Dissertationes Forestales 33

Multiplication of hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) from cuttings

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Academic dissertation

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ABSTRACT

The primary aim of the present study was to find an efficient and simple method of vegetative propagation for producing large numbers of hybrid aspen (*Populus tremuloides* L. x *P. tremula* Michx.) plants for forest plantations. The key objectives were to investigate the main physiological factors that affect the ability of cuttings to regenerate and to determine whether these factors could be manipulated by different growth conditions. In addition, clonal variation in traits related to propagation success was examined. According to our results, with the stem cutting method, depending on the clone, it is possible to obtain only 1–8 plants from one stock plant per year. With the root cutting method the corresponding values for two-year-old stock plants are 81–207 plants. The difference in number of cuttings between one- and two-year-old stock plants is so pronounced that it is economically feasible to grow stock plants for two years. There is no reason to use much older stock plants as a source of cuttings, as it has been observed that rooting ability diminishes as root diameter increases. Clonal variation is the most important individual factor in propagation of hybrid aspen. The fact that the efficiently sprouted clones also rooted best facilitates the selection of clones for large-scale propagation. In practice, root cuttings taken from all parts of the root system of hybrid aspen were capable of producing new shoots and roots. However, for efficient rooting it is important to use roots smaller than one centimeter in diameter. Both rooting and sprouting, as well as sprouting rate, were increased by high soil temperature; in our studies the highest temperature tested (30ºC) was the best. Light accelerated the sprouting of root cuttings, but they rooted best in dark conditions. Rooting is essential because without roots the sprouted cutting cannot survive long. For aspen the criteria for clone selection are primarily fiber qualities and growth rate, but ability to regenerate efficiently is also essential. For large-scale propagation it is very important to find clones from which many cuttings per stock plant can be obtained. In light of production costs, however, it is even more important that the regeneration ability of the produced cuttings be high.

Keywords: vegetative propagation, regeneration ability, stem cuttting, root cutting.

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Vantaa, November 2006

Niina Stenvall

LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles and manuscripts, which are referred to in the text by their Roman numerals:

I Yu, Q., Mäntylä, N. and Salonen M. 2001. Rooting of hybrid clones of *Populus tremula* L. x *P. tremuloides* Michx. by stem cuttings derived from micropropagated plants. Scandinavian Journal of Forest Research 16: 238-245.

II Stenvall, N., Haapala, T. and Pulkkinen, P. 2004. Effect of genotype, age and treatment of stock plants on propagation of hybrid aspen (*Populus tremula* x *Populus tremuloides*) by root cuttings. Scandinavian Journal of Forest Research 19: 303-311.

III Stenvall, N., Haapala, T. and Pulkkinen, P. 2006. The role of a root cutting´s diameter and location on the regeneration ability of hybrid aspen. Forest Ecology and Management. (In press).

IV Stenvall, N., Aarlahti, S., Haapala, T. and Pulkkinen, P. 2005. The effect of soil temperature and light on sprouting and rooting of root cuttings of hybrid aspen clones. Canadian Journal of Forest Research 35: 2671-2678.

V Stenvall, N., Piisilä, M. and Pulkkinen, P. Does the variation of root carbohydrates explain the clonal differences in sprouting efficiency of hybrid aspen root cuttings? (Submitted manuscript).

CONTRIBUTIONS

	I	II	III	IV	V
Original idea	MS	PP, NS	NS, PP	NS	NS
Study design	MS, NS	NS, PP	NS	NS, SA	NS
Data gathering	NS, QY	NS, *	NS, *	NS, SA	NS, MP
Analyses	QY, NS	NS, TH	NS	NS, SA	NS, MP
Manuscript preparation	QY	NS, TH	NS, TH	NS, TH	NS

MP: Maria Piisilä, MS: Maija Salonen, NS: Niina Stenvall, PP: Pertti Pulkkinen, QY: Qibin Yu, SA: Säde Aarlahti, TH: Tapani Haapala, * : several research assistants

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INTRODUCTION

Biology of *Populus* species: European (*Populus tremula* L.) and hybrid aspen (*P. tremula* x *P. tremuloides* Michx.)

Species of the genus *Populus* (Salicaceae) are widely distributed throughout the northern hemisphere (Eckenwalder 1996). The number of species ranges from 22 to 85 plus hundreds of hybrids, varieties and cultivars, because within the genus occur extensive interspecific hybridization and high morphological variation (Eckenwalder 1996). *Populus* species are economically and ecologically important because of their suitable wood properties (i.e for paper making and timber), fast growth rate and high biodiversity of organisms dependent on living or decaying trees of *Populus* (Angelstam and Mikusinski 1994, Zsuffa et al. 1996, Hammond 1997, Hazell et al. 1998, Martikainen 2001, Ranua 2001, Rautio et al. 2001). *Populus* species have also become a very important model organism for the study of tree biology (Stettler et al. 1996). European aspen is a broad-leaved, dioecious tree species that is widely distributed in Eurasia and also in North Africa. Aspen is one of the fastest growing tree species in Finland and the northern hemisphere, although in these areas it is at the northern fringe of its geographical range (Eckenwalder 1996, Hynynen and Karlsson 2002). Hybrid aspen is a man-made hybrid between European aspen and the closely related North American quaking aspen (*P. tremuloides*). Hybrid aspen grows even faster than European aspen and in Fennoscandia can reach a height of 20 meters in just 25 years (Hynynen and Karlsson 2002).

Aspen can reproduce both sexually and asexually (Eckenwalder 1996), but successful sexual reproduction is infrequent despite the abundant crop of viable seeds (Bärring 1988, Worrell 1995). Germination and seedling establishment require sufficient moisture conditions (Latva-Karjanmaa et al. 2003); and the viability of aspen seeds decreases quite rapidly (Fechner et al. 1981). On the other hand, the asexual reproduction of aspen from root suckers is efficient (Bärring 1988, Worrel 1995). Due to clonal expansion, aspen can be competitive in all kinds of forests (Eckenwalder 1996), but in mixed forests in Finland it is a minority species, and does not usually form large stands (Anon. 2005).

Adventitious organogenesis in aspen

Endogenous control of adventitious organogenesis

The physiological and morphological factors related to the regeneration process depend on the species, genotype, age of the stock plant and the characteristics of the plant part (Lowell and White 1986, De Klerk 2000, Hartmann et al. 2002). Control of the ability to regenerate is associated mainly with changes in levels of endogenous plant hormones and metabolites (Haissig 1974b, Jarvis 1986, De Klerk 2000).

The main regulators of the formation of new shoots and roots in plant tissue are plant hormones, and the most important of these is usually the ratio of auxin and cytokinins (Skoog and Miller 1965, Sachs 1991, Hausman et al. 1997, De Klerk 2000). Auxin is synthesized in both the aerial portions of the plant (especially in the apical meristems and young developing leaves) and in the roots (Cline 1991, Ljung et al. 2005). The auxin derived from the shoot is transported basipetally towards the root system (Cline 1991, Casimiro et al. 2003, Friml 2003). There can also be auxin transport within the root system from the root apex and other parts of the roots (Ljung et al. 2005). Auxin effectively inhibits the outgrowth of axillary buds (Cline

1991, Horvath 1999, Wan 2006), a phenomenon called apical dominance (Eliasson 1961, Farmer 1962a, Eliasson 1971c, Cline 1991, Ridge 1991). Apical dominance can be disturbed by stress, such as grazing or cutting off the apical bud, or changes in environmental conditions. Lowered concentration of auxin will lead to frequent burst of axillary buds from the stem or initiation of shoot primordia from the root system (Farmer 1962a, Eliasson 1971c, Schier 1973c, Horvath 1999, Wan 2006). Auxin is also related to adventitious rooting of cuttings; it is necessary in the beginning of root initiation and again in elongation of root primordia, but not between or after these periods (Jarvis 1986, Hausman et al.1997). Cytokinins are synthesized mainly in the root system and transferred via the xylem to other parts of the plant (Van Staden and Davey 1979, Letham 1994). Shoot meristems can also synthesize cytokinins, but the production of these compounds is distributed more locally (Böhner and Gatz 2001, Shimizu-Sato and Mori 2001, Sakakibara 2006). Cytokinins mainly inhibit root initiation and promote shoot initiation (Winton 1971, Wolter 1968, Van Staden and Harty 1988, Werner et al. 2001, Schmülling 2002). The influence of cytokinins may thus depend on their concentration and on the stage of the root initiation process (Okoro and Grace 1978, Jarvis 1986, Van Staden and Harty 1988, Cline 1999, Schmülling 2002). Cytokinins may also affect the rooting process indirectly through effects on rejuvenation and carbohydrate mobilization (Hartmann et al. 2002). In general, a high auxin-cytokinin ratio in plant tissue promotes initiation of adventitious roots, and a low auxin-cytokinin ratio favours formation of adventitious buds (Skoog and Miller 1965, Schier 1976, Schier 1981, Sachs 1991, Werner et al. 2001).

The effect of other plant hormones is variable: they can stimulate and inhibit regeneration by themselves or act as synergists or antagonists together with other plant hormones (De Klerk 2000). Gibberellic acid has been shown to inhibit the first cell divisions of adventitious root formation, but it has also induced rooting in stem cuttings taken from etiolated plants (Hansen 1988). According to Schier (1973a), exogenous gibberellic acid (GA_3) inhibites initiation of the first cell divisions of shoot primordia in the roots of aspen but later stimulates elongation of the adventitious shoot. Observations on abscisic acid and ethylene are also contradictory (Schier 1973d, Schier and Campbell 1978, Davies and Sankla 1988, Mudge 1988, De Klerk 2000). This may be due to the variable hormone interactions in different plant tissues, as well as interactions between genotypes and species and environmental conditions (Schier 1973a, Schier 1973b, Schier 1973d, De Klerk 2000, Hartmann et al. 2002). In addition, the requirements for shoot or root initiation can be entirely different from the conditions needed for their further development and elongation (Schier 1973a, Schier 1973b).

Adventitious shoot and root development

Development of adventitious shoots in the stem usually starts from axillary buds in the nodal area (Hartmann et al. 2002). The buds burst and start growing as a new shoot after the apical dominance is broken and the inhibitory effect of auxin decreases (Cline 1991). The root primordia develop from the xylem or the cambium cells of the stem (Fig. 1, Schier 1973b, Haissig 1974a).

The origin of shoot development (sprouting) from roots differs between species of poplar. In the roots of *P. angustifolia, P. deltoides* and *P. balsamifera* the shoot develops from preexisting suppressed buds (Schier and Campbell 1976). These buds (also called adventitious buds) arise from systems of primary tissue in the root periderm (Schier and Campbell 1976), which develops in the outer cortex during secondary growth of the root (Fig. 2, Fahn 1987). In roots of *P. tremuloides*, the new shoots usually originate from the phellogen (cork cambium), which develops from cells of the pericycle (Fig. 2, Schier 1973b, Fahn 1987). Meristems that

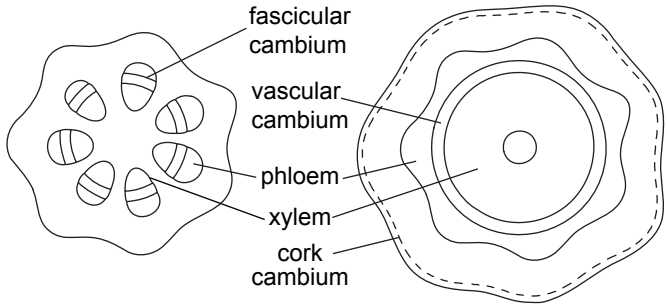

Figure 1. Cross section of a stem (Modified from Ridge 1991).

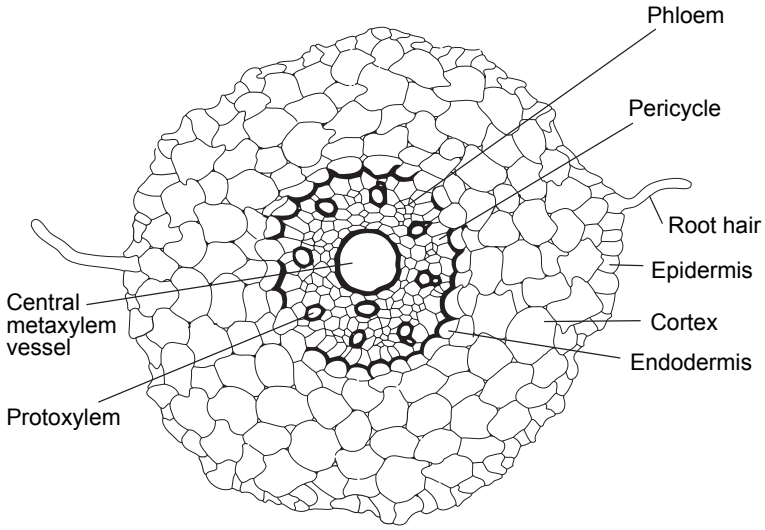

Figure 2. Cross section of a young root (Modified from Fahn 1987).

are initiated in the phellogen during secondary growth may develop into primordial buds and then elongate into shoots (Schier 1973b). The young meristems are usually indeterminate (Hartmann et al. 2002); but in the case of aspen, primordia morphogenic plasticity has not been found. The primordial buds of aspen develop into shoots rather than into roots (Schier 1973b). The primordia can be in various stages of morphogenesis: some may contain only undifferentiated meristematic cells, and others may contain already formed tissue structures (Schier 1973b). The development of primordia may not be continuous because the physiological requirements for initiation, growth and elongation are variable. In general, development is arrested at the primordial stage or after the formation of a bud (Schier 1973b). The primordia can be in the functional stage for several years, but the total length of time that the meristem can remain in the primordial stage is unknown (Schier 1973a, Schier 1981). Development of

aspen primordia is not dependent on the life cycle of the plant or on the specific time of the root ontogeny (Schier and Campbell 1976, Schier 1981).

Adventitious roots of aspen root cuttings originate from primordia produced by meristematic cells in the root periderm (Schier 1973b). Randomly adventitious roots can also originate from the base of a new shoot (Hartmann et al. 2002). In general, root primordia are not initiated until during the propagation process (Schier and Campbell 1976, Hartmann et al. 2002).

Adventitious shoots and roots can also emerge from the callus (Eliasson 1961, Schier and Campbell 1976), which is a mass of actively dividing undifferentiated cells that parenchyma tissues produce, for example, after wounding or hormone treatment (Hartmann et al. 2002).

Methods for large-scale vegetative propagation of aspen

Aspen can be propagated on a large scale by micropropagation and with stem and root cuttings. There are several different methods of micropropagation: embryos, organs, protoplasts or single cells can be cultured in vitro (Bonga 1985). Micropropagation is rabid, but laborious and technically demanding, requires hi-tech facilities, and is thus relatively costly (Vasil 1994). While micropropagation is a reliable and efficient method for propagation of aspen (Winton 1971, Ahuja 1983, Ahuja 1984), the high propagation costs have prevented large-scale commercial use of this technique in Finland (Pulkkinen 2001). However, it may be possible to reduce the labor input and costs of micropropagation by using bioreactor systems (Akita et al. 1994, Vasil 1994). In the bioreactors, large masses of regenerable cells, tissues or organs are grown in liquid media. They are processed, sorted and distributed automatically and then allowed to develop into rooted plantlets (Vasil 1994). One of the most used methods in commercial plant production is cutting propagation (Hartmann et al. 2002). Cuttings are pieces of plant each of which is capable of regenerating into a complete plant. Cuttings are generally made from stems or roots, but the preferred type of cutting depends on both the species and the propagation conditions (Mahlstede and Haber 1957, Hartmann et al. 2002). Hartwood cuttings of aspen are very difficult to root, but leafy softwood cuttings can be used in propagation of several *Populus* species. In addition to European aspen, the closely related species quaking aspen (*P. tremuloides*) and white poplar (*P. alba*) can also be propagated by root cuttings (Hartmann et al. 2002). The limiting factors of the cutting technique are usually the low level of adventitious rooting and the number of cuttings per stock plant (Hartmann et al. 2002).

Main characteristics of the stock plant in relation to the success of vegetative cutting propagation

Genotype

Among aspen trees, large variation is found in many characteristics related to the success of vegetative regeneration (Farmer 1962a, Tew 1970, Schier and Campbell 1976, Schier and Campbell 1980, Ahuja 1983, Zsuffa 1992, David et al. 2001, Haapala et al. 2004). The genotype affects the production and regeneration ability of all types of cuttings. Due to the different growth rates of different genotypes (Yu et al. 2001b), the amount of cutting production can be extremely variable: the fastest growing plants generally have the largest

root system and tallest shoots (Pregitzer and Friend 1996). The rooting ability of forest tree cuttings is also genetically controlled (Ying and Bagley 1977, Haissig 1986, Haissig and Riemenschnieder 1988).

Physiological condition

The physiological condition of the stock plant is important in determining the amount and regeneration ability of cuttings (Hartmann et al. 2002, Frey 2003). One of the most important factors is the time when the cuttings are taken. There is clear seasonal variation in the level of carbohydrates and hormones, which are mainly responsible for the ability of the cuttings to regenerate (Eliasson 1971b, Schier 1973c, Schier and Zasada 1973, Frey 2003). Regulation of carbohydrate mobilization is thought to be controlled mainly by hormones, but the exact mechanism is not known (Nanda and Anand 1970, Haissig 1986, Loescher et al. 1990). In the case of aspen, carbohydrate concentrations in the roots are highest during dormancy and start to diminish in spring, when carbohydrates are transported to shoots for bud burst and vegetative growth. The concentration of carbohydrates in roots starts to increase again late in the growing season after the shoot growth has ended and transport of storage carbohydrates into the roots begins (Kramer and Kozlowski 1960, Schier and Zasada 1973, Isebrands and Nelson 1983, Loescher et al. 1990). The mobilization rate and the ratio of carbohydrates can also change in response to climatic conditions (Fege and Brown 1984, Bonicel et al. 1987, Nguyen et al. 1990). In near-zero or lower temperatures the level of carbohydrates is more stable than in warm conditions due to the decreased activity of the enzymes that degrade carbohydrates (Nanda and Anand 1970, Fege and Brown 1984, Hartmann et al. 2002).

Stock plants must be healthy and vigorous: possible diseases or virus infections can decrease growth of the shoot and roots and thus the amount of cutting production. The ability of cuttings to regenerate from unhealthy stock plants can be weak (Hartmann et al. 2002, Frey 2003). Furthermore, the water balance of the stock plant can affect the regeneration of cuttings. There is evidence that cuttings taken from fully turgid stock plants root best, but this has not been tested rigorously (Loach 1988a). To improve rooting of stem cuttings, several species of stock plants have been grown in low light intensities (Biran and Halevy 1973, Eliasson and Brunes 1980, Maynard and Bassuk 1988). Certain rooting inhibitors have been found to be more abundant in tissues grown in intense light compared to tissues grown at lower levels of light (Eliasson 1971a, Eliasson and Brunes 1980). However, irradiance to the stock plants must be sufficient, because vigorously growing stock plants can produce more cuttings with high rooting ability than suffering plants can (Biran and Halevy 1973, Moe and Andersen 1988). The effect of photoperiod and light quality on the regeneration vigour of the cuttings during the growth of stock plants has been investigated very little (Moe and Andersen 1988, Hartmann et al. 2002).

The physiological condition of a stock plant can also be modified. Hormonal and cutting treatments of stock plants have been used to increase the production of cuttings and to enhance their ability to regenerate. According to Gombkoto and Bercsek (1979), in 12-year-old trees of *Populus*, root pruning and hormone treatment (α-naphtylenic acid) can greatly increase root formation. Moreover, the indole-3-butyric acid (IBA) treatment to one-year-old peach (*Prunus*) trees considerably increased the number of new roots (Starbuck and Preczewski 1986). Decapitation (cutting off the shoot) or girdling (removing parts of the bark) of the shoot blocks the upward and downward stream of transpiration in the plant (Schier 1978b, Hartmann et al. 2002). After decapitation of dormant stock plants, the ability of the roots to

regenerate may improve due to the accumulation of carbohydrates and cytokinins (Schier 1978b).

Age

Age of the stock plant influences the ability of cuttings to regenerate as well as the number of cuttings obtained (Haissig 1974b, Hackett 1988, Hartmann et al. 2002). Due to their generally larger root system and longer shoot, older stock plants produce cuttings more abundantly than younger plants do (Pregitzer and Friend 1996). However, cuttings from older plants may have lower potential to form adventitious roots than those taken from younger plants (Mahlstede and Haber 1957, Haissig 1974b, Bonga 1985, Hackett 1988). This has frequently been observed in the cuttings of many species of woody plant (Hackett 1988). According to Muhs (1992), it is unclear whether the reduced rooting ability of hybrid aspen cuttings is due to lignification or to some other physiological change related to the senescence of the plant. During physiological aging of the plant, the production of rooting inhibitors may increase or otherwise reduce the production of rooting cofactors or promoters (e.g. phenols, Hackett 1988).

It may be possible to manipulate the stage of maturation of the stock plant by using pruning and hormone treatments. Severe annual pruning of the stock plant shoot may change the auxin response of the cuttings and produce a supply of new, young shoots, which can be used for cutting propagation (Blazich 1988, Hackett 1988). Although gibberellic acid has induced juveline growth in ivy (*Hedera*), information on the use of hormone treatments to rejuvenalisation is limited (Hartmann et al. 2002).

Main characteristics and growth conditions of the cutting that affect its ability to regenerate

Carbohydrate content

Regeneration of cuttings requires energy (Haissig 1974c, Haissig 1986). Carbohydrate and hormonal content is related to the size of the cutting (length and diameter) and to the original location of the cutting on the stock plant (Hartmann et al. 2002). Degradation of those carbohydrates that are the main source of energy in cuttings is controlled mainly by hormones or indirectly by physiological factors related to growth conditions (Nanda and Anand 1970, Haissig 1974c, Loescher et al. 1990, Frey 2003).

The diameter of the root defines mainly the carbohydrate content of the cutting: the thickest cuttings have the largest nutritional reserves (Wargo 1976, Dirr and Heuser 1987, Nguyen et al. 1990, Kolb and McCormick 1991). In general, the length of a root cutting in greenhouse conditions should be 2–6 cm (Hartmann et al. 2002). In the root system the diameter and location of cuttings are related to each other: the roots are usually thickest near the root collar, becoming thinner towards the distal end of the root system (Pregitzer and Friend 1996). Thorpe and Murashige (1970) have shown that stimulation of primordial buds occurs only where enough starch has accumulated. The carbohydrate content enables survival of the cutting by providing nourishment for development of the emerging sprout until it is ready for photosynthesis, but does not have a clear role in the rooting process (Schier and Zasada 1973, Schier 1976, Haissig 1986, Davis 1988, Veierskov 1988). Very thin root cuttings

may lack sufficient nutritional reserves or lack the ability to regenerate shoot primordia and support their further growth (Kramer and Kozlowski 1960).

The carbohydrate content of a softwood stem cutting may not be essential for its ability to regenerate. The stem cutting is ready for photosynthesis due to the presence of the old leaf from the stock plant and to the existence of the axillary bud, which is ready for shoot growth (Hartmann et al. 2002). However, it is not known whether the stimulatory effect of the old leaf and the growing adventitious shoot on rooting is caused by photosynthesis, retranslocation of carbohydrates, auxin or some other substance (Eliasson 1971d, Davis 1988, Hartmann et al. 2002).

Hormone relations

The ability of cuttings taken from different parts of the stock plant to regenerate may differ due to apical dominance and strong polarity of the shoot and roots (Eliasson 1969, Eliasson 1971c, Steneker and Walters 1971, Hartmann et al. 2002). Due to polarity, the new roots from a root cutting are initiated at the distal end of the cutting, and shoots sprout near the proximal end (Maini 1968, Schier and Campbell 1976). In stem cuttings the shoot is formed at the distal end, and the roots develop at the proximal end of the cutting (Hartmann et al. 2002). These phenomena are caused by different levels of the plant hormone auxin, which is transported from the apex towards the root tips (Maini 1968, Eliasson 1971c, Schier 1978a). When the plant segments are cut, the physiological balance is disturbed. This may cause redistribution of auxin and a response to different growth factors (Eliasson 1969). According to Eliasson (1969), the stem cuttings of aspen taken from the upper growing parts of the stock plant contained larger amounts of rooting inhibitors than did cuttings taken from the basal part of the stem or from the roots. However, a cutting from the upper stem parts also had the highest level of auxin (Eliasson 1969). According to Schier (1978a), the original location of the root cutting in the root system has no effect on regeneration ability.

The ability of cuttings to regenerate can also be manipulated by hormonal treatments. In general, the root production is stimulated and hastened by natural (indoleacetic acid; IAA) or synthetic auxins (e.g. indolebutyric acid; IBA and naphthaleneacetic acid; NAA). The best auxin and its optimum concentration are dependent on the both species or genotype and the growth conditions. At a high concentration of auxin, bud development is inhibited even if root formation is adequate. In rooting of stem cuttings, hormone treatments are common; but they are rarely used in root cutting propagation (Blazich 1988, Hartmann et al. 2002).

Stage of maturation

The stage of maturation in different parts of the plant is determined by the pattern of bud development. The parts near the base of the plant are the oldest in chronological age, but are less mature (Hartmann et al. 2002). The mature tissues may have higher carbohydrate content than the juvenile ones, but due to other internal factors, they may regenerate slowly and poorly (Mahlstede and Haber 1957, Farmer 1962a, Ali and Westwood 1968, Haissig 1974b, Hackett 1988). Softwood cuttings, usually with a single node and leaf, have been used in propagation of aspen and other difficult-to-root species, because the woody stem cuttings root more slower and less easily (Okoro and Grace 1976, Muhs 1992, Hartmann et al. 2002). On the contrary, roots can retain their juvenility, and thus have a high regeneration capacity for a long time (Bonga 1985).

Propagation conditions

The optimal propagation conditions for efficient and rapid adventitious shoot and root formation of cuttings may differ greatly (Hartmann et al. 2002). By manipulating the propagation conditions, it may be possible to improve parts of the regeneration process. Light is not essential for sprouting of root cuttings, but it is necessary for good shoot growth after the sprout has emerged (Davis 1988, Loach 1988a). Growing cuttings in different photoperiods and light qualities has been shown to produce conflicting effects on regeneration ability (Hartmann et al. 2002). In the rooting process, however, light may be an important inhibiting factor (Shapiro 1958, Eliasson 1971a). Eliasson and Brunes (1980) have shown that irradiation of the base of aspen stem cuttings prevents rooting almost entirely. Thus information on the complex mechanism of the physiological and anatomical changes occuring in response to light is limited (Eliasson and Brunes 1980, Maynard and Bassuk 1988).

The water status of cuttings is a balance between uptake of water and losses due to transpiration. The uptake of water by cuttings is mainly related to the water content of the growth medium, but stem cuttings can also absorb water through the leaves (Loach 1988a). The growth medium must thus provide moisture for the cuttings, but oxygen is also required in the rooting process; thus the medium must be aerated (Hartmann et al. 2002). High air humidity helps to maintain sufficient moisture and decreases the risk of drying in cuttings and newly formed shoots (Loach 1988a). If the cuttings become dry for any length of time, the cuttings will not root, even if they are later rehydrated (Hartmann et al. 2002). High air temperature increases the loss of water from the leaves of stem cuttings (Loach 1988a). High air temperature also tends to promote development of the shoot before roots are formed in cuttings. Johansson and Lundh (1988) showed that *P. tremula* root cuttings sprouted more efficiently when grown at 25°C air temperature than at 10°C. To improve both rooting and shooting efficiency, increased soil temperature (with bottom heat) is widely used in stem and root cutting propagation of many species (Maini and Horton 1966, Steneker 1974, Hartmann et al. 2002). High soil temperature may induce root initation in cuttings, but lower soil temperatures are generally beneficial for elongation of roots (Hartmann et al. 2002).

AIMS OF THE THESIS

During the last decade, especially in Finland, the paper industry has shown considerable interest in the wood properties of hybrid aspen and in using its fibers for pulping (Karlsson and Holm 2001, Ranua 2001). Wood properties of hybrid aspen, such as short fibers, quadrangular fiber form for high opacity, and low lignin content make hybrid aspen very suitable material for production of high quality paper. The white wood of hybrid aspen decreases the need for environmentally harmful bleaching chemicals (Ranua 2001, Rautio et al. 2001). The quality traits of fiber vary greatly among hybrid aspen trees due to differences in genetic make up, which has been shown by high heritability values (Pulkkinen 2001, Rautio et al. 2001, Yu et al. 2001a). The use of vegetative methods of propagation makes it easier to obtain uniform plant material with the desired characteristics for the paper industry. With these methods it is also possible to exploit genetic variation in order to obtain the most suitable clonal raw material for all plantings (Pulkkinen 2001).

The main aim of the present study was to find an efficient and simple method of vegetative propagation for producing large numbers of hybrid aspen plants for forest plantations. The key objectives were to investigate the main physiological factors that affect the ability of cuttings to regenerate and to determine whether these factors could be manipulated by different growth conditions. In addition, clonal variation in traits related to propagation success was examined.

Specific aims of the study were:

1) To test the effects of genotype on the ability of stem and root cuttings to regenerate (**I–V**).

2) To study seasonal fluctuation and clonal differences in levels of soluble carbohydrates and starch, and the relationship between soluble carbohydrate content and ability of roots to regenerate (**V**).

3) To investigate whether the root diameter and location of the root cutting have affect sprouting and rooting efficiency (**III**).

4) To test whether increased soil temperature and light/dark conditions affect the regeneration efficiency and rate of root cuttings (**IV**).

5) To investigate the root structure of hybrid aspen clones and the number of cuttings in one- and two-year-old stock plants, and to determine whether the number of root cuttings can be manipulated by hormonal or cutting treatments of stock plants (**II, III**).

MATERIAL AND METHODS

Plant material

The clones used in this study (**I–V**) were mainly hybrid aspens originating from crosses between *Populus tremula* females from southern Finland (latitudes 60–63°N) and *P. tremuloides* males from Canada (latitudes 45–53°N). There were two exceptions: one of the clones originated from a cross between a *P. tremuloides* female originating from Canada but grown in Sweden (57°N) and a *P. tremula* male from Finland (60°N); another clone was a pure *P. tremula* from Finland (60°N). The age of the trees used as ortets varied between 18 and 44 years. The clones were selected from a group of aspen clones that have been propagated commercially in Finland for several years. These clones have previously been selected from experimental trials or stands primarily because of their good phenotype and secondly due to their suitable wood quality. These clones have also been micropropagated in a large scale. In a study of stem cutting technique (**I**), ten of these clones were used; and in studies of root cutting method (**II–IV**), five of them were used. In the study of carbohydrate content of roots (**V**), two vigorously and two poorly regenerating clones were selected based on our earlier results on root cutting method (**II–IV**). The plant material was raised and the experiments were conducted in Haapastensyrjä Tree Breeding Station of the Finnish Forest Reseach Institute in southern Finland (60°36´N, 24°36´E).

Stem cutting method (I)

The stem cuttings were taken from micropropagated stock plants that were grown after rooting for 22–40 days in a greenhouse. The second harvest was taken from the same stock plants after 51–55 days regrowth. Stem cuttings consisted of a single 3–5 cm long node. To reduce water stress, about half of the leaf surface of a cutting was cut off. Neither the unlignified apical part of the stem nor the basal stub with two or three axillary buds was used as a source of cuttings. The cuttings were treated by dipping their base into a solution of IBA (indole-3-butyric acid) (concentration either 0.6 or 1.2 mM). Cuttings were inserted into rooting trays filled with a 1:1 mixture of fertilized peat and vermiculite (Kekkilä, Finland). The experiments were performed in a greenhouse with relative humidity maintained at 90% and temperature kept at about 20°C under natural light conditions. The mean number of cuttings produced per stock plant, rooting percentage, rooting time, number of roots, and length of the longest root were measured. Cuttings were considered to be rooted when the minimum root length was 1 cm. The presence of callus and lateral roots was recorded. The height of the axillary shoot of the stem cutting was measured at the time of transplantation and again in autumn.

Root cutting method (II–V)

Detailed information on the stock plants' growth conditions can be found in papers **II–V**. In general, the stock plants were grown for two years in a greenhouse that had frame partly covered by plastic. To study the effect of age of the stock plant, one-year-old plants were also used (**II**). In that study the stock plants were subjected to treatments that induced root formation, i.e. cutting off different amounts of root mass, hormone treatments: 0.25 mM IBA (indole-3-butyric acid) and 0.025 mM NAA (α-napthaleneacetic acid), and delaying bud burst

in the spring (shoots were cut down after leaf fall, **II**). To study the carbohydrate content of roots in different conditions (**V**), the stock plants were either grown in the field in natural conditions for two years or were put into the freezer (-18°C) after growing in the field for one year.

The cuttings were taken from healthy roots, diameter 2–10 millimetres, and cut into pieces 3–4 centimetres long. To study the effect of location and diameter of the cutting on regeneration ability, the distance from the proximal end of the root cutting to the root collar of the stock plant was recorded, and diameter at the midpoint of each cutting was measured (**III**). The root cuttings were placed horizontally on a 1:1 peat-sand mixture (Kekkilä Oy) in plastic containers and covered with a double layer of polypropylene fabric (Kesko Oyj) or with a 0.5–1.0 cm layer of peat-sand medium.

The root cutting experiments were carried out either in a growing chamber (**III–V**) or in a greenhouse (**II**). The mean temperature in the growing room was about 18°C and in the greenhouse 15–25°C. Relative humidity was maintained at 90%. Day length in the growing room was 18 hours, which imitates the day length in southern Finland in summer. The experiments were irrigated with automatic sprayers, but no fertilisers were used. To determine the effects of soil temperature and light on sprouting and rooting efficiency of root cuttings (**IV**), four soil temperatures (18, 22, 26 and 30°C) and light or dark conditions were used.

The root cutting was considered to have sprouted and rooted when one or more buds of the cutting had begun to form a new shoot or root. The sprouting and rooting percentages were calculated per variable group (e.g. clone, size class of cutting or soil temperature) by dividing the number of sprouted or rooted cuttings by the sum of all cuttings of the group in question. The terms sprouting and rooting efficiency are used as synonyms for the sprouting and rooting percentage of cuttings. The interval between the start of the experiment and the first day when a sprout or root was identified used as a measure of sprouting or rooting time (synonyms sprouting and rooting rate) of the cutting. The locations of new roots in the cuttings (proximal, central or distal area of the cutting) were also recorded (**III**).

Carbohydrate analysis (V)

The root samples (1–2 g) for the carbohydrate analysis (**V**) were collected monthly throughout the year and were frozen immediately after collection. Before the analyses, the frozen root samples were dried for five days in 40°C to achieve a constant weight, ground in a mill for 20s, and stored in airtight plastic bags at 4°C. Soluble carbohydrates (fructose, glucose and sucrose) were extracted from the samples with 80% ethanol, and the fraction was dissolved to 500 µl of the silylation reagent (pyridine:trimethylsilylimidazole (TMSI) 100:21) and analysed by gas-liquid chromatography (Hewlett Packard 6890 Series GL System). The method of analysis was based on the enzymatic procedure described by Karkalas (1985). First, the soluble carbohydrates were extracted with 75% isopropanol, and the insoluble starch was hydrolysed to glucose by amyloglucosidase (Sigma A-7420). Second, the glucose oxidase-peroxidase (Sigma G-6641) was added to the hydrolysed starch to form a light-absorbing complex suitable for colorimetric analyses (spectrophotometer UV-2401 PC, Schimadzu, Japan).

Statistical analyses

The data were analysed using the SAS (**I**, SAS Institute Inc., Cary, NC, USA) or SPSS (**II–V**, SPSS Inc., Chicago, IL, USA) statistical package. Statistical significances of clone and treatment means were analysed by ANOVA (PROC GLM), followed by pairwise comparisons using Tukey´s post hoc test (at the 5% level of risk). When necessary, the data were transformed to meet the assumptions of valid statistical tests. Pearson's correlation test was used to investigate the correlations between the measured variables (**I, III, IV, V**). Linear regression analysis was used to study clonal differences in root structure (**III**).

RESULTS AND DISCUSSION

Sprouting ability

The sprouting ability of root cuttings varied significantly between clones (**II–V**). This result is in agreement with other studies on vegetative propagation of aspen (Farmer 1962a, Zufa 1971, Schier 1974, Schier and Campbell 1980, Ahuja 1983, Dreesen and Harrington 1999). The level of sprouting efficiency varied between experiments, but the ranking of the clones according to their sprouting ability was relatively constant (Fig. 3). The average sprouting result can be higher and more constant than the natural range of sprouting efficiency among aspen genotypes, because the clones studied were selected from a group of clones that could be micropropagated successfully, and thus they had already shown an ability to propagate vegetatively.

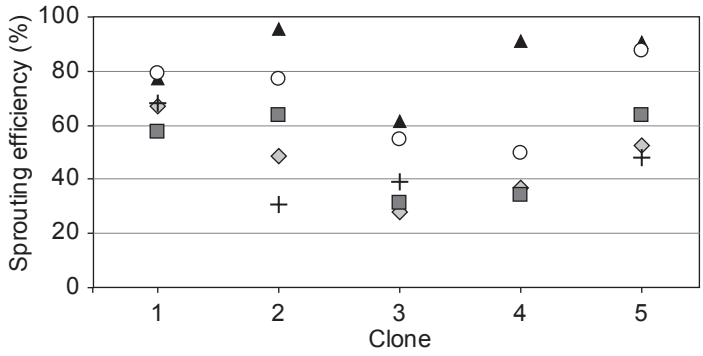

Figure 3. Sprouting efficiency of the root cuttings of five hybrid aspen clones in five experiments (indicated by different symbols).

The clones that showed good sprouting results in all experiments (**II–V**) also had the highest concentration of total carbohydrate reserves (**V**). The sprouting efficiency of root cuttings fluctuated with season: sprouting was most efficient when the concentrations of non-structural carbohydrate levels in the roots were highest, i.e. in winter and spring (**V**). A similar seasonal fluctuation of carbohydrates (Bonicel et al. 1987, Schier and Zasada 1973, Johannson 1993, Landhäusser and Lieffers 2003) and clonal variation in carbohydrate content of *Populus* roots (Tew 1970, Schier and Johnston 1971, Isebrands and Nelson 1983, Nguyen et al. 1990) have also been found in other studies. The clonal variation in carbohydrate content may be due to the different growth rhythms, rates and strengths of transferring photosynthates to the roots (Schier and Johnston 1971, Nelson et al. 1982, Isebrands and Nelson 1983). In addition, the relationship between the carbohydrate level and sprouting efficiency of root cuttings in our experiment (**V**) is generally in agreement with earlier results (Johansson 1993, Iwasa and Kubo 1997, Landhäusser and Lieffers 1997).

Clonal differences in the regeneration efficiency of roots have primarily been explained to be dependent on starch or total non-structural carbohydrate (fructose, glucose, sucrose and starch) content (Schier and Zasada 1973, Landhäusser and Lieffers 1997). However, the greatest differences between clones were in sucrose concentration, and sucrose was also the only carbohydrate that was strongly correlated with the sprouting efficiency of root cuttings (**V**). Throughout the year the most efficiently sprouted clone had the highest sucrose level, and the most poorly sprouted clone the lowest sucrose level. There was also variation in fructose and glucose concentrations, but these did not explain the clonal differences in sprouting efficiency. However, the carbohydrate content alone cannot explain the regeneration efficiency of cuttings. The stock plants that were frozen throughout the year had high, and almost stable, concentrations of carbohydrates even though the sprouting efficiency of their cuttings was very low in summer and autumn (**V**). The reason for this may be that hormonal signals controlling the seasonal physiological phenomena may not function until conditions, e.g. temperature, are suitable (Nanda and Anand 1970, Schier 1973c).

The sprouting efficiency of root cuttings did not differ between one- and two-year-old stock plants (**II**). However, decapitation of one-year-old stock plants clearly improved the sprouting efficiency of root cuttings compared to the very low sprouting of untreated stock plants. Thus the effect was not the same in two-year-old stock plants. One reason for this could be the variation in the physiological condition of one- and two-year-old stock plants: root cuttings from one-year-old stock plants were taken, on average, one to two weeks later than those from two-year-old stock plants. By that time the buds of the untreated stock plants had already swollen or even started to burst. Decapitation treatment could prevent carbohydrate transport from the roots, and the physiological condition for sprouting was better in cuttings of decapitated stock plants. Bud burst in the spring may have changed the hormonal relations and carbohydrate reserves of the roots, which was observed in our experiment (**V**) and also in other studies (Farmer 1962a, Schier 1973c, Schier and Zasada 1973, Schier 1978b, Landhäusser and Lieffers 1997).

The diameter and location of the root cutting did not affect sprouting efficiency (**III**). This means that even the thinnest cuttings had adequate regeneration potential, and the carbohydrate content of root cuttings was not a limiting factor for sprouting efficiency. Starr (1971) and Schier (1978a) reported similar results for *P. tremuloides* root cuttings, and they found no differences in sprouting efficiency between cuttings of a diameter from 0.6 cm to 5.0 cm or between cuttings taken sequentially along the root length. However, Farmer (1962b) found that *P. tremuloides* root cuttings that were 0.3–1.0 cm in diameter sprouted more efficiently than those that were 1.5–2.5 cm thick.

The sprouting of root cuttings was at the same level in both light and dark conditions, but increased soil temperature improved sprouting efficiency (**IV**). Sprouting (79%) was most efficient with a soil temperature of 30°C. Maini and Horton (1966) found that temperatures of 18°C and 24°C increased the sprouting of *P. tremuloides* root cuttings compared to a temperature of 15°C. However, sprouting decreased progressively at higher temperatures of 31°C and 35°C. Adventitious shoot and root development is basically division of cells. The high temperature may increase the number of dividing meristematic cells, affect the rate of division or increase the length of the cells, thus speeding up the initiation of new shoots or roots (Kester 1970, Dykeman 1976). High temperature may also affect this process indirectly by activating or inhibiting hormones or enzymes related to hormone activations, and due to this, the optimal temperature for shoot and root initiation or growth may be different (Gifford 1967, Kester 1970).

Sprouting rate

Average sprouting time of root cuttings differed between experiments; it was 17, 19 and 26 days in studies **IV, III** and **II**, respectively. The first shoots emerged on root cuttings within a week. The sprouting rate differed between clones: the maximum difference between the fastest and slowest clones was, on average, six days (**III, IV**). Schier and Campbell (1976) reported similar results for root cuttings of twenty *P. tremuloides* clones: the time required for sprouting varied between 16–22 days.

The diameter and location of the root cutting were significantly correlated with sprouting time: thicker root cuttings originating near the root collar produced shoots faster than thinner cuttings from the distal parts of the root system (**III**). Ede et al. (1997) found also that thick root cuttings of the empress tree (*Paulownia*) sprouted faster than thinner cuttings did, but the effect of the location in the root system was not clear. However, in their experiment the roots were generally only 30 cm long.

Cuttings sprouted faster in light (average 16 days) than in dark (average 19 days, **IV**). However, the half-centimeter thick peat cover in dark conditions may have caused a slight delay, about one day, in the observations of shoot initials. The sprouting time correlated negatively with soil temperatures (**IV**). Sprouting was fastest (15 days) at a soil temperature of 30°C and slowest (19 days) at 18°C. With *P. tremuloides* similar results of faster sprouting at high temperatures have also been found by Maini and Horton (1966), Zasada and Schier (1973) and Fraser et al. (2002).

Rooting ability

The rooting efficiency of root cuttings in different clones varied from 9% to 67% (**III, IV**). In the study of Schier and Campbell (1976) the rooting of *P. tremuloides* root cuttings in different clones varied from 0 to 10%. Few studies have investigated the rooting efficiency of aspen root cuttings, because the sprouts are usually cut off and rooted as stem cuttings. In our experiment the rooting of stem cuttings in different clones varied from 10 to 76% (**I**). Schier (1974) and Schier and Campbell (1980) obtained similar results with aspen stem cuttings: the rooting efficiency of the various clones was 25–90% and 12–85%, respectively.

Average rooting efficiency of stem cuttings was 53% in the first harvest and 27% in the second (**I**). The low rooting efficiency in the second harvest may be due to seasonal variation

in ability to regenerate, as has been observed in our other experiments (**II, V**). Blake and Atkinson (1986) and Vieitez and Peña (1968) also noted that rooting of poplar and willow (*Salix*) stem cuttings was very low in late summer and autumn due to unfavorable hormonal content. Although Schier (1980) found that treating aspen stem cuttings with a mixture of four rooting hormones significantly improved rooting efficiency and the number of roots, the seasonal difference in hormonal content may also be the reason that IBA treatment (1.2 mM) improved the rooting efficiency of stem cuttings only at the second harvest in late summer (**I**). During early summer the endogenous concentration of auxin may be favourable for rooting, but later in the summer some genotypes with low level of endogenous auxin may respond to treatment with exogenous auxin (Loach 1988b).

The diameter of the root cutting strongly affected its ability to root (**III**). The thinnest (0.15–0.30 cm) root cuttings had the highest (34%) rooting efficiency and the largest cuttings (0.61–1 cm) were the poorest (12%) rooting efficiency. *Populus tremuloides* root cuttings that were 1–2 cm in diameter had, on average, only 2% rooting efficiency (Schier and Campbell 1976). The very low rooting efficiency of the thick root cuttings may be due to ageing of the roots. According to Bonga (1985), roots remain juvenile for a long time. However, morphological changes during secondary growth of the root may prevent growth of adventitious roots. Woody roots can become covered with a layer of thin-walled corky cells like those found in woody stems (Kramer and Kozlowski 1960). However, ageing of the roots did not affect the sprouting efficiency of cuttings: thick roots had a rapid and high rate of sprouting (**III**). This result suggests that differences in hormonal relations or other compounds may inhibit root formation during root senescence (Haissig 1974b). As almost all (92%) new roots developed at the distal end of the cuttings, the root cuttings showed strong polarity (**III**). Based on previous results with *P. tremuloides* roots (Steneker and Walters 1971, Schier and Campbell 1976, Schier 1978a), the polarity of cuttings was expected.

The rooting efficiency of root cuttings was increased in dark propagation conditions with high soil temperature (**IV**). The average rooting efficiency in light was 24% and in dark 49%. Light has been shown to have an inhibitory effect on initiation of the lateral root primordia of *Populus nigra* cuttings (Shapiro 1958) and also on other species (Eliasson 1980, Golaz and Pilet 1985). The molecular and biochemical background of the inhibitory effect of light is not yet clear, but there are suggestions that light may destroy auxins, change the ratio of promoting and inhibiting phenolics, or activate auxin oxidases (Christensen et al. 1980, Eliasson 1980, Maynard 1995). The highest rooting percentage (52%) in our study occurred at the highest soil temperature used (30°C). The least efficient soil temperature for rooting was 18°C, at which 29% of the root cuttings rooted. In many studies, increased soil temperature has been found to have a beneficial effect on rooting of cuttings (e.g. Gifford 1967, Dykeman 1976, Kester 1970).

Rooting rate

Average rooting rate in different experiments varied from 22 (**IV**) to 38 (**III**) days. Average rooting time for root cuttings was always longer than the sprouting time (**III, IV**). This result may indicate that the sprouting process promotes initiation of adventitious rooting. When the cutting has sprouted, the carbohydrate supply from the leaves may support root elongation (Eliasson 1968). Although the sprouting time of root cuttings differed clearly between clones (**II, III, IV**), for rooting time there were no differences between clones (**III, IV**). The rooting time of stem cuttings in different clones varied from 24 to 42 days (**I**). Okoro and Grace

(1976) observed that the average rooting time of aspen stem cuttings was 24 days. Neither the characteristics of the root cuttings (diameter class or location, **III**) nor the IBA treatments (0.6 and 1.2 mM) of stem cuttings affected the rooting time (**III, I**).

The propagation conditions were also ineffective in manipulating the rooting time of root cuttings (**IV**). The effect of light did not change the rooting rate of cuttings: rooting in light and in dark took the same time (22 days). Due to the inhibitory effect of light, it might be expected that the rooting process would be slowed down as it was in the study of Shapiro (1958) with *P. nigra* cuttings. Although increased soil temperature improved sprouting and rooting efficiency and accelerated the sprouting rate, it did not affect the rooting rate of cuttings (**IV**).

Number of cuttings

The number of cuttings in the stem cutting method used here was relatively low: from 6 to 13 cuttings per stock plant (**I**). In the study of Haapala et al. (2004) the result from the hybrid aspen method of stem cutting was at the same level. The use of the stem cutting method in large scale propagation is limited, because the multiplication coefficient is low. The stem cuttings of aspen cannot be taken from larger plants, because in lignified shoots the rooting ability decreases (Hartmann et al. 2002).

The number of root cuttings differed markedly between clones (**II, III**). Almost twice as many root cuttings could have been taken from the stock plants of the best clones compared to the poorest clones. The variation in numbers of cuttings between clones may be due to differences in the structure of the root system (**III**). The number of roots was relatively similar, but the length of the roots varied greatly between clones. The clone with the shortest roots naturally gave the smallest number of root cuttings. However, the clone with the longest roots did not produce the greatest number of root cuttings. Naturally, the two-year-old stock plants had a larger root system, which can be cut into more root pieces, than the roots of younger and smaller stock plants. Two-year-old stock plants had, on average, twenty times more root cuttings per stock plant than the one-year-old stock plants (**II**). Pruning the roots of the stock plant and hormone treatments did not increase the number of root cuttings per stock plant (**II**). However, the result could be different if the root mass of the treated stock plants had been studied after one or two years' growth, and the plant had had a longer time to change the shoot : root ratio.

CONCLUSIONS

Clonal variation is the most important individual factor in propagation of hybrid aspen. The fact that the efficiently sprouted clones also rooted best facilitates the selection of clones for large-scale propagation. It is very important to find clones from which a large number of cuttings per stock plant can be obtained. However, even more important in light of production costs is the good ability of the produced cuttings to regenerate. Much work and money are wasted if many cuttings are propagated and only a few of them are capable of regenerating into plants. Clone selection must also be based on several propagation tests, because the yearly variation in growth conditions of the stock plant may affect the results. In the case of aspen the criteria for clone selection are primarily the fiber qualities and growth rate, but ability to regenerate efficiently is also essential. It is possible that the properties of the wood may have some effect on the success of clonal propagation, and perhaps not all clones are suitable for large-scale propagation (Pulkkinen 2001).

According to our results, depending on the clone, it is possible to obtain only 1–8 plants from one stock plant per year with the stem cutting method. With the root cutting method the comparable values are 81–207 plants. The considerable difference between these methods in number of plants produced indicates that in large-scale propagation of plants the use of root cutting method is preferred. The difference in number of root cuttings between one- and two-year-old stock plants is so pronounced that it is economically feasible to grow stock plants for two years. There is no reason to use much older stock plants as a source of cuttings, as it has been observed that rooting ability diminishes as the root diameter increases.

Root cuttings taken from all parts of the root system of hybrid aspen were capable of producing new shoots and roots. However, for efficient rooting it is important to use roots smaller than one centimeter in diameter. Rooting and sprouting as well as sprouting rate were increased by high soil temperature; in our studies the highest temperature tested (30°C) was the best. With increased soil temperature it is also possible to narrow the large variation in sprouting rate among clones, which can cause problems in the propagation process if the sampled clones represent both extremes of sprouting rate. Light accelerated the sprouting of root cuttings, but they rooted best in the dark. Rooting is essential because the sprouted cutting cannot survive long without roots. Dark conditions (covering the cutting) also help to keep the soil and cutting moist thus protecting the cutting from drying, especially at high soil temperatures. In general, sprouting was best in winter and spring when the carbohydrate level in roots was the highest. Cutting the stock plant shoot off (decapitation) may be helpful in delaying bud burst in spring, which rapidly reduces sprouting ability.

REFERENCES

Anonymous. 2005. Metsätilastollinen vuosikirja 2005. Finnish Statistical Yearbook of Forestry 2005. Finnish Forest Research Institute. Vammalan kirjapaino Oy. Vammala. pp. 424.

Ahuja, M.R. 1983. Somatic cell differentiation and rapid clonal propagation of aspen. Silvae Genet. 32 (3-4): 131-135.

— 1984. A commercially feasible micropropagation method for aspen. Silva Genet. 33 (4-5): 174-176.

Akita, M., Shigeoka, T., Koizuimi, Y and Kawamura, M. 1994. Mass propagation of shoots of *Stevia rebaudiana* in a large scale bioreactor. Plant Cell Rep. 13: 180-183.

Ali, N. and Westwood, M.N. 1968. Juvenility as related to chemical content and rooting of stem cuttings of *Pyrus* species. Proc. Amer. Soc. Hort. Sci. 93: 77-82.

Angelstam, P. and Mikusinski, G. 1994. Woodpecker assemblages in natural and managed boreal and hemiboreal forests – a review. Ann. Zool. Fenn. 31: 157-172.

Biran, I. and Halevy, A.H. 1973. Stock plant shading and rooting of dahlia cuttings. Scientia Horticulturae 1: 125-131.

Blake, T.J. and Atkinson, S.M. 1986. The physiological role of abscisic acid in the rooting of poplar and aspen stump sprouts. Physiol. Plant. 67: 638-643.

Blazich, F.A. 1988. Chemicals and formulations used to promote adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 132-149.

Bonga, J.M. 1985. Vegetative propagation in relation to juvenility, maturity, and rejuvenation. In Bonga, J.M. and Durzan, D.J. (eds.) Tissue culture in forest trees. Martinus Nijhoff Publishers, Netherlands. ISBN 90-247-2660-3. pp. 387-412.

Bonicel A., Haddad, G. and Gagnaire, J. 1987. Seasonal variations of starch and major soluble sugars in the different organs of young poplars. Plant Phys. and Biochem. 25 (4): 451-459.

Bärring, U. 1988. On the reproduction of aspen (*Populus tremula* L.) with emphasis on its suckering bility. Scan. J. For. Res. 3: 229-240.

Böhner, S. and Gatz, C. 2001. Characterization of novel target promoters for the dexamethasone-indusible/tetracycline-repressible regulator TGV using liciferase and isopentenyl transferase as sensitive reporter genes. Mol. Gen., Genet. 264: 860-870.

Casimiro, I., Beeckman, T., Graham, N., Bhalerao, R., Zhang, H., Casero, P., Sandberg, G. and Bennett, M.J. 2003. Dissecting *Arabidopsis* lateral root development. Trends Plant Sci. 4: 165-171.

Christensen, M.C., Eriksen, E.N. and Andersen, A.S. 1980. Interaction of stock plant irradiance and auxin in the propagation of apple rootstocks by cuttings. Scientia Holticulturae 12: 11-17.

Cline, M.G. 1991. Apical dominance. Bot. Rev. 57: 318-358.

David, A.J., Zasada, J.C., Gilmore, D.W. and Landhäusser, S.M. 2001. Current trends in the management of aspen and mixed aspen forests for sustainable production. For. hron. 77 (3): 525-532.

Davis, D.T. 1988. Photosynthesis during adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 79-87.

— and Sankla, N. 1988. Effect of shoot growth retardants and inhibitors on adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 174-184.

De Klerk, G.-J. 2000. Adventitious organogenesis. In Spier, R. (ed.) The Encyclopedia of cell technology. John Wiley & Sons, Inc. New York. pp. 12-24. ISBN: 0471161233.

Dirr, M.A. and Heuser, Jr., C.W. 1987. The reference manual of woody plant propagation: from seed to tissue culture. Varsity Press, Inc. Athens, Ga. 239 pp.ISBN 0-942375-00-9.

Dreesen, D.R. and Harrigton, J.T. 1999. Vegetative propagation of aspen, narrowleaf cottonwood, and riparian trees and shrubs. In Landis, T.D., Barnett, J.P., tech. coords. National proceedings: forest and conservation nursery associations-1998. Gen. Tech. Rep. SRS-25. Asheville, NC. pp. 129-137.

Dykeman, B. 1976. Temperature relationship in root initiation and development of cuttings. Int. Plant Prop. Soc. 26: 201-207.

Eckenwalder, J.E. 1996. Systematics and evolution of *Populus*. In Stettler, R.F., Bradshaw, H.D., Jr., Heilman, P.E. and Hinckey, T.M. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Canada. pp. 7-32.

Ede, F.J., Auger, M. and Green, G.A. 1997. Optimizing root cutting success in *Paulownia* spp. J. Hort. Sci. 72(2): 179-185.

Eliasson, L. 1961. The influence of growth substances on the formation of shoots from aspen roots. Physiol. Plant. 14: 150-156.

— 1968. Dependence of root growth on photosynthesis in *Populus tremula*. Physiol. Plant. 21: 806-810.

— 1969. Growth regulators in *Populus tremula* I. Distribution of auxin and growth inhibitors. Physiol. Plant. 22: 1288-1301.

— 1971a. Growth regulators in *Populus tremula* II. Effect of light on inhibitor content in root suckers. Physiol. Plant. 24: 205-208.

— 1971b. Growth regulators in *Populus tremula* III. Variation of auxin and inhibitor level in roots in relation to root sucker formation. Physiol. Plant. 25: 118-121.

— 1971c. Growth regulators in *Populus tremula*. IV. Apical dominance and suckering in young plants. Physiol. Plant. 25: 263-267.

— 1971d. Adverse effect of shoot growth on root growth in rooted cuttings of aspen. Physiol. Plant. 25: 268-272.

— 1980. Interaction of light and auxin in regulation of rooting in pea stem cuttings. Physiol. Plant. 48: 78-82.

— and Brunes, L. 1980. Light effects on root formation in aspen and willow cutting. Physiol. Plant. 48: 261-265.

Fahn, A. 1987. Plant anatomy. 3rd ed. Wheaton & Co. Ltd. Exeter. p. 544.

Farmer, Jr., R.E. 1962a. Aspen root sucker formation and apical dominance. For. Sci. 4: 403-410.

— 1962b. Depth and diameter of the parent roots of aspen suckers. Univ. Michigan, Michigan For. 23. 4 pp.

Fechner, G., Burr, K. and Myers, J.F. 1981. Effects of storage, temperature, and moisture stress on seed germination and early development of trembling aspen. Can. J. For. Res. 11: 718-722.

Fege, A.S and Brown, G.N. 1984. Carbohydrate distribution in dormant *Populus* shoots and hardwood cuttings. Forest Sci. 30 (4): 999-1010.

Fraser, E.C., Lieffers, V.J., Landhäusser, S.M. and Frey, B.R. 2002. Soil nutrition and temperature as drivers of root suckering in trembling aspen. Can. J. For. Res. 32: 1685-1691.

Frey, B.R., Lieffers, V.J., Landhäusser, S.M., Gomeau, P.G. and Greenway, K.J. 2003. An analysis of sucker regeneration of trembling aspen. Can. J. For. Res. 33: 1169-1179.

Friml, J. 2003. Auxin transport: shaping the plant. Curr. Opin. Plant Biol. 6: 7-12.
Gifford, G.F. 1967. The influence of growth media, temperatures, and light intensities on aspen root and top growth. USDA For. Serv. Gen. Tech. Rep. INT-67. Intermt. For. and Range Exp. Stn., Ogden, Utah. 4 pp.
Golaz, F.W. and Pilet, P-E. 1985. Light and decapitation effects on *in vitro* rooting in maize root segments. Plant Physiol. 79: 377-380.
Gombkoto, G. and Bercsek, P. 1979. Stimulation of rooting of old trees before transplantation. Symposium on growth regulators in floriculture 14.–19.5.1979 in Poland. Tech. Comm. Int. Soc. Hortic. Acta Hortic.91: 491-500.
Haapala, T., Pakkanen, A. and Pulkkinen, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* x *P. tremuloides*). Forest Ecology and Management 193: 345-354.
Hackett, W.P. 1988. Donor plant maturation and adventitious root formation. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 11-28.
Haissig, B.E. 1974a. Origins of adventitious roots. New Zealand Journal of Forestry. Sci. 4: 299-310.
— 1974b. Influences of auxins and auxin synergists on adventitious root primordium initiation and development. N. Z. J. of For. Sci. 4: 311-323
— 1974c. Metabolism during adventitious root primordium initiation and development. N. Z. J. of For. Sci. 4: 324-337.
— 1986. Metabolic processes in adventitious rooting of cuttings. In Jackson, M.B. (ed.) New root formation in plants and cuttings. Martinus Nijhoff Pub. Dordrecht/Boston/Lancaster. pp. 141-189.
— and Riemenschnieder, D.E. 1988. Genetic effects on adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 47-60.
Hammond, H.E.J. 1997. Arthropod diversity from *Populus* coarse woody material in north-central Alberta: a review of taxa and collection methods. Can. Entolol. 129: 1009-1033.
Hansen, J. 1988. Influence of gibberellins on adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 162-173.
Hartmann, H.T., Kester, D.E., Davies Jr., F.T. and Geneve, R.L. 2002. Hartmann and Kester´s plant propagation: principles and practices. 7th ed. Pearson education, New Jersey. ISBN 0-13-679235-9.
Hausman, J.F., Evers, D., Kevers, C. and Gaspar, T. 1997. Internal controls of root induction in Poplar shoots raised *in vitro*. Angew. Bot. 71: 104-107.
Hazell, P., Kellner, O., Rydin, H. and Gustafsson, L. 1998. Presence and abundance of four epifytic bryophytes in relation to density of aspen (*Populus tremula*) and other stand characteristics. For. Ecol. Manage. 107: 147-158.
Horvath, D.P. 1999. Role of mature leaves in inhibition of root bud growth in *Euphorbia esula* L. Weed Science 47: 544-550.
Hynynen, J. and Karlsson, K. 2002. Intensive management of hybrid aspen in Finland. In Hynynen, J. and Sanaslahti, A. (eds.). Management and utilization of broadleaved tree species in Nordic and Baltic countries – Birch, aspen and alder. Finnish Forest Research Institute. Research Papers 847: 99-100.

Isebrands, J.G. and Nelson, N.D. 1983. Distribution of [^{14}C]-labeled photosynthates within intensively cultured *Populus* clones during the establishment year. Physiol. Plant. 59: 9-18.

Iwasa, Y. and Kubo, T. 1997. Optimal size of storage for recovery after unpredictable disturbances. Evol. Ecol. 11: 41-65.

Jarvis, B.C. 1986. Endogenous control of adventitious rooting in non-woody cuttings. In Jackson, M.B. (ed.) New root formation in plants and cuttings. Martinus Nijhoff Pub. Dordrecht/Boston/Lancaster. pp. 191-222.

Johansson, T. 1993. Seasonal changes in contents of root starch and soluble carbohydrates in 4-6 year old *Betula pubescens* and *Populus tremula*. Scan. J. For. Res. 8: 94-106.

— and Lundh, J-E. 1988. Sucker production from root cuttings of *Populus tremula* in relation to growing conditions. Scan. J. For. Res. 3: 75-82.

Karkalas, J. 1985. An improved enzymic method for the determination of native and modified starch. J. Sci. Food Agric. 36: 1019–1027.

Karlsson, K. and Holm, S. 2001. Industrial aspen plantations in Finland. In Pulkkinen, P., Tigerstedt, P.M.A. and Viirros, R. (eds.) Aspen in paper making. University of Helsinki, Department of Applied Biology. Publication No 5: 5-8.

Kester, D.E. 1970. Temperature and plant propagation. Int. Plant Prop. Soc. 20: 153-156.

Kolb, T.E. and McCormick, L.H. 1991. Relationship between total nonstructural carbohydrate concentration and root diameter in sugar maple. For. Sci. 1: 343-346.

Kramer, P.J. and Kozlowski, T.T. 1960. Physiology of trees. McGraw-Hill Book Company, New York. 642 pp.

Landhäusser, S.M. and Lieffers, V.J. 1997. Seasonal changes in carbohydrate storage and regrowth in rhizomes and stems of four boreal forest shrubs: applications in *Picea glauca* understorey regeneration. Scan. J. For. Res. 12: 27-32.

— and Lieffers, V.J. 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees 17: 471-476.

Latva-Karjanmaa, T., Suvanto, L., Leinonen, K. and Rita, H. 2003. Emergence and survival of *Populus tremula* seedlings under varying moisture conditions. Can. J. For. Res. 33: 2081-2088.

Letham, D.S. 1994. Cytokinins as phytohormones–sites of biosynthesis, translocation and function of translocated cytokinin. In Mok, D.W.S. and Mok, M.C. (eds.) Cytokinins–chemistry, activity, and function. CRC Press. Boca Raton. Ann Arbor. London. pp. 57–80.

Ljung, K., Hull, A.K., Celenza, J., Yamada, M., Estelle, M., Normanly, J. and Sandberg, G. 2005. Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. Plant Cell 17: 1090-1104.

Loach, K. 1988a. Water relations and adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 102-116.

— 1988b. Hormone applications and adventitious root formation in cuttings – a critical review. Acta Hortic. 227: 126-133.

Loescher, W.H., McCamant, T., and Keller, J.D. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25(3): 274-281.

Lowell, P.H. and White, J. 1986. Anatomical changes during adventitious root formation. In Jakcson, M.B. (ed.) New root formation in plants and cuttings. Martinus Nijhoff Pub. Dordrecht/Boston/Lancaster. pp. 111-140.

Mahlstede, J.P. and Haber, E.S. 1957. Plant Propagation. J. Wiley & Sons. New York. pp. 191-225.

Maini, J.S. 1968. The relationship between the origin of adventitious buds and the orientation of *Populus tremuloides* root cuttings. Bull. Ecol. Soc. Amer. 49 (2): 81-82

— and Horton, K.W. 1966. Vegetative propagation of *Populus* spp. 1. Influence of temperature on formation and initial growth of aspen suckers. Can. J. Bot. 44: 1183-1189.

Martikainen, P. 2001. Conservation of threatened saproxylic beetles: significance of retained aspen *Populus tremula* on clear-cut areas. Ecol. Bull. 49: 205-218.

Maynard, B.K. 1995. Propagating cuttings. Part III. How light affects the rooting of cuttings in the propagation bench. Am. Nurseryman. March 1: 32-37.

— and Bassuk, N.L. 1988. Etiolation and banding effects on adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 29-46.

Moe, R. and Andersen, A.S. 1988. Stock plant environment and subsequent adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 214-234.

Mudge, K.W. 1988. Effect of ethylene on rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 150-161.

Muhs, H-J. 1992. Macro- and microvegetative propagation as a tool in tree breeding as demonstrated by case studies of aspen and cashew and regulations for marketing clonal material. In Baker, F.W.G. (ed.) Rapid propagation of fast-growing woody species. Redwood Press ltd. Melksham. pp. 71-85. ISBN 0-85198-742-7.

Nanda, K.K. and Anand, V.K. 1970. Seasonal changes in auxin effects on rooting of stem cuttings of *Populus nigra* and its relationship with mobilization of starch. Physiol. Plant. 23: 99-107.

Nelson, N.D., Dickmann, D.I. and Gottschalk, K.W. 1982. Autumnal photosynthesis in short-rotation intensively cultured *Populus* clones. Photosynthetica 16(3): 321-333.

Nguyen, P.V., Dickmann, D.I., Pregitzer, K.S. and Hendrick, R. 1990. Late-season changes in allocation of starch and sugar to shoots, coarse roots, and fine roots in two hybrid poplar clones. Tree Physiol. 7: 95-105.

Okoro, O.O. and Grace, J. 1976. The physiology of rooting *Populus* cuttings I. Carbohydrates and photosynthesis. Physiol. Plant. 36: 133-138.

— and Grace, J. 1978. The physiology of rooting *Populus* cuttings II. Cytokinin activity in leafless hardwood cuttings. Physiol. Plant. 44: 167-170.

Pregitzer, K.S. and Friend, A.L. 1996. The structure and function of *Populus* root systems. In: Stettler, R.F., Bradshaw, H.D., Jr., Heilman, P.E. and Hinckey, T.M. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Canada. pp. 331-354.

Pulkkinen, P. 2001. The effect of wood properties to the possibilities of vegetative propagation of hybrid aspen. In Pulkkinen, P., Tigerstedt, P.M.A. and Viirros, R. (eds.) Aspen in paper making. University of Helsinki, Department of Applied Biology. Publication No 5: 34-39

Ranua, J. 2001. Industrial use of aspen fibres. In Pulkkinen, P., Tigerstedt, P.M.A. and Viirros, R. (eds.) Aspen in paper making. University of Helsinki, Department of Applied Biology. Publication No 5: 1-4

Rautio, M., Kangas, T., Auterinen, T., Álen, R. and Pulkkinen, P. 2001. Wood quality components of hybrid aspen for paper making. In Pulkkinen, P., Tigerstedt, P.M.A. and

Viirros, R. (eds.) Aspen in paper making. University of Helsinki, Department of Applied Biology. Publication No 5: 19-26.
Ridge, I. (ed.) 1991. Plant physiology. Biology: Form and function. The Open University. Walton Hall, Milton Keynes. pp. 12-62. ISBN 0-340-53186-X.
Sachs, T. 1991. Pattern formation in plant tissues. Cambridge University Press. New York. 234 p.
Sakakibara, H. 2006. Cytokinins: activity, biosynthesis, and translocation. Annu. Rev. Plant Biol. 57: 431-449.
Schier, G.A. 1973a. Effect of gibberellic acid and an inhibitor of gibberellin action on suckering from aspen root cuttings. Can. J. For. Res. 3: 39-44.
— 1973b. Origin and development of aspen root suckers. Can. J. For. Res. 3: 45-53.
— 1973c. Seasonal variation in sucker production from excised roots of *Populus tremuloides* and the role of endogenous auxin. Can. J. For. Res. 3: 459-461.
— 1973d. Effect of abscisic acid on sucker development and callus formation on excised roots of *Populus tremuloides*. Physiol Plant. 28: 143-145.
— 1974. Vegetative propagation of aspen: clonal variation in suckering from root cuttings and in rooting of sucker cuttings. Can. J. For. Res. 4: 565-567.
— 1976. Physiological and environmental factors controlling vegetative regeneration of aspen. USDA For. Serv. Gen. Tech. Rep. Rocky Mountain. 29: 20-23.
— 1978a. Variation in suckering capacity among and within lateral roots of an aspen clone. USDA For. Serv., Research note INT-241. Intermt. For. and Range Exp. Stn., Ogden, Utah. 7 pp.
— 1978b. Root suckering in young aspen, girdled, defoliated, and decapitated at various seasons. In Hollis, C.A. and Squillance, A.E. (eds.) Proceedings Fifth North American forest biology workshop. University of Florida, Gainesville, and USDA For. Serv., Southeastern For. Exp. St., Asheville, N.C. p. 259-267.
— 1980. Rooting stem cuttings from aspen seedlings. USDA Forest Service INT-282. Intermountain Forest and Range Experiment Station, Ogden, Utah. 4p.
— 1981. Physiological research on adventitious shoot development in aspen roots. USDA For. Serv. Gen. Tech. Rep. INT-107. Intermt. For. and Range Exp. Stn., Ogden, Utah. 12 pp.
— and Campbell, R.B. 1976. Differences among *Populus* species in ability to form adventitious shoots and roots. Can. J. For. Res. 6: 253-261.
— and Campbell, R.B. 1978. Effect of ethephon on suckering of excisted roots and rooting of cuttings in trembling aspen. For. Sci. 24: 66-72
— and Campbell, R.B. 1980. Variation among healthy and deteriorating aspen clones. USDA Forest Service Research Paper INT-264, Intermountain Forest and Range Experiment Station, Ogden, Utah. 12p.
— and Johnston, R.S. 1971. Clonal variation in total nonstructural carbohydrates of trembling aspen roots in three Utah areas. Can. J. For. Res. 1: 252-255.
— and Zasada, J.C. 1973. Role of carbohydrate reserves in the development of root suckers in *Populus tremuloides*. Can. J. For. Res. 3: 243-250.
Shapiro, S. 1958. The role of light in the growth of root primordia in the stem of the Lompardy Poplar. In Thimann, K.V (ed.) The Physiology of Forest Trees. Ronald Press. New York. pp. 445-465.
Shimizu-Sato, S. and Mori, H. 2001. Control of outgrowth and dormancy in axillary buds. Plant Physiol. 127: 1405-1413.

Skoog, F. and Miller, C.O. 1965. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. In Bell, E. (ed.) Molecular and cellular aspects of development. Harper and Row. New York. pp. 481-494.

Schmülling, T. 2002. New insights into the functions of cytokinins in plant development. J. Plant Growth Regul. 21: 40-49.

Starbuck, C.J. and Preczewski, J.L. 1986. Effect of root applied IBA on root and shoot growth of dwarf peach trees. J. Environ. Hort. 4(3): 80-82.

Starr, G.H. 1971. Propagation of aspen trees from lateral roots. Journal of Forestry 12: 866-867.

Steneker, G.A. 1974. Factors affecting the suckering of trembling aspen. Forest Chr. February 32-34.

— and Walters, M.A. 1971.Effect of root length upon the suckering of trembling aspen. Environment Canada. For. Serv. North. For. Res. Cent. Inf. Rep. A-X-46.

Stettler, R.F., Bradshaw, H.D., Jr., Heilman, P.E. and Hinckey, T.M. 1996. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Canada. p. 539.

Tew, R.K. 1970. Root carbohydrate reserves in vegetative reproduction of aspen. Forest Sci. 16: 318-320.

Thorpe, T.A. and Murashige, T. 1970. Some histochemical changes underlying shoot initiation in tobacco callus cultures. Can. J. Bot. 48: 277-285.

Van Staden, J. and Davey, J.E. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ. 2: 93–106.

— and Harty, A.R. 1988. Cytokinins and adventitious root formation. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 185-201.

Vasil, I.K. 1994. Automation of plant propagation. Plant cell and Organ culture 39: 105-108.

Veierskov, B. 1988. Relations between carbohydrates and adventitious rooting. In Davis, T.D., Haissig, B.E. and Sankhla, N. (eds.) Adventitious root formation in cuttings. Dioscorides Press. Portland. pp. 70-78.

Vieitez, E. and Peña, J. 1968. Seasonal rhythm of rooting of *Salix atrocinerea* cuttings. Physiol. Plant. 21: 544-555.

Wargo, P.M. 1976. Variation of starch content among and within roots of red and white oak trees. For. Sci. 22: 468-471.

Wan, X., Landhäusser, S.M., Lieffers, V.J. and Zwiazek, J.J. 2006. Signals controlling root suckering and adventitious shoot formation in aspen (*Populus tremuloides*). Tree Physiol. 26: 681-687.

Werner, T., Motyka, V., Strnad, M., Schmülling, T. 2001. Regulation of plant growth by cytokinin. Proc. Natl. Acad. Sci. USA. 98: 10487-10492.

Winton, L.L. 1971. Plantlets from aspen tissue cultures. Science 160: 1234-1235.

Wolter, K.E. 1968. Root and shoot initiation on aspen callus cultures. Nature. 219: 509-510.

Worrel, R. 1995. European aspen (*Populus tremula* L.): a review with particular reference to Scotland. I Distribution, ecology and genetic variation. Forestry 68 (2): 93-105.

Ying, Ch. Ch. and Bagley, W.T. 1977. Variation in rooting capability of *Populus deltoides*. Silvae Gen. 26(5-6): 204-207.

Yu, Q., Pulkkinen, P., Rautio, M., Haapanen, M., Alén, R., Stener, L.G., Beuker, E. and Tigerstedt, P.M.A. 2001a. Genetic control of wood physicochemical properties, growth, and phenology in hybrid aspen clones. Can. J. For. Res. 31: 1348-1356.

Yu, Q. and Haapanen, M. 2001b. Growth and phenology of hybrid aspen clones (*Populus tremula* L. x *Populus tremuloides* Michx.). Silva Fenn. 35 (1): 15-25.

Zasada, J.C. and Schier, G.A. 1973. Aspen root suckering in Alaska: Effect of clone, collection date, and temperature. Northwest Science 2 (47): 100-104.

Zufa, L. 1971. A rapid method for vegetative propagation of aspens and their hybrids. Forestry Chronicle February 36-39.

Zsuffa, L. 1992. Experience in vegetative propagation of *Populus* and *Salix* and problems related to clonal strategies. In Baker, F.W.G. (ed.) Rapid propagation of fast-growing woody species. Redwood Press ltd. Melksham. pp. 86-97. ISBN 0-85198-742-7.

—, Giordano, E., Pryor, L.D. and Stettler, R.F. 1996. Trends in polar culture: some global and regional perspectives. In Stettler, R.F., Bradshaw, H.D., Jr., Heilman, P.E. and Hinckey, T.M. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Canada. pp. 515-539.