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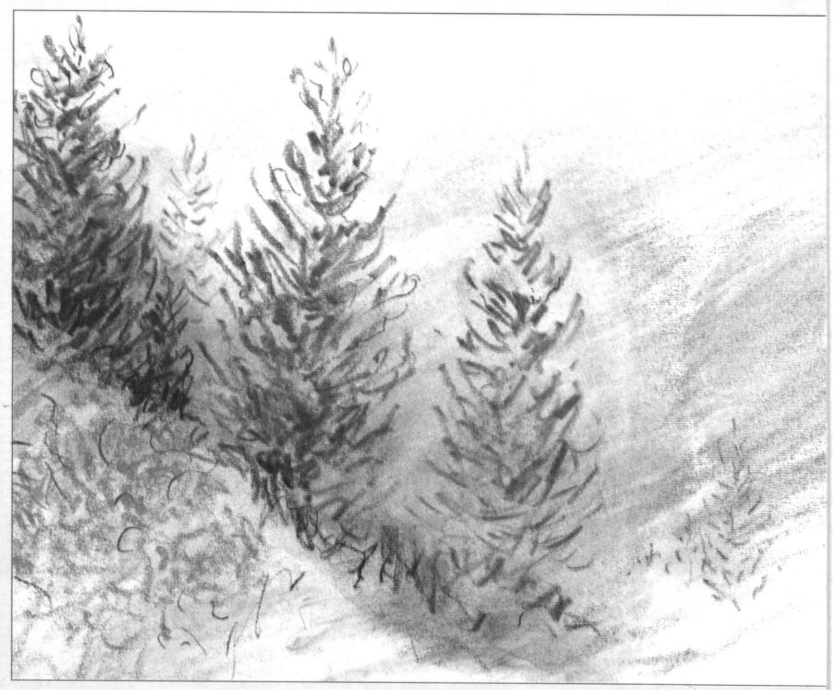

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Editorial Office Unioninkatu 40 A, FIN-00170 Helsinki, Finland
Phone +358 0 857 051, Fax +358 0 625 308, E-mail silva.fennica@metla.fi, WWW Home Page http://www.metla.fi/publish/silva/

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Growth, Crown Structure and Seed Production of Birch Seedlings, Grafts and Micropropagated Plants

Anneli Viherä-Aarnio and Leena Ryynänen

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Growth, crown structure, flowering and seed production of silver birch seedlings, grafts and micropropagated plants was compared during four years in a polythene greenhouse experiment. The growth of the seedlings was clearly the most vigorous and that of the grafts the weakest, the micropropagated plants being intermediate. The seedlings had the highest and the grafts the lowest number of branches before cutting the tops of the plants, but the differences between the material types were no more significant after cutting the tops. The grafts had significantly shorter and thinner branches than the seedlings and the micropropagated plants, whereas the differences in branch length and branch thickness between the latter two groups were not significant. The grafts started flowering at the age of two years, one year earlier than the other two types of material. At the age of four years the micropropagated plants had abundant seed production, about 75 % of that of the seedlings and about two times higher than that of the grafts. Thus, the micropropagated plants can be used instead of grafts when establishing polythene greenhouse seed orchards of birch.

Keywords *Betula pendula* Roth, micropropagation, grafts, seedlings, seed orchards, flowering, seed production.
Authors' addresses *Viherä-Aarnio*, The Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland; *Ryynänen*, The Finnish Forest Research Institute, Punkaharju Research Station, Finlandiantie 18, FIN-58450 Punkaharju, Finland **Fax** to *Viherä-Aarnio* +358 0 857 2575 **E-mail** anneli.vihera-aarnio@metla.fi
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1 Introduction

Cultivation of the native birches, silver birch (*Betula pendula* Roth) and pubescent birch (*B. pubescens* Ehrh.) in Finland has increased considerably in recent years. Birch is now planted on nearly 20 % of the annual area of artificial regeneration (Aarne 1993). Using polythene greenhouse seed orchards, the mass production of genetically improved birch seed can be arranged at low cost and minimum space consumption (Lepistö 1973). Most of the birch seed orchards are multi-clonal orchards. Bi-clonal seed orchards have also been established in order to produce full-sib families for practical cultivation (Hagqvist 1991).

A seed orchard can be established with the grafts of tested plus trees or seedlings selected from the best progenies. The weaker growth, branches and seed production of grafts compared to seedlings has sometimes been a problem. On the other hand, seedlings cannot be used in biclonal seed orchards, because seedlings do not have the same genotype as the parent trees. If specific parental combinations are detected by means of progeny testing, the same combination is to be reproduced using the original parental genotypes.

A modern method for cloning, micropropagation via tissue culture has several advantages compared to conventional methods of clonal propagation, i.e., cutting propagation and grafting. There is, however, very little information on, for example, the growth habit and flowering of the micropropagated birch plants in both natural and greenhouse conditions. According to Huhtinen and Yahyaoglu (1974) micropropagated plants of an early flowering type of *Betula pendula* were vigorously growing due to abundant lateral shoots. In the study of Meier-Dinkel (1989) the 2-year-old micropropagated plants from both juvenile and mature material of *B. platyphylla* var. *japonica* × *B. pendula* showed characteristics of seedlings, such as vigorous orthotrophic growth and high rooting capacity. The 2-year-old micropropagated plants of Meier-Dinkel (1989) derived from both juvenile and mature hybrid birch showed absence of flowering. According to Brand and Lineberger (1992) micropropagated birch plants displayed many characteristics of seedlings. The degree of rejuvenation of their less than one-year-old material varied with characteristic. All the studies mentioned above have been made on very young material, and knowledge about the later development of micropropagated material of birch is lacking.

The aim of this study was to compare the growth, crown structure, flowering and seed production of seedlings, grafts and micropropagated plants of silver birch during a period of several years. In addition, the aim was to determine whether micropropagated plants can be used instead of seedlings or grafts in birch seed orchards designed for the mass production of genetically improved seed.

2 Material and Methods

The material consisted of seedlings, grafts and micropropagated plants originated from the same trees. The material was collected from a silver birch stand at Punkaharju, in south-eastern Finland (61°49'N, 29°18'E, 90 m asl). The stand (0.58 ha) had been planted with seedlings of local origin in 1932. Twigs were collected from 10 random trees in the stand for tissue culture in winter 1988 and for grafting in early spring 1989. Open-pollinated seed from the same trees had been collected in 1985.

Propagation by tissue culture started at Punkaharju Research Station in 1988, where potting of micropropagated plants, after 13–16 passages, grafting and sowing were done in spring 1989, too. The method of tissue culture for adult birch trees took place according to normal routine practice using vegetative buds as tissue material for the micropropagation of birch in the laboratory (Ryynänen and Ryynänen 1986). WPM (Lloyd and McCown 1980) with BAP 4,4 μM was used for cultivation. Before rooting the shoots were subcultured in MS medium (Murashige and Skoog 1962) made to half-strength with 2,2 μM BAP and 2,85 μM IAA. The rooting of shoots took place in the same MS medium without any growth regulators. The rooted shoots about 20 mm high were potted and the seed was sown in peat:perlite mixture (1:2) at the same time, in the middle of April. Grafting was done by using one-year-old and 30 cm high seedlings as rootstocks. The scions from each mother tree were grafted onto an open-pollinated progeny of the same tree. The material was grown in an unheated greenhouse under natural light during spring–summer 1989 and in an open nursery during winter 1989–90. In May 1990 the plants were transplanted into 8 liter pots with fertilized peat in the greenhouse.

The experiment was established in a 50 m long, 16 m wide and 6 m high, polythene arched hall in June 1990. The growing conditions in the experiment were maintained as similar as possible to the conditions in a polythene greenhouse seed orchard (Pirttilä and Saarela 1989). The soil at the growing site of the experiment was glacial till, covered with a 30 cm layer of peat. The peat was limed during the establishment of the trial and fertilized thereafter with NPK fertilizer (7-5-15) in August 1990 and 1991 as well as with NPK fertilizer (10-7-14) in spring 1991–93. Natural light was used and the temperature during the summer was maintained at +25 °C.

The experiment was carried out using 10 different genotypes (clones), each represented by two micropropagated plants, two grafts and two seedlings originating from open-pollination of these clones. Thus, the material is not genetically completely constant across plant types, because the seedlings are the offspring of the trees from which twigs were collected for grafting and tissue culture. The growing area was subdivided into two blocks according to the direction of sunlight, and groups of the different types of material were planted randomly in the blocks, so that each block included one individual/type of propagation/genotype. Thus each material type was represented by 20 plants altogether.

The growth, crown structure, development of flowering and the seed crops were followed in the experiment. The height of the plants was measured in spring 1991. After the plants had reached the ceiling of the polythene hall in August 1991, they were topped to the height of 3.5 m. In 1991 and 1993, diameter at breast height (dbh) and the number of living branches were measured. In addition, the length, thickness and angle of three branches were measured at two different heights along the stem, i.e., altogether six sample branches per individual. Before topping the plants, the sample branches were measured at the relative heights of 0.5 and 0.75. After topping the upper branches were measured at the height of 3.0 m.

Observations on flowering were made over a four year period 1990–93. The number of male catkins and seed catkins were counted and seed production in grams/plant weighed. In 1993, male flowering was measured as the number of male catkins per branch from the same sample branches as used for measuring the branch size.

The significance of the differences between the three types of material was tested using a two-way analysis of variance (general linear model) and Tukey's test. The SAS/STAT statistical package was used. A mixed model was applied using material type as fixed and block as random effects and type III estimable functions. Before the analysis of variance, a SQRT-transformation was done to the number of male catkins in 1992 as well as seed catkins and seed production in 1993, whereas LOG-transformed values were used for the analysis of variance of male catkins in 1993 and seed catkins as well as seed production in 1992.

3 Results

3.1 Growth and Crown Structure

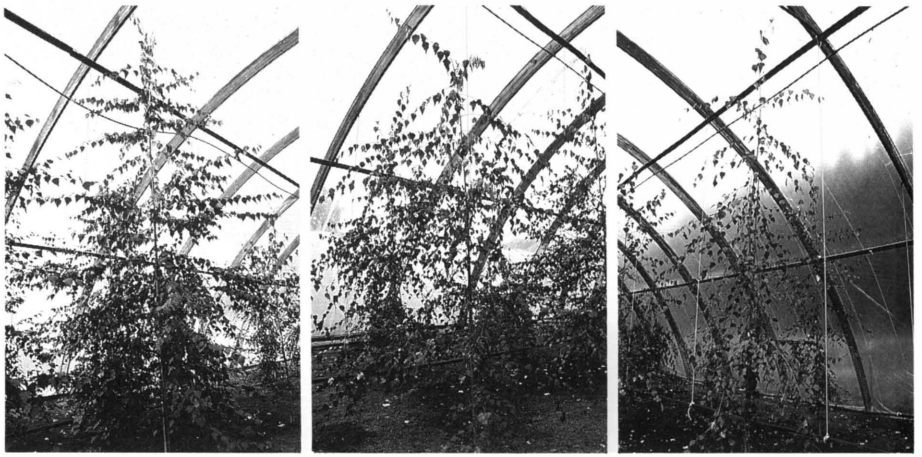
In spring 1991, when the plants were two years old, there were highly significant differences ($p < 0.003$) in the mean height between material types (Table 1). The height growth of the seedlings was clearly the most vigorous and that of the grafts the weakest, the micropropagated plants being intermediate. Differences in the dbh were highly significant ($p < 0.0001$). The seedlings had the thickest and the grafts the thinnest stems. There were also highly significant differences ($p < 0.0001$) in the number of living branches between the material types. The seedlings had the highest and the grafts the lowest number of branches. Differences in branch length and branch thickness were also highly significant ($p < 0.0001$). The grafts had significantly shorter and thinner branches than the seedlings and micropropagated plants. The differences between the latter two groups were not significant.

Table 1. Crown structure of 2-year-old seedlings, micropropagated plants and grafts of silver birch. Different letters indicate significant differences between material types (Tukey's test, $p < 0.05$).

Trait	Material type									Analysis of variance		
	Seedling			Micropropagated plant			Graft					
	$\bar{x}$	s.d.		$\bar{x}$	s.d.		$\bar{x}$	s.d.		Material type	Block	Error
Height, cm	286	28.9	a	261	28.1	ab	244	51.1	b	F = 6.46 DF = 2 p < 0.003	F = 0.99 DF = 1 p < 0.3235	DF = 56 MS = 14.13
d.b.h., mm	16.1	3.4	a	11.4	3.7	b	7.8	3.1	c	F = 29.36 DF = 2 p < 0.0001	F = 2.75 DF = 1 p < 0.1031	DF = 55 MS = 11.31
Number of living branches	30.3	4.6	a	20.3	7.6	b	14.6	3.5	c	F = 41.37 DF = 2 p < 0.0001	F = 0.49 DF = 1 p < 0.4863	DF = 56 MS = 30.54
Branch length, cm	66.5	24.3	a	66.9	23	a	33.3	28.8	b	F = 24.56 DF = 2 p < 0.0001	F = 0.07 DF = 1 p < 0.7975	DF = 56 MS = 303.53
Branch thickness, mm	5.5	1.6	a	5.2	1.5	a	2.9	1.6	b	F = 29.62 DF = 2 p < 0.0001	F = 1.52 DF = 1 p < 0.2222	DF = 56 MS = 1.30
Branch angle, °	54	13.3	a	56	14.5	a	61	18.1	a	F = 1.16 DF = 2 p < 0.3196	F = 0.74 DF = 1 p < 0.3927	DF = 56 MS = 114.83

Table 2. Crown structure of 4-year-old seedlings, micropropagated plants and grafts of silver birch. Different letters indicate significant differences between material types (Tukey's test, $p < 0.05$).

Trait	Material type									Analysis of variance		
	Seedling			Micropropagated plant			Graft					
	$\bar{x}$	s.d.		$\bar{x}$	s.d.		$\bar{x}$	s.d.		Material type	Block	Error
d.b.h., mm	63.3	8.1	a	54.9	8.6	b	39.5	7	c	F = 51,28 DF = 2 p < 0.0001	F = 7,07 DF = 1 p < 0.0102	DF = 56 MS = 56,61
Number of living branches	45	5.3	a	44.1	7	a	41.7	4.6	a	F = 1,87 DF = 2 p < 0.1636	F = 2,71 DF = 1 p < 0.1053	DF = 56 MS = 31,88
Branch length, cm	163.1	32.9	a	164.4	37.8	a	143.8	40.7	b	F = 4,45 DF = 2 p < 0.0161	F = 0,77 DF = 1 p < 0.3848	DF = 56 MS = 599,72
Branch thickness, mm	11.3	3.3	a	11.2	3	a	8.7	2.6	b	F = 12,67 DF = 2 p < 0.0001	F = 0,01 DF = 1 p < 0.9071	DF = 56 MS = 3,37
Branch angle, °	85	12	a	86	16	a	84	17	a	F = 0,12 DF = 2 p < 0.8847	F = 0,01 DF = 1 p < 0.9284	DF = 56 MS = 90,84



a b c

Fig. 1. The three material types originating from the same plus tree (E 5383) in summer 1991 at the age of two years. **(a)** seedling **(b)** micropropagated plant **(c)** graft. Photo J. Lehto.

The grafts had slightly larger branch angle than the other two groups, but the differences were not significant (Fig. 1).

In spring 1993, when the plants were four years old, dbh had increased but the highly significant differences ($p < 0.0001$) between the different material types remained (Table 2). The seedlings had the thickest stems, the grafts the thinnest and the micropropagated plants were intermediate. The number of living branches was now almost the same in the three material types, and the differences were no longer statistically significant ($p < 0.1636$). The branches of the grafts were still significantly shorter ($p < 0.0161$) and thinner ($p < 0.0001$) than those of the seedlings and micropropagated plants. The three material types all had about as wide branch angle, and the differences were not significant ($p < 0.8847$).

3.2 Flowering and Seed Production

At the age of two years from grafting, flowering occurred on the grafts. Female catkins developed into seed on the grafts of two of the clones. Male catkins developed on the grafts of 9 clones, a total of 135 catkins for the whole experiment.

Both male and female flowering occurred on all three material types in the third year (Table 3). Seedlings had the lowest number of male catkins. The mean number of male catkins in both the micropropagated plants and grafts was more than two times higher than that of the seedlings. The differences between the material types were highly significant ($p < 0.0045$). In the same year, the seedlings produced the highest number of seed catkins and the grafts the lowest, whereas the micropropagated plants were intermediate. Differences between the material types were not, however, statistically significant ($p < 0.0554$).

At the age of four years, flowering was very abundant compared to the previous year (Table 3). Flowering and seed production in natural birch stands was also extremely heavy in 1993. Due to the very heavy flowering, male flowering was measured as the number of male catkins per branch from six sample branches per individual. Micropropagated plants had the highest number of male catkins, seedlings the lowest and the grafts were intermediate. Differences between

Table 3. Number of male catkins and seed catkins in seedlings, micropropagated plants and grafts of silver birch. Different letters indicate significant differences between material types (Tukey's test, $p < 0.05$).

Trait	Material type									Analysis of variance		
	Seedling			Micropropagated plant			Graft			Material type	Block	Error
	$\bar{x}$	s.d.		$\bar{x}$	s.d.		$\bar{x}$	s.d.				
Male catkins												
3-year-old, catkins/plant (1992)	52	150	b	127	125	a	127	108	a	F = 5.96 DF = 2 p < 0.0045	F = 0.53 DF = 1 p < 0.4711	DF = 56 MS = 36.37
4-year-old, catkins/branch (1993)	48	60	a	81	98	a	62	80	a	F = 2.39 DF = 2 p < 0.1006	F = 5.14 DF = 1 p < 0.0272	DF = 56 MS = 0.62
Seed catkins												
3-year-old, catkins/plant (1992)	341	435	a	287	304	a	105	149	a	F = 3.05 DF = 2 p < 0.0554	F = 0 DF = 1 p < 0.9707	DF = 56 MS = 3.44
4-year-old, catkins/plant (1993)	3739	3775	a	3016	2242	a	1454	1091	b	F = 5.68 DF = 2 p < 0.0057	F = 0 DF = 1 p < 0.9844	DF = 56 MS = 451.35

Table 4. Seed production of seedlings, micropropagated plants and grafts of silver birch. Different letters indicate significant differences between material types (Tukey's test, $p < 0.05$).

Trait	Material type									Analysis of variance		
	Seedling			Micropropagated plant			Graft			Material type	Block	Error
	$\bar{x}$	s.d.		$\bar{x}$	s.d.		$\bar{x}$	s.d.				
3-year-old, seeds g/plant (1992)	72.4	97.2	a	66.7	74.8	a	25.7	41.9	a	F = 3.02 DF = 2 p < 0.0566	F = 0.02 DF = 1 p < 0.8917	DF = 56 MS = 2.58
4-year-old, seeds g/plant (1993)	807.9	760.7	a	609.5	386.2	ab	326	261.3	b	F = 5.92 DF = 2 p < 0.0047	F = 0.36 DF = 1 p < 0.5490	DF = 56 MS = 88.99

the groups were not, however, statistically significant ($p < 0.1006$).

The female flowering and production of seed catkins of all the three material types at the age of four years was more than ten times higher than that of the previous year (Table 3). Production of seed catkins/plant was the highest in seedlings and the lowest in grafts. Difference between these two groups was statistically highly significant ($p < 0.0057$). The mean seed catkin production of the micropropagated plants was intermediate, but closer to that of the seedlings. The seedlings and the micropropagated plants differed significantly from the grafts.

The production of seeds followed the same pattern as the production of seed catkins (Table 4). In 1992 differences between the material types were not significant ($p < 0.0566$), the within-group variation being high. In 1993 the seed production of seedlings was highest and that of the grafts the lowest, the micropropagated plants being intermediate. Compared to the seedlings, the seed production of the micropropagated material was 75.4 % and that of the grafts 40.4 %.

4 Discussion

The micropropagated plants were intermediate between seedlings and grafts regarding the height and diameter growth and number of branches. With respect to the size of branches and seed production, the micropropagated plants were closer to seedlings than grafts. The closer similarity between the micropropagated plants and the seedlings suggests that the micropropagated material had been rejuvenated. Rejuvenation is defined as a decrease, to a varying degree, in the characteristics of an adult tree that happens in the micropropagated plant during vegetative propagation (Bonga 1987). These results suggest that grafts were phenotypically less rejuvenated than micropropagated plants. There can be several explanations to this phenomenon. The 5 cm long twig used for grafting consists of several differentiated tissues compared to the tiny bud explant used for micropropagation consisting mostly of primary meristem. Subsequently correlative controls of neighbouring cells and other tissues of the tree are reduced to a minimum by taking this kind of smallest possible explant (Bonga 1987). It is also possible that repetitive subculture of the micropropagated plants, 13–16 passages before potting, have increased stepwise the degree of rejuvenation (Franclet et al. 1987).

Rejuvenation of birch (*Betula* sp.) by micropropagation has been reported previously by Brand and Lineberger (1992). In their study, micropropagated plants displayed many characteristics of seedlings. However, their micropropagated plants developed mature phenology more rapidly than plants grown from seed, and they concluded that the level of juvenility regained by the micropropagated plants may not be equivalent to that of seedlings. The degree of rejuvenation varied with characteristic. Brand and Lineberger (1992) examined the bark colour, incidence of antocyanin and pubescens of stems of less than one-year-old plants. Their material was originally obtained from 15-year-old grafted seed orchard clones.

In our study, which was done on older trees, attention was directed to the vigour and the growth habit of the plants, characteristics which are closely connected to the magnitude of seed production. According to Huhtinen and Yahyaoglu (1974) micropropagated plants of an early-flowering type of *Betula pendula* were vigorously growing due to abundant lateral shoots. Our results, however, show that seedlings are more vigorous and have even more branches.

There are several studies where micropropagated and grafted apple trees (*Malus domestica* Borkh.) have been compared in terms of growth habit and yield. In the study by Zimmerman and Miller (1991), micropropagated trees were equivalent in vigour to grafts. According to the results of Larsen and Higgins (1993), micropropagated apple trees were more vigorously growing than grafts, but varied with cultivar.

In our study the grafts started to flower at the age of two years, one year earlier than the micropropagated plants and seedlings. According to Brand and Lineberger (1992) flowering ability of birch was one of the first features to be lost during rejuvenation and one of the last to be regained with maturation. The absence of flowering by two-year-old micropropagated hybrid birch (*Betula platyphylla* var. *japonica* × *B. pendula*) is also mentioned by Meier-Dinkel (1989). In an experiment with apple trees, the flowering of micropropagated plants was delayed one to two years compared to grafts (Zimmerman and Miller 1991). Rosati and Gaggioli (1989) found that the rejuvenation of micropropagated apple trees lasted for some six years, because the pattern of flowering of micropropagated and grafted trees did not resemble each other until the sixth year. According to our results, micropropagated trees lost their juvenility as regards flowering in the same year of growth as the seedlings. At the age of four the micropropagated plants still resembled seedlings more than grafts with respect to crown structure and seed production. The seed production by the micropropagated plants is expected to continue to resemble that of the seedlings. The early development of male flowering of the micropropagated plants was, however, closer to that of the grafts.

The delayed flowering of micropropagated birch we observed probably has no practical importance on total seed production since, in the first year, even the flowering of the grafts was low. The variation within material types was large. This was probably due to differences between the clones, a factor to be further investigated. In the third year, seed production of the micropropagated plants and seedlings was already more than two times greater than that of the grafts. Evidently the size and ramification of the branches has a considerable effect on seed production; the longer and the more ramified the branches are, the more there are potential sites for male and seed catkins. According to the results by Larsen and Higgins (1993), micropropagated apple trees can also overcome some effects of delayed early fruit bearing because of the generally larger tree size compared with grafts.

Birch seedlings may start flowering at the age of two or three years in unheated greenhouses (Kärki 1976). Through increasing the photoperiod, regulating temperature, increasing CO_2 concentrations, fertilization, irrigation and increasing air humidity, flowering can be induced even earlier (Longman and Wareing 1959). Using this kind of intensive treatment, seedlings have been made to produce seed already at the age of eight months (Kärki 1976, Holopainen and Pirttilä 1978). Because they resemble seedlings more than grafts in many respects, micropropagated plants can probably also be induced to flower earlier through such intensive treatments.

The phase of heavy flowering and seed production of birch seed orchards in polythene greenhouses has been shown to last for about six years, whereafter flowering decreases (Tyystjärvi and Pirttilä 1984). We intend to follow our experiment in the coming years in order to gain more information on the length of the production time and the total seed crop as the different plant types age. But at this stage, our results suggest that the use of micropropagated birch plants instead of grafts in polythene greenhouse seed orchards is promising.

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