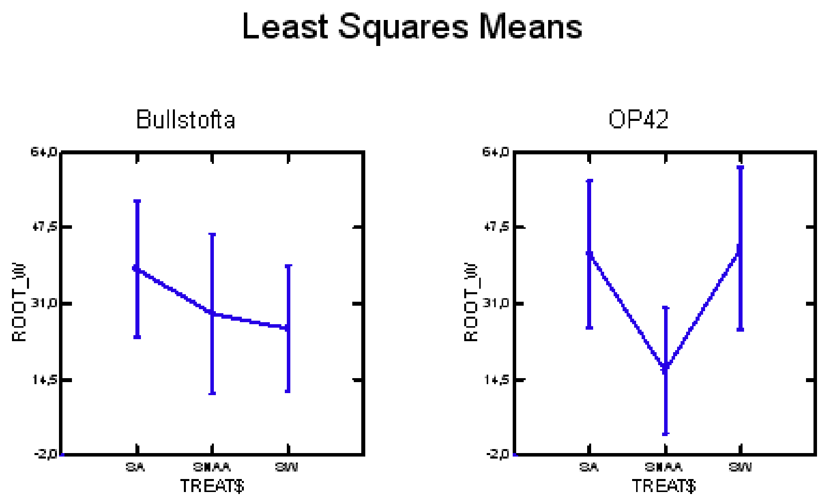

Figure 23. Least squares means for the dependent variable of root weight. The xlabel includes the three solutions for soaking the cuttings: allgrow solution (SA), solution of NAA (SNAA) and water solution (SW). The left figure is for Bullstofta clone and the right is for OP42 clone.

The results for leaf area showed no significant differences between treatments and clones (table 9). The cutting length had significant impact on the obtained F and P values (0.042 < 0.05) for treatment and clones.

Table 9. ANOVA table for leaf area

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	0,751	2	0,376	1,417	0,258
CLONES	0,786	1	0,786	2,965	0,095
TREATS*CLONES	0,573	2	0,286	1,080	0,352
CUTTING LENGTH	1,195	1	1,195	4,508	0,042
Error	8,219	31	0,265		

The root/shoot ratio is the relationship between weight of biomass stored in bellow and above-ground plant parts. The weight of cutting is not included in this relationship. In this study there were no significant differences between treatments and clones. A significant clone-treatment effect was related to opposite pattern of response for the two clones to NAA treatment (figure 24).

Table 10. ANOVA table for the root/shoot ratio

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	0,042	2	0,021	0,494	0,615
CLONES	0,002	1	0,002	0,053	0,820
TREATS*CLONES	1,074	2	0,537	12,592	0,000
CUTTING LENGTH	0,107	1	0,107	2,515	0,124
Error	1,195	28	0,043		

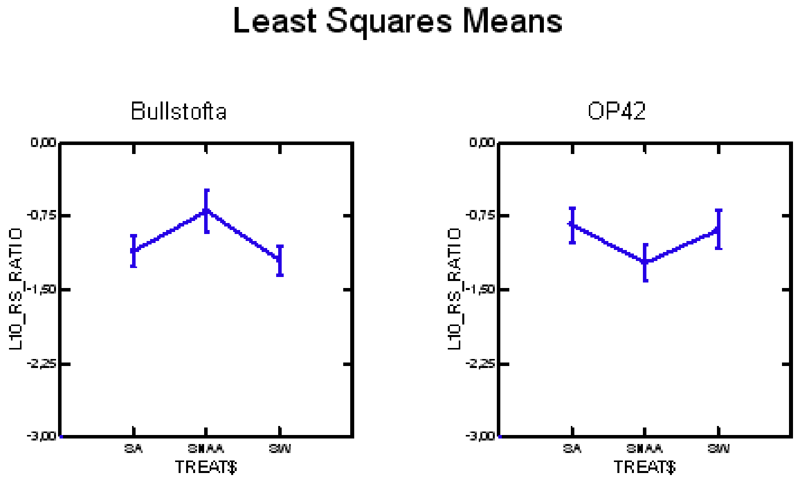

Figure 24. Least squares means for the dependent variable of root/shoot ratio. The xlabel includes the three solutions for soaking the cuttings: allgrow solution (SA), solution of NAA (SNAA) and water solution (SW). The left figure is for Bullstofta clone and the right is for OP42 clone.

The specific leaf area (SLA) is the relationship between leaf area and leaf weight and is often used in growth analysis as an important characteristic of a species or variety. However, this parameter is also affected by different growth conditions, especially available light. Being to a large extent an inherent characteristic, SLA was not affected by treatment or cutting length. The differences in SLA between the two clones are significant, (table11, figure 25).

Table 11. ANOVA table for the specific leaf area

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	32,716	2	16,358	1,066	0,357
CLONES	168,518	1	168,518	10,986	0,002
TREATS*CLONES	25,440	2	12,720	0,829	0,446
CUTTING LENGTH	0,608	1	0,608	0,040	0,844
Error	475,509	31	15,339		

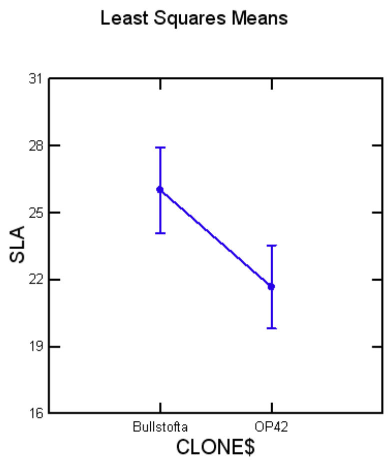

Figure 25. Least squares means for the dependent variable of the specific leaf area. The xlabel represents the two used clones: Bullsofta and OP42.

Net assimilation rate showed no significant differences for any of the treatments. However, a relatively low P-value for clone suggests that also net assimilation rate might be used as a parameter of measuring differences between different poplar varieties (table 12).

Table 12. ANOVA table for the net assimilation rate

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-ratio**	**p-value**
TREATS	175,547	2	87,773	0,777	0,469
CLONES	348,780	1	348,780	3,086	0,089
TREATS*CLONES	161,932	2	80,966	0,716	0,496
CUTTING LENGTH	7,847	1	7,847	0,069	0,794
Error	3 503,278	31	113,009		

A positive effect of soaking poplar cuttings into NAA solution (or other rooting hormones) is well-known in the literature (Nordine, 1984). In this trial however, no positive effects of pre-treatment with NAA were observed. The pattern of root/shoot ratio in the two clones implies the necessity to test the different concentrations of rooting medium on several clones. This parameter is also important in practical plant propagation as the goal is to produce the plants with larger root/shoot ratio given the desired size of above-ground part of plants.

Conclusions

The success of plantation establishment using poplar cuttings depends mainly on the ability of the cuttings to develop early and vigorous root system.

Normally, pre-soaking stimulates rooting of cuttings of poplar clones increasing the plant survival. In some cases, individual clones do not respond significantly to soaking.

In the greenhouse trial conducted within the frames of this study the results do not support the hypothesis regarding the positive effects of soaking cuttings in NAA.

Another consideration is that the high concentration of added fertilizers might have damaged the roots and caused a negative effect of NAA. According to Hudson & Dale (1975), the application of high concentration of hormones or growth regulators can affect negatively root development.

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ANNEXE 1. Results of the first harvest

Nr	Clone	Treatment	Cutting length mm	Cutting diameter mm	Root no. Callus	Root no. Cutting	Root length mm	Root tip no	Leaf no.	LA cm^2	Shoot length mm	Cutting Weight mg	Root Weight mg	Leaf Weight mg	Shoot Weight mg
181	OP42	SNAA	52	11	0	1	76	No data	2	38,7	27	1829	12	198	24
182	Bullstofta	SNAA	54	10	0	0	0	No data	3	36,6	33	1528	24	95	21
183	OP42	SNAA	61	10	0	2	85	No data	2	33,6	21	1519	0	169	25
184	Bullstofta	SNAA	55	10	0	2	36	No data	1	0	8	911	4	0	11
185	OP42	SNAA	53	11	0	0	0	No data	4	23,2	17	1494	0	118	14
186	OP42	SA	57	10	0	2	122	No data	8	84,3	81	2008	30	420	68
187	Bullstofta	SA	47	12	2	4	93	No data	5	56,1	45	1148	22	286	45
188	OP42	SA	56	10	0	5	156	No data	4	59,8	29	1862	42	315	29
189	Bullstofta	SA	55	8	2	5	138	No data	9	73	149	601	39	293	72
190	Bullstofta	SA	59	11	3	6	94	No data	11	74,5	115	1626	41	317	65
191	OP42	SA	49	8	0	4	147	No data	4	22,8	19	886	30	118	19
192	OP42	SA	54	9	1	6	154	No data	5	29,8	38	1217	33	120	22
193	Bullstofta	SA	55	10	0	14	152	No data	12	99,4	161	1437	55	368	94
194	Bullstofta	SA	53	12	0	3	81	No data	12	70,2	118	1724	15	254	58
195	OP42	SA	52	12	1	10	238	No data	11	110,7	151	2339	82	502	108
196	Bullstofta	SNAA	58	10	5	6	139	No data	5	31,9	21	1243	60	211	40
197	OP42	SNAA	60	11	0	4	123	No data	9	76,6	76	1908	18	363	55
198	Bullstofta	SNAA	59	12	0	3	62	No data	5	60,1	46	2166	0	221	38
199	Bullstofta	SNAA	59	10	4	9	137	No data	13	150,7	193	1307	60	607	165
200	OP42	SNAA	58	10	1	4	157	No data	8	66,8	70	1825	38	403	49

201	Bullstofta	SW	57	10	1	9	111	No data	12	134	188	1228	23	451	117
202	Bullstofta	SW	55	10	1	5	47	No data	10	70	109	1225	12	283	55
203	OP42	SW	69	5	0	12	118	No data	10	115,2	184	501	59	508	128
204	Bullstofta	SW	58	10	0	3	143	No data	12	138,7	153	1208	33	504	117
205	OP42	SW	59	9	0	5	126	No data	12	97,5	132	1164	51	436	87
206	Bullstofta	SW	55	10	4	0	33	No data	7	37,3	43	1292	6	137	27
207	OP42	SW	52	7	0	5	144	No data	9	80,4	135	467	43	305	67
208	OP42	SW	54	9	0	5	132	No data	10	52,1	102	1181	46	253	60
209	OP42	SW	56	6	0	4	136	No data	6	37,4	60	584	23	134	21
210	Bullstofta	SW	52	10	2	6	137	No data	6	46,9	77	1209	34	203	60
211	Bullstofta	SW	57	10	0	2	58	No data	5	40	46	1030	9	166	35
212	OP42	SNAA	53	11	0	0	0	No data	2	20	21	1594	0	99	0
213	Bullstofta	SW	56	10	2	8	125	No data	11	98	148	1020	41	325	83
214	Bullstofta	SA	56	11	1	4	160	No data	10	129,5	194	1477	44	507	123
215	Bullstofta	SA	53	9	0	9	138	No data	12	124,4	215	492	48	437	129
216	OP42	SNAA	56	11	2	1	56	No data	8	75,4	99	2076	13	314	58
217	OP42	SNAA	49	11	5	10	89	No data	7	68,1	59	1783	49	379	38
218	OP42	SA	55	8	2	4	129	No data	4	20,6	22	1133	28	96	15
219	Bullstofta	SW	56	9	3	7	129	No data	9	69,1	112	866	48	285	53

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