Micropropagated Silver Birches (*Betula pendula*) in the Field – Performance and Clonal Differences

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Micropropagated and seed-born silver birches (*Betula pendula* Roth) were compared for survival, height growth and occurrence of biotic damage (voles, hares, mooses, stem lesions and cankers) in field trials in southern Finland. The material consisted of 11 clones and 10 different lots of seedlings growing in 10 field trials, established in clear-cut forest cultivation areas. The plants were 6–7 years old. The micropropagated and seed-born material types did not significantly differ from each other as regards survival, height growth and frequencies of damage caused by biotic agents. Large and significant differences were, however, detected in survival, height and frequencies of all types of biotic damage between single clones. Careful selection and testing of birch clones in field conditions is recommended before wide-scale commercial micropropagation and practical forest cultivation takes place.

Keywords *Betula pendula* Roth, micropropagation, clone, clonal variation, field testing, herbivory
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1 Introduction

Clonal propagation of birch (*Betula* spp.) via tissue culture has been possible since the 1970's (Huhtinen and Yahyaogly 1974, Huhtinen 1976). As reviewed by Meier-Dinkel (1992), research on tissue culture has been done with a number of species and varieties of birches. The first micropropagated birches that could be established in soil substrate were with *B. pendula* (Huhtinen and Yahyaogly 1974, Huhtinen 1976, Chalupa 1981a), *B. pendula* f. *purpurea* (Simola 1985), *B. pendula* var. *carelica* (Ryynänen and Ryynänen 1986), *B. platyphylla* var. *szechuanica* (McCown and Amos 1979), *B. pubescens* (Chalupa 1981b) and *B. papyrifera* (Minocha et al. 1986).

Micropropagation of complete plants from mature trees, which is an important aim from the point of view of forest tree breeding, has first been reported for *B. pendula* (Chalupa 1981a, Särkilahti 1988, Welander 1988) and on *B. pendula*

var. *carelica* (Ryynänen and Ryynänen 1986). The first steps towards large-scale production of micropropagated material were the *in vitro* propagation of several hundred plants of juvenile *B. platyphylla* var. *szechuanica* reported by McCown and Amos (1979) and of mature *B. pendula* var. *carelica* reported by Ryynänen and Ryynänen (1986).

In Finland, methods for the micropropagation of birch were developed in the 1980's. As a result, both juvenile and mature trees can be used as starting material (Simola 1985, Ryynänen and Ryynänen 1986, Särkilahti 1988). The practical application of micropropagation in forestry started in 1986–88 when a joint project to propagate birch on a large-scale was set up by three companies. The first clonal propagated silver birch plantlets were sold to forest owners in spring 1989. At the beginning of the 1990's more than one million clonal propagated birches were produced for forest cultivation (Pekkarinen 1993). However, production was finished in 1994 as it was considered that large-scale production was unprofitable. Nevertheless, micropropagation of birch remains a valuable tool in research (e.g. Lemmetyinen et al. 1998), tree breeding (Viherä-Aarnio and Ryynänen 1994, 1995, Häggman and Oksa 1999), and gene conservation (Ryynänen 1999).

Although micropropagation of birch can be routinely used in research, tree breeding and for large-scale production of reproductive material, relatively few results concerning the performance of micropropagated birches in field conditions have been published. In the nursery, the early development of micropropagated plants and seedlings of *B. platyphylla* var. *szechuanica* was followed by McCown and Amos (1979) and the performance of micropropagated material of *B. pendula* was reported by Jokinen and Törmälä (1991). Meier-Dinkel (1992) compared the performance of clones of *B. pendula*, *B. pubescens* and *B. platyphylla* var. *japonica* × *B. pendula* in field experiments in Germany. Viherä-Aarnio (1994) reported the first results on the performance of micropropagated silver birch clones in a six-year-old field trial in Finland. Viherä-Aarnio and Ryynänen (1994, 1995) also compared seedborn, micropropagated and grafted plants of silver birch as regards growth, crown structure, flowering and seed production during the first four years in a polythene greenhouse seed orchard. Jones et al. (1996) monitored micropropagated and seedling trees of silver birch up to the age of seven years as regards height, girth, bark and flowering.

Experiments in controlled environments have provided much information concerning individual birch clones and variation among clones in, for example, response to salt and mycorrhization (Catinus et al. 1990), sensitivity and response to tropospheric ozone (Pääkkönen et al. 1993, 1995), production of primary and secondary metabolites (Lavola et al. 1994), susceptibility to fungal diseases (Poteri and Rousi 1996) and palatability to herbivores (Jia et al. 1997, Rousi et al. 1997). Very little, however, is known about the importance of clonal variation in field conditions. Rousi et al. (1997) assessed the vole damage of silver birch clones in 2-year-old field trials and Stener (1999) reported results from Swedish field tests concerning height, height growth, diameter and five different quality traits of 83 ten-year-old silver birch clones.

In this paper we compare micropropagated and seed-born silver birches and examine clonal differences within the micropropagated material in field conditions of forest cultivation areas.

2 Material and Methods

2.1 Field Trials

The material consists of three different trial series: 1336/1–4, 1443/1–3 and 1444/1–3 (altogether 10 trials), which were established in 1989 and 1992 by the Finnish Forest Reseach Institute in co-operation with the Enso-Gutzeit Company in southeastern Finland between latitudes 61º and 63ºN (Table 1).

The trials include 11 different silver birch clones and 10 different lots of seedlings (Table 2, 3 and 4). Both the clones and seedlots were of southern Finnish origin (between latitudes 61° and 63°N). The clones had been selected for large-scale propagation programme within progenies from open pollination or controlled crossings of plus trees, within progenies from seed

Table 1. Site and experimental characteristics of the field trials included in the study.

Trial number		Planting year	Location	Lat.	Long.	Alt.	Forest type [1)]	Number of entries and blocks	Number of plants/plot	Total area, ha
1336	1	1989	Jyväskylä	62°15'N,	26°02'E,	140 m	MT	6/5	49	0.6
	2	"	Liperi	62°40'	29°34'	150	OMT	"	"	"
	3	"	Imatra	61°10'	28°52'	73	OMT	"	"	"
	4	"	Savonranta	62°07'	29°09'	165	MT	"	"	"
1443	1	1992	Ruokolahti	61°20'	28°55'	125	MT	12/9	9	0.6
	2	"	Ruokolahti	61°20'	28°55'	125	MT	11/9	"	"
	3	"	Taipalsaari	61°07'	28°14'	87	OMT	"	"	"
1444	1	1992	Ruokolahti	61°19'	28°56'	100	MT, OMT	7/5	219	4.3
	2	"	Juankoski	63°14'	28°27'	167	OMT	"	200	4.2
	3	"	Tuupovaara	62°23'	30°54'	155	MT	"	"	"

[1)] According to Cajander (1949)

orchards and among phenotypically selected plus trees. The seedling lots were families related to the clones or corresponding geographical origins. Details of the material in trial series 1336 are reported by Viherä-Aarnio (1994).

Micropropagation of the material was done by the Kemira and Hortus companies according to the axillary micropropagation method with no callus phase. The micropropagated plantlets were grown further at the Enso-Gutzeit Ukonniemi nursery at Imatra. Details of the propagation method as well as raising the micropropagated plantlets and the seedlings have been reported earlier by Viherä-Aarnio (1994). In trial series 1336 and 1443 one-year-old material was used whereas in trial series 1444, the age of the micropropagated plants and seedlings was two years. In all trials, both the micropropagated and seedling material was containerized.

All trials were established on clear-cut, moist upland sites classified as *Myrtillus* or *Oxalis-Myrtillus* site types according to the Finnish classification (Cajander 1949). Before planting, site preparation was done by ploughing or harrowing. A randomized block design was used in all trials, but the number of blocks and plot size varied between different trial series (Table 1). Spacing of 2 × 2 m was used in series 1336, and spacing 2.5 × 2.5 m in the rest of the trials.

2.2 Measurements

The survival (%), height and relative frequency (%) of trees suffering from damage by moose (*Alces alces*), hare (*Lepus* sp.) or vole (*Microtus* sp.) was assessed, as well as the relative frequency of plants suffering from a stem lesion or canker caused by fungi. The measurements of trial series 1336/1–4 were made in autumn 1993, of trial series 1443/1–3 in autumn 1996 and of series 1444/1–3 in spring 1997. The age of the trees at time of measurement were six years (trials 1336/1–4 and 1443/1–3) and seven years (trials 1444/1–3).

In winter 1992–93, the vole population was high in some parts of eastern Finland (Kaikusalo and Henttonen 1992), and trial series 1443/1–3 and trial 1444/1 suffered very severe browsing by voles. Vole damage was therefore assessed in spring 1993, one year after planting when the plants were two and three years old. Four classes of damage severity were used: healthy plant (1), less than half of the circumference eaten (2), more than half of the circumference eaten (3), or the whole circumference eaten or plant cut (4). In this paper, damage classes 2–4 were combined to form the category "vole damage".

2.3 Statistical Analysis

Mean and standard deviation for each variable were calculated for each experimental lot. An analysis of variance on plot means followed by

Table 2. Characteristics of the material in trial series 1336/1–4.

Material type	Clone or seedlot	Origin	Selected from / type of seedlot
Clone	KL 2M	Plus tree E2818 Valkeakoski (61°12'N, 24°00'E, 90 m asl)	Open pollinated progeny of plus tree
"	KL 4M	Plus tree E 4052 Sysmä (61°21'N, 25°40'E, 90 m asl)	"
"	KL 1M	Plus tree E 173 Imatra (61°15'N, 28°50'E, 100 m asl)	"
Seedlings	P27-73-0992	Taipalsaari, plus stand 992 (61°21'N, 28°15'E, 85 m asl)	Stand seed
"	P27-87-0001	Lieksa (63°19'N, 30°01'E, 130 m asl)	"
"	P27-87-0005	(E 1970 × E 1980) open pollination in progeny trial 542/6 at Jyväskylä (62°08'N, 25°43'E, 85 m asl)	Open pollinated seed of one family

Table 3. Characteristics of the material in trial series 1443/1–3.

Material type	Clone or seedlot	Origin	Selected from / type of seedlot
Clone	JR1/1	E1970 Kangasala × E 1980 Nummi-Pusula	Control pollinated progeny of plus trees
"	V5818	V590 Loppi × V554 Rautalampi	"
"	V5832	V540 Rautalampi × pollen mixture (S/C Finland)	"
"	V5279	V599 (Loppi × Rautalampi) × pollen mixture (S/C Finland)	"
"	K2674	K2674 Eno (62°47'N, 30°05'E, 120 m asl)	Plus tree in natural stand
"	E9702	Stand 687 Kangasala (61°25'N, 24°09'E, 50 m asl)	Stand seed
Seedlings	R01-90-0261	E1970 Kangasala × E1980 Nummi-Pusula	Controlled pollination of plus trees
"	R01-77-3449	V590 Loppi × V 554 Rautalampi	"
"	P27-88-0142	V5832 open pollination in progeny trial 611/1 at Röykkä	Open pollination of plus tree
"	P27-88-0141	V5279 open pollination in seed orchard 364 at Oitti	"
"	P27-89-0122	Eno, plus stand 777 (62°46'N, 30°11'E, 120 m asl)	Stand seed
"	P27-89-0010	Puumala (61°32'N, 28°11'E)	"

Table 4. Characteristics of the material in trial series 1444/1–3.

Material type	Clone or seedlot	Origin	Selected from / type of seedlot
Clone	JR1/2	E1970 Kangasala × E1980 Nummi-Pusula	Control pollinated progeny of plus trees
"	KL 4M	E4052 Sysmä (61°21'N, 25°40'E, 90 m asl)	Open pollinated progeny of plus tree
"	V5818	V590 Loppi × V554 Rautalampi	Control pollinated progeny of plus trees
"	V5834	V596 Loppi × seed orchard 288	Open pollinated progeny of plus tree
"	JR1/2, KL 4M, V5818, V5834 [1)]		
Seedlings	P27-89-0010	Puumala (61°32'N, 28°11'E)	Stand seed
"	P27-89-0105	Outokumpu (62°43'N, 29°01'E)	"

[1)] Random mixture of clones with equal number of plants/clone)

Tukey tests were used to test the significance of the differences between different experimental lots, trials, blocks (within trial) and experimental lot × trial interaction based on linear model (1):

$$x_{ijk} = \mu + a_i + b_j + c_{k(j)} + ab_{ij} + e_{ijk} \quad (1)$$

where x_{ijk} = mean value of experimental lot i in trial j and block k, μ = overall mean, a_i = fixed effect of experimental lot i, b_j = fixed effect of trial j, $c_{k(j)}$ = random effect of block k (within j^{th} trial), ab_{ij} = fixed effect of interaction between experimental lot i and trial j, e_{ijk} = residuals.

The percentage values (survival and damage by various agents) were *arcus sin* transformed before the ANOVA was performed. In cases where the frequencies of damage caused by different biotic damage agents varied greatly between individual trials in a trial series, the results from only trials with remarkably high frequencies were considered and the ANOVA performed separately on them using another linear model (2):

$$x_{ij} = \mu + a_i + b_j + e_{ij} \quad (2)$$

where x_{ij} = mean value of experimental lot i in block j, μ = overall mean, a_i = fixed effect of experimental lot i, b_j = random effect of block j, e_{ij} = residuals.

The differences between micropropagated and seed-born material, and between clone and corresponding seedling lot in trial series 1443/1–3, were tested by pairwise comparisons with corresponding contrasts. The SAS/STAT™ statistical package was used (SAS Institute Inc. 1989).

3 Results

3.1 Survival and Height

The average survival of trees in trial series 1336 varied from 60% (Imatra) to 94% (Jyväskylä) (Fig. 1). The average height of the six-year-old trees in different trials varied from 1.5 m (Savonranta) to 3.3 m (Jyväskylä). In trial series 1443, the average survival of trees varied from 70% (Taipalsaari) to 79% (Ruokolahti), and the average height from 2.0 m (Ruokolahti) to 2.3 m (Taipalsaari) (Fig. 2). Average survival of trees after seven years in trial series 1444 varied from 64% (Ruokolahti) to 85% (Juankoski), and average height from 1.9 m (Ruokolahti) to 3.0 m (Juankoski) (Fig. 3). Variation among different trial sites within all three trial series was significant ($p < 0.05$) as regards survival and height and, in some cases there was a significant lot × trial site interaction (Table 5 and 6).

There was no significant difference ($p < 0.7791$, $p < 0.6937$ and $p < 0.7538$ for 1336, 1443 and 1444, respectively) between the height of micropropagated and seed-born trees in all trials (Figs. 1, 2 and 3). Neither was there a significant dif-

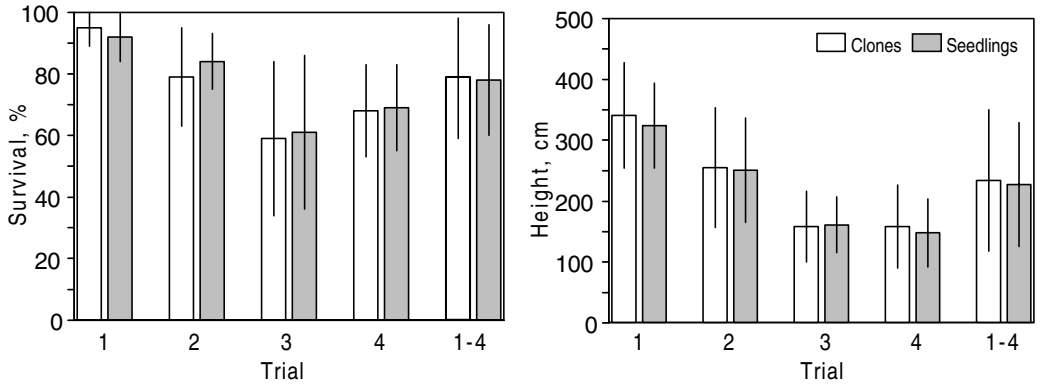

Fig. 1. Survival (%) and height (cm) of micropropagated and seed-born material (mean and S.D.) in trial series 1336/1–4. Trial 1 = Jyväskylä, 2 = Liperi, 3 = Imatra, 4 = Savonranta.

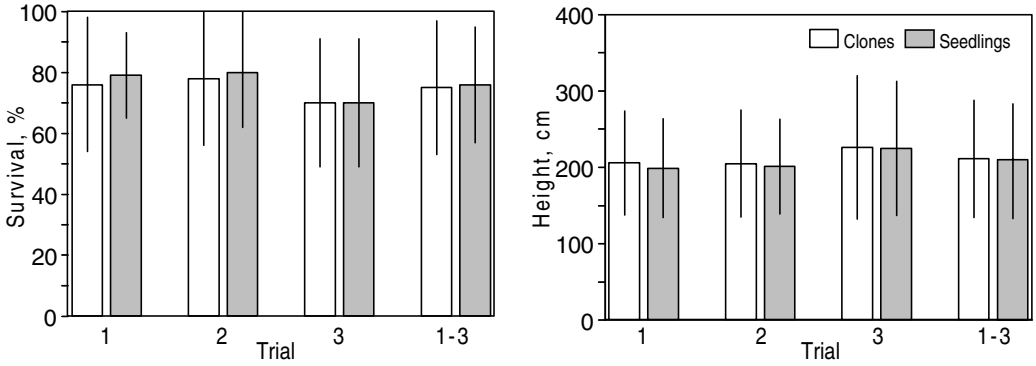

Fig. 2. Survival (%) and height (cm) of micropropagated and seed-born material (mean and S.D.) in trial series 1443/1–3. Trial 1 = Ruokolahti, 2 = Ruokolahti, 3 = Taipalsaari.

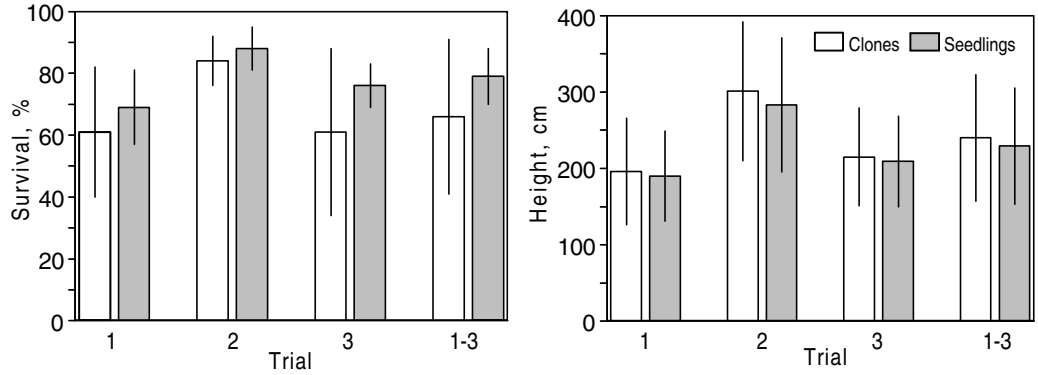

Fig. 3. Survival (%) and height (cm) of micropropagated and seed-born material (mean and S.D.) in trial series 1444/1–3. Trial 1 = Ruokolahti, 2 = Juankoski, 3 = Tuupovaara.

ference between the survival of micropropagated and seed-born trees in trial series 1336 ($p < 0.395$) and series 1443 ($p < 0.5526$). The survival of micropropagated and seed-born trees in trial series 1444 did significantly differ ($p < 0.0001$), but the significance disappeared when the analysis was performed without the poorly performing clone V5834 ($p < 0.1641$).

In all three trial series there were statistically highly significant differences between the experimental lots in terms of survival and height (Table 5 and 6). In trial series 1336, clone KL 1M performed significantly worse than the other lots (Fig. 4). The selected clones KL 2M and KL 4M performed well, but did not differ significantly from the corresponding stand origin. Although the ranking of lots varied slightly from site to site, the worst lots were the same at all sites (e.g. KL 1M in survival and height as well as E1970 × E1980 open pollination in survival) and the best lots (KL 2M and KL 4M) performed well in all trials. The tallest tree in the trial series 1336 (5.3 m) was recorded within clone KL 2M at Jyväskylä.

In trial series 1443, the selected clones V5818 and V5279 were the best performing lots as regards both survival and height, whereas the clones K2674 and E9702 were the poorest (Fig. 5). The difference between each clone and its corresponding seedling lot (i.e. related family or a corresponding stand origin) was tested pairwise. A statistically significant difference ($p < 0.0001$) existed in height between the clone V5818 and the family V590 × V554 as well as between the clone E9702 and stand origin of Puumala ($p < 0.0027$). In survival, there was a significant dif-

Table 5. ANOVA table of survival (%) in trial series 1336, 1443 and 1444.

Trial series	Site	Lot	Block (within trial)	Lot x site	Error
1336/1–4	F = 71.96 DF = 3 p<0.0001	F = 38.21 DF = 5 p<0.0001	F = 2.51 DF = 16 p<0.0037	F = 4.80 DF = 15 p<0.0001	DF = 80 MS = 0.0117
1443/1–3	F = 7.61 DF = 2 p<0.0006	F = 4.17 DF = 11 p<0.0001	F = 3.87 DF = 24 p<0.0001	F = 0.69 DF = 20 p<0.8352	DF = 245 MS = 0.0627
1444/1–3	F = 56.71 DF = 2 p<0.0001	F = 64.82 DF = 6 p<0.0001	F = 3.80 DF = 10 p<0.0005	F = 5.13 DF = 12 p<0.0001	DF = 60 MS = 0.0079

Table 6. ANOVA table of height in trial series 1336, 1443 and 1444.

Trial series	Site	Lot	Block (within trial)	Lot x site	Error
1336/1–4	F = 408.11 DF = 3 p<0.0001	F = 30.54 DF = 5 p<0.0001	F = 4.79 DF = 16 p<0.0001	F = 2.76 DF = 15 p<0.0018	DF = 80 MS = 5.995
1443/1–3	F = 12.87 DF = 2 p<0.0001	F = 11.96 DF =11 p<0.0001	F = 5.54 DF =24 p<0.0001	F = 1.04 DF = 20 p<0.4102	DF = 245 MS =1334.91
1444/1–3	F = 105.20 DF = 2 p<0.0001	F = 10.61 DF = 6 p<0.0001	F = 2.65 DF = 10 p<0.0094	F = 1.69 DF = 12 p<0.0915	DF = 60 MS = 593.55

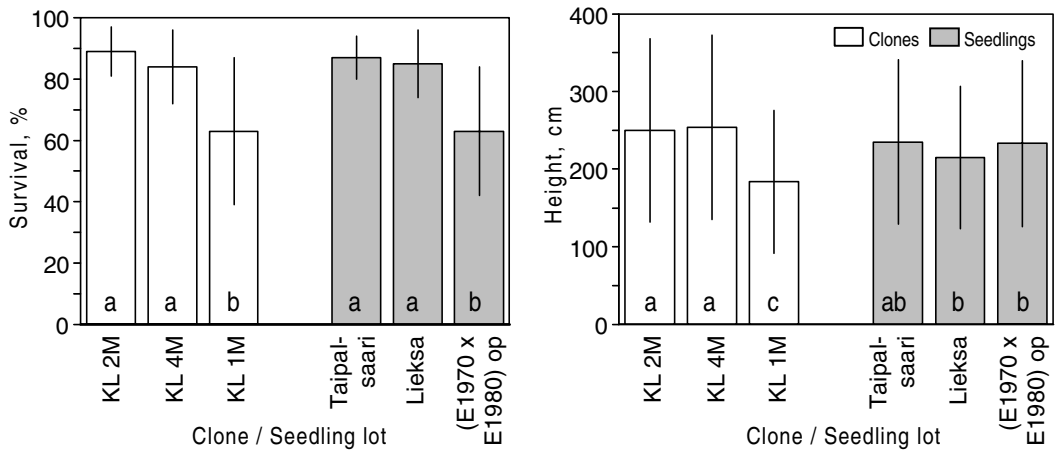

Fig. 4. Survival (%) and height (cm) of different clones and seedling lots (mean and S.D.) in trial series 1336/1–4. Means marked with a different letter are significantly different (Tukey's test, $p < 0.05$).

ference between the clone K2674 and the stand origin from Eno ($p < 0.0102$), as well as between the clone E9702 and Puumala stand origin ($p < 0.0262$). The tallest tree in trial series 1443 (5.8 m) was observed in clone JR1/1 at Ruokolahti.

In trial series 1444, the variation in the height and survival among different clones and seed origins was considerable and differences highly significant (Fig. 6, Tables 5 and 6). The best lot was clone V5818 for both survival and height. Both seedling lots in trial series 1444 had high survival and rather good height growth, and the performance of even the best clone (V5818) did not differ significantly from that of the seedling lots. The V5834 clone had the weakest performance, its average survival being less than 30%. The survival of the clone

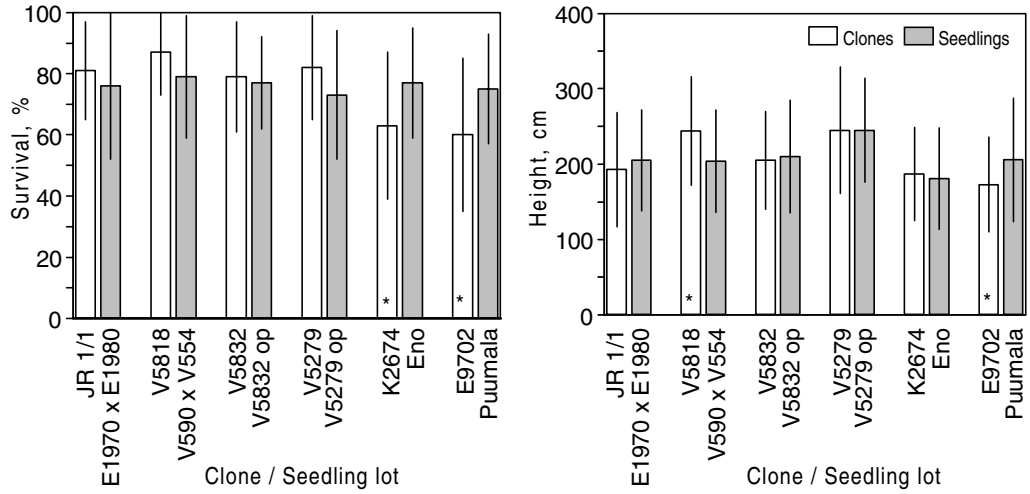

Fig. 5. Survival (%) and height (cm) of different clones and seedling lots (mean and S.D.) in trial series 1443/1–3. Clones marked with an asterisk (*) differ significantly from their comparison seedling lots ($p < 0.05$).

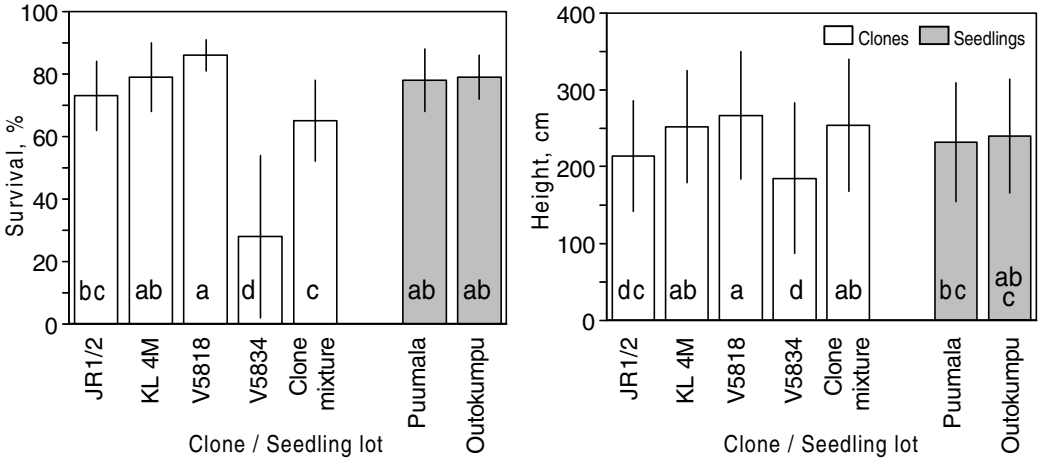

Fig. 6. Survival (%) and height (cm) of different clones and seedling lots (mean and S.D.) in trial series 1444/1–3. Means marked with a different letter are significantly different (Tukey's test, $p < 0.05$).

mixture of four clones (clone V5834 included) also remained rather low. Although there were some differences in the survival ranking of the lots in different sites, it was the same lots (V5834 and clone mixture) that had lowest survival on all sites in trial series 1444. The ranking of lot height at Juankoski was also somewhat different compared to the two other sites in trial series 1444, but the lot × trial site interaction was not, however, significant (Table 6).

3.2 Biotic Damages

3.2.1 Vole Damage

Damage caused by voles was observed in trial series 1443/1–3 and 1444/1–3 in spring 1993, the following year after planting, when the plants were two and three years old, respectively.

The damage by voles in trial series 1443 was severe, the average proportion of trees damaged

Table 7. Frequency of plants (%) damaged by voles (mean and S.D.) in micropropagated and seed-born material in trial series 1443/1–3 in June 1993 at the age of 2 years.

Material type	Trial 1 Ruokolahti	Trial 2 Ruokolahti	Trial 3 Taipalsaari	Trials 1–3
Clones	59 (*28*)	50 (*27*)	59 (*21*)	56 (*26*)
Seedlings	55 (*26*)	52 (*32*)	52 (*22*)	53 (*27*)
Average	57 (*27*)	51 (*29*)	55 (*22*)	54 (*26*)

Table 8. Frequency of plants (%) damaged by voles (mean and S.D.) in micropropagated and seed-born material in trial series 1444/1–3 in June 1993 at the age of 3 years.

Material type	Trial 1 Ruokolahti	Trial 2 Juankoski	Trial 3 Tuupovaara	Trials 1–3
Clones	29 (*14*)	1 (*1*)	2 (*3*)	11 (*15*)
Seedlings	52 (*21*)	1 (*2*)	1 (*1*)	18 (*27*)
Average	35 (*19*)	1 (*1*)	2 (*3*)	13 (*19*)

being 54% (Table 7). Some 56% of the micropropagated trees were damaged and 53% of the seed-born trees. The difference was not significant ($p < 0.2389$).

In trial series 1444, noticeable vole damage was observed only at Ruokolahti (1444/1), whereas the frequencies of vole damage at Juankoski and Tuupovaara were very low (Table 8). The analyses of variance was therefore only performed within the Ruokolahti trial, where the frequency of trees damaged by voles was 29% within the micropropagated material and 52% within the seed-born material. The difference between the two material types was statistically significant ($p < 0.0003$).

In trial series 1336, considerable vole damage was observed only at Savonranta, where 14% of the micropropagated trees and 18% of the seed-born trees were damaged (Table 9). The difference between the two material types was not statistically significant ($p < 0.4391$).

There were significant differences in the frequency of vole damage between clones and seedling lots in trial series 1443 (Table 10). The clones K2674 and E9702 had the highest frequency of vole damage (Fig. 7) and the lowest frequency was observed in the clone V5279 as well as in

Table 9. Frequency of plants (%) damaged by voles (mean and S.D.) in micropropagated and seed-born material in trial series 1336/2–4 in September 1993 at the age of 6 years.

Material type	Trial 2 Liperi	Trial 3 Imatra	Trial 4 Savonranta	Trials 2–4
Clones	2 (*5*)	7 (*8*)	14 (*10*)	8 (*9*)
Seedlings	1 (*3*)	4 (*8*)	18 (*11*)	8 (*10*)
Average	1 (*4*)	6 (*8*)	16 (*10*)	8 (*10*)

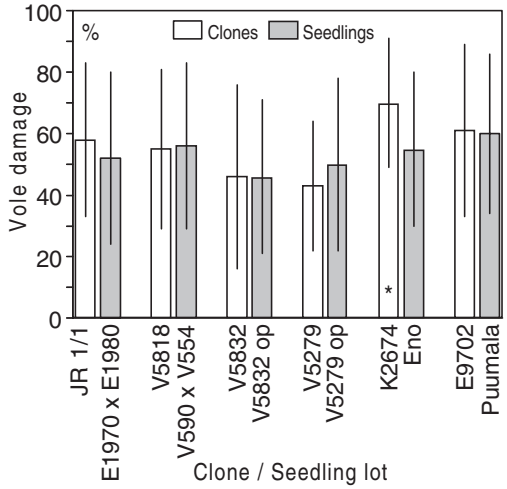

Fig. 7. Frequency of plants (%) damaged by voles in different clones and seedling lots (mean and S.D.) in trial series 1443/1–3. Clones marked with an asterisk (*) differ significantly from their comparison seedling lots ($p < 0.05$).

the clone V5832 and its open pollinated progeny (V5832 op). Pairwise comparisons between clone and related seedling lot revealed that only the clone K2674 and corresponding stand seed origin of Eno differed significantly ($p < 0.0160$).

In trial 1444/1 (Ruokolahti), the two stand seed origins had the highest vole damage frequency (58 and 45%) and the clone V5818 the lowest (18%) (Fig. 8). The differences between the lots were statistically significant (Table 10).

Vole damage among the clones at trial 1336/4 (Savonranta) varied from 9 to 23%, but the differences between the lots were not significant (Table 10).

Table 10. ANOVA table of biotic damages in trial series 1336, 1443 and 1444.

Damage type	Trial/ trial series	Site	Lot	Block (within trial)	Lot × site	Error
Vole	1336/4		F = 1.38 DF = 5 p<0.2735	F = 1.2 DF = 4 p<0.3408		DF = 20 MS = 0.0198
"	1443/1–3	F = 1.77 DF = 2 p<0.1719	F = 2.74 DF = 11 p<0.0023	F = 5.17 DF = 24 p<0.0001	F = 1.94 DF = 20 p<0.0107	DF = 245 MS = 0.0759
"	1444/1		F = 4.89 DF = 6 p<0.0022	F = 2.09 DF = 4 p<0.1142		DF = 24 MS = 0.0244
Moose	1336/4		F = 0.87 DF = 5 p<0.5188	F = 1.77 DF = 4 p<0.1749		DF = 20 MS = 0.0435
"	1443/3		F = 1.33 DF = 10 p<0.2304	F = 13.18 DF = 8 p<0.0001		DF =80 MS =0.0975
"	1444/1–3	F = 33.13 DF = 2 p<0.0001	F = 3.61 DF = 6 p<0.0040	F = 18.81 DF = 10 p<0.0001	F = 1.55 DF = 12 p<0.1319	DF = 60 MS = 0.0172
Stem lesions	1336/2–4	F = 166.16 DF = 2 p<0.0001	F = 7.50 DF = 5 p<0.0001	F = 1.51 DF = 12 p<0.1460	F = 4.16 DF = 10 p<0.0002	DF = 60 MS = 0.0186
"	1443/1–3	F = 24.14 DF = 2 p<0.0001	F = 2.51 DF = 11 p<0.0053	F = 3.35 DF = 24 p<0.0001	F = 1.06 DF = 20 p<0.3952	DF = 245 MS = 0.0923
"	1444/1–3	F = 90.54 DF = 2 p<0.0001	F = 26.38 DF = 6 p<0.0001	F = 3.91 DF = 10 p<0.0004	F = 2.84 DF = 12 p<0.0038	DF = 60 MS = 0.0133

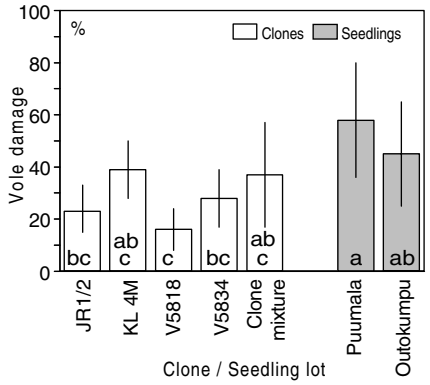

Fig. 8. Frequency of plants (%) damaged by voles in different clones and seedling lots (mean and S.D.) in trial 1444/1 Ruokolahti. Means marked with a different letter are significantly different (Tukey's test, $p < 0.05$).

3.2.2 Moose Damage

Moose had browsed a significant number of trees at only the Savonranta trial (38%) in series 1336 (Table 11) and the Taipalsaari trial (24%) in series 1443 (Table 12). The difference between the micropropagated and seed-born material was not significant in either of the two trials ($p < 0.3584$ and 0.3213, respectively). In the large trial series 1444/1–3, all three trials were quite heavily browsed. On the average, 32% of the micropropagated trees and 29% seed-born trees were damaged (Table 13). The difference was not significant ($p < 0.2403$).

Differences among the lots at Savonranta (1336/4) and Taipalsaari (1443/3) trials were not

Table 11. Frequency of plants (%) damaged by moose (mean and S.D.) in micropropagated and seed-born material in trial series 1336/2–4 in September 1993 at the age of 6 years.

Material type	Trial 2 Liperi	Trial 3 Imatra	Trial 4 Savonranta	Trials 2–4
Clones	6 (*14*)	0	36 (*21*)	14 (*21*)
Seedlings	6 (*15*)	0.5 (*1.3*)	41 (*13*)	16 (*21*)
Average	6 (*14*)	0.2 (*0.9*)	38 (*18*)	15 (*21*)

Table 12. Frequency of plants (%) damaged by moose (mean and S.D.) in micropropagated and seed-born material in trial series 1443/1–3 in September 1996 at the age of 6 years.

Material type	Trial 1 Ruokolahti	Trial 2 Ruokolahti	Trial 3 Taipalsaari	Trials 1–3
Clones	0	0.4 (*3*)	24 (*29*)	7 (*19*)
Seedlings	0	0.2 (*1.7*)	26 (*30*)	9 (*22*)
Average	0	0.3 (*2.3*)	24 (*30*)	8 (*21*)

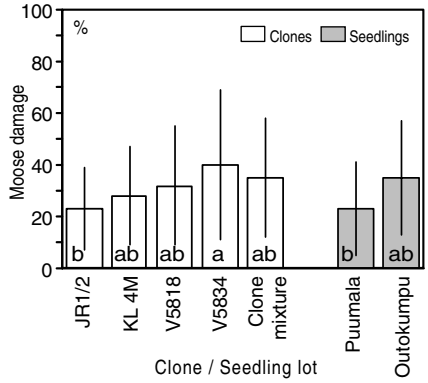

Fig. 9. Frequency of plants (%) damaged by moose in different clones and seedling lots (mean and S.D.) in trial series 1444/1–3.

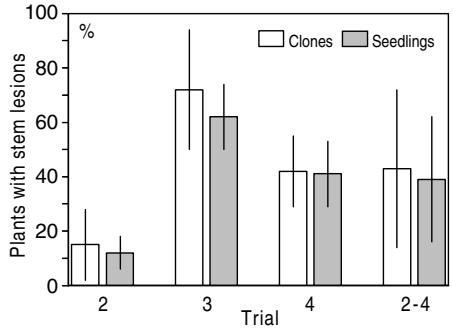

Fig.10. Frequency of plants (%) with stem lesions in micropropagated and seed-born material (mean and S.D.) in trial series 1336/2–4. Trial 2 = Liperi, 3 = Imatra, 4 = Savonranta.

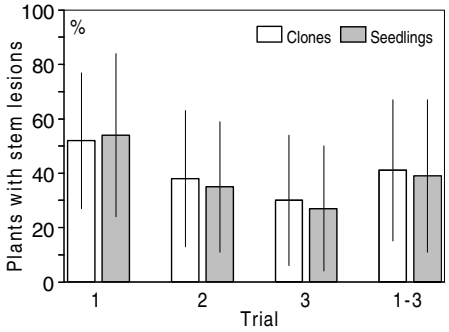

Fig.11. Frequency of plants (%) with stem lesions in micropropagated and seed-born material (mean and S.D.) in trial series 1443/1–3. Trial 1 = Ruokolahti, 2 = Ruokolahti, 3 = Taipalsaari

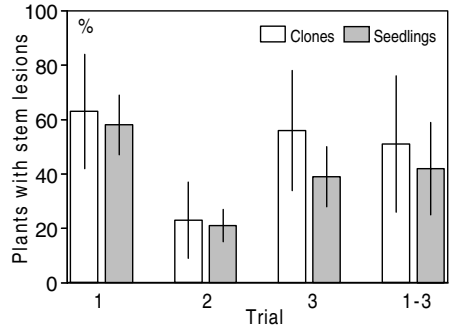

Fig.12. Frequency of plants (%) with stem lesions in micropropagated and seed-born material (mean and S.D.) in trial series 1444/1–3. Trial 1 = Ruokolahti, 2 = Juankoski, 3 = Tuupovaara.

Table 13. Frequency of plants (%) damaged by moose (mean and S.D.) in micropropagated and seed-born material in trial series 1444/1–3 in spring 1997 at the age of 7 years.

Material type	Trial 1 Ruokolahti	Trial 2 Juankoski	Trial 3 Tuupovaara	Trials 1–3
Clones	33 (*22*)	16 (*10*)	40 (*23*)	32 (*22*)
Seedlings	40 (*21*)	8 (*5*)	31 (*18*)	29 (*21*)
Average	35 (*22*)	14 (*9*)	37 (*22*)	31 (*22*)

statistically significant, but the differences in the trial series 1444/1–3 were significant (Table 10). The clone V5834 and the clone mixture had the highest frequencies of trees damaged by moose, whereas the clone JR 1/2 and Puumala stand origin had the lowest frequencies (Fig. 9). The ranking of damage by lots was the same at all trial sites, and no significant interaction between the lot × trial site was detected.

3.2.3 Stem Lesions

In all three trial series the average frequencies of trees suffering from stem lesions were rather high. There was, however, wide and statistically significant variation between sites within each series (Figs. 10, 11 and 12, Table 10). The frequency of stem lesions only differed significantly ($p < 0.0313$) between micropropagated and the seed-born trees in trial series 1336. The differ-

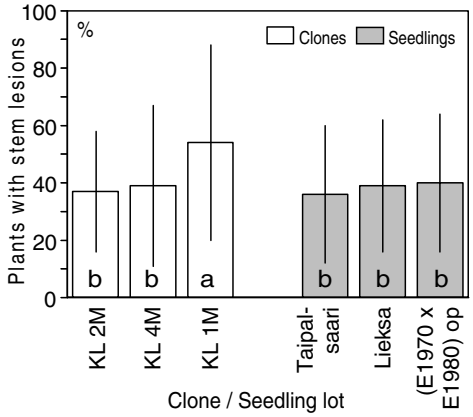

Fig.13. Frequency of plants (%) with stem lesions in different clones and seedling lots (mean and S.D.) in trial series 1336/2–4.

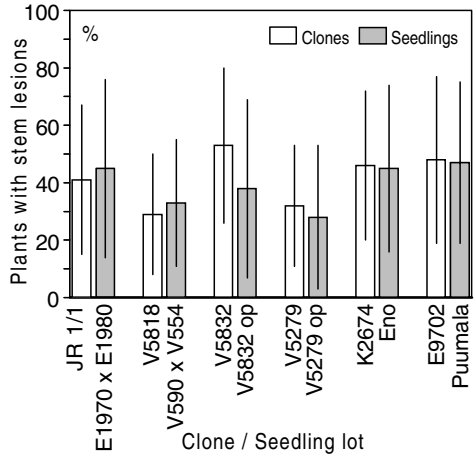

Fig.14. Frequency of plants (%) with stem lesions in different clones and seedling lots (mean and S.D.) in trial series 1443/1–3.

ence between the two material types was also statistically significant ($p < 0.0018$) in trial series 1444/1–3 (Fig. 12), but when the most susceptible clone V5834 was dropped, the difference was no longer significant ($p < 0.8262$).

Statistically significant differences were detected among the lots in all three trial series regarding the frequency of stem lesions (Table 10). Clone KL 1M had the highest frequency of stem lesions in trial series 1336 (Fig. 13), and the clone V5832 in 1443. Clones V5818 and V5279 with their related seedling lots had the lowest frequency of lesions (Fig. 14). Pairwise comparisons, however, revealed that none of the clones differed significantly from their corresponding seedling lots. Clone V5834 had the highest frequency of stem lesions (80%) and the clone V5818 the least in trial series 1444 (Fig. 15). The ranking of the clones on the basis of stem lesion frequency was similar in all trials, with V5834 performing the worst.

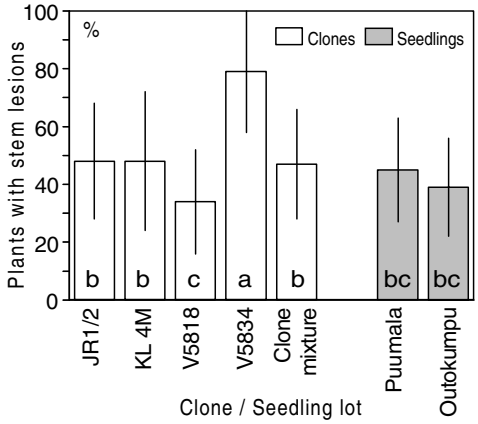

Fig. 15. Frequency of plants (%) with stem lesions in different clones and seedling lots (mean and S.D.) in trial series 1444/1–3.

4 Discussion

There were no significant differences between micropropagated and seed-born silver birches in growth and field performance in our study. Earlier studies, where these two material types are compared are few, and they mainly include rather limited materials from genetic point of view.

McCown and Amos (1979) compared the growth of seedlings and micropropagated birches in the field and found that both had identical growth rates in the spring and summer, but the micropropagated plants stopped growth one month earlier than the seedlings. This resulted in the micropropagated plants having a smaller size than the seedlings. The earlier cessation of growth in the micropropagated material may have been

due to genetic differences. Jokinen and Törmälä (1991) followed the growth of four micropropagated silver birch clones and two seed-born lots during the first growing season in the nursery, and reported that the micropropagated plants grew somewhat faster. This difference, however, was almost certainly due to genetic differences and not a general advantage of micropropagation or successful clonal selection.

In a study by Meier-Dinkel (1992) the growth of micropropagated plants of *B. pubescens* (three clones) in the lowlands of North Germany was good. In another trial at higher elevation the growth of micropropagated plants of *B. pendula* and *B. pubescens* was much slower, which was explained by poorer growth conditions and sensitive response to transplanting of the two-year-old plants. However, the trials of Meier-Dinkel (1992) did not include seed-born lots. In the same study, hybrid birches (*B. platyphylla* var. *japonica* × *B. pendula*) propagated *in vitro* from mature genotypes, showed a vigorous orthotrophic growth typical to seedlings. According to McCown (1989) micropropagated birch closely resemble seedlings in its overall phenotypic characteristics. On the other hand, micropropagation can be used to markedly increase the uniformity of tree crops, while at the same time provide transplants with growth characteristics typical of seedlings.

Viherä-Aarnio and Ryynänen (1995) compared silver birch seedlings, grafts and micropropagated plants as regards growth, crown structure, flowering and seed production during the first four years in a polythene greenhouse experiment with ten different genotypes. At the age of two years, the growth of the seedlings was the most vigorous and that of the grafts the lowest, the micropropagated plants being intermediate. The difference between the seed-born and the micropropagated plants was, however, not significant. The seedlings had significantly higher number of branches than the micropropagated plants, whereas the differences in branch length, branch thickness and seed production between these two groups were not significant. The closer similarity between the micropropagated plants and the seedlings suggests that the micropropagated material had been rejuvenated. Various juvenile morphological and biochemical features have also been reported among micropropagated plants from mature trees of *Betula* species by Brand and Lineberger (1992a, 1992b). However, Viherä-Aarnio and Ryynänen (1995) found, that micropropagated plants were closer to the grafts than the seedlings with respect to the male flowering, indicating that all features of the micropropagated trees may not be juvenile (Jones et al. 1996).

Jones et al. (1996) compared the field performance of silver birch trees produced by micropropagation with that of seedlings during seven years. Material for micropropagation was collected from a 20-year-old tree, which in turn had been produced by grafting a shoot from a 40-year-old tree. In their study, micropropagated trees grew at similar rate to seedling trees and no obvious mutant types were observed. The micropropagated trees were more uniform in height and trunk girth than seedlings. The high uniformity of micropropagated plants also shown by McCown and Amos (1979) could not be seen in our results, probably due to the highly variable environmental conditions between and within our trial sites.

The material of this study was quite extensive, with over 29 000 trees at the time of establishment on nearly 17 ha. As far as the authors are aware, this is the most extensive material reported on micropropagated birch. Although the material is limited genetically (11 clones and 10 seedling lots), it enabled us to compare, micropropagated and seed-born material in field conditions. Variation between and within trial sites in environmetal factors, (e.g. topography, soil type, moisture conditions, ground vegetation) was wide and reflected in the variation in the growth of the trees. The trial sites, however, represent the range of field conditions in southern Finland.

When comparisons of this kind are made, it must be remembered, however, that the selection of genotypes included in the study has an effect on the results. Comparisons should be done between clones and seedling lots as closely related as possible. The performance of the micropropagated material, in particular, is dependent on the clonal propagation of good or poor genotypes by chance. For example, clone V5834 included in trial series 1444 was an exceptionally poorly performing clone. Its low survival lowered the average survival of the whole micropropagated material group. When this clone was excluded from the analysis, the difference between the

plant types was no longer significant. Thus the use of micropropagated material does not automatically infer a general advantage over seed-born plants; any benefits or risks are dependent on the selection of successful clones to be multiplied.

Statistically significant variation among clones in survival and growth was detected in all three trial series included in this study (Tables 5 and 6, Figs. 4, 5 and 6). Reports from clone tests of birch, where several clones are compared in field conditions, are few. Meier-Dinkel (1992) was the first to report significant differences between different clones of *B. pubescens* in field trials as regards height growth. Viherä-Aarnio (1994) reported preliminary results from one of the trials included in this study, and found significant variation between one exceptionally poorly performing clone and other clones. Considerable clonal variation with respect to growth and stem quality traits was reported by Stener (1999) in a study consisting of 83 birch clones, which had been selected as plus trees in central Sweden. Significant differences were also detected in the seed production of different silver birch clones in a greenhouse experiment by Viherä-Aarnio and Ryynänen (1994).

Micropropagation can be used to clonally multiply superior genotypes, both for commercial production and for breeding purposes, but the selection of clones to be propagated is of crucial importance. In this study even the best performing selected clones seldom differed from seedling lots used as comparison, even if the seedling lots were stand origins. The trial series 1443 was especially planned for comparing a clone with a corresponding seedling lot, i.e. the family from which the clone was selected (1), progeny from open pollination of the clone itself (2) or stand seed of the same geographical origin as the clone (3). In only one case was there a significant difference between the clone and its comparison lot (clone V5818 and the family V590 × V554) with respect to height. In contrast, clone K2674, which was multiplied from a phenotypically selected plus tree from Eno, had significantly lower survival than stand seed origin (seed mixture of several mother trees) from the same stand.

The low genetic gain of clonal selection to be seen in this study is evidently due to the selection background of the material. Selection of clones for the propagation programme was done by different organizations and by personnel with varying expertise. Furthermore, the conditions where selection was made varied from mature natural stands to progeny tests. Even the selection criteria varied from case to case, although good growth and good stem quality were usually emphasized.

Although the reports from birch clone tests in the field are rather few, different types of experiments in controlled environments have been made. Clonal differences have been reported in sensitivity and response to tropospheric ozone (Pääkkönen et al. 1993, 1995), production of primary and secondary metabolites (Lavola et al. 1994), susceptibility to fungal diseases (Poteri and Rousi 1996), and palatability to herbivores (Jia et al. 1997, Rousi et al. 1997). Since part of the clones used in these experiments are the same as included in our study, it is possible to compare field performance of a clone and its behaviour in controlled tests. On the basis of the examples given below, it is not possible to see any clear correlation between the field performance of a clone and its single traits measured in controlled environments.

In their studies on the sensitivity and response of birch clones to ozone, Pääkkönen et al. (1993, 1995) used five clones, of which V5818, KL2M, V5834 and JR1/1 (KL7M) were the same as in our study. The clone KL2M was found to be the most tolerant one, while V5818 and V5834 were intermediate with respect to their sensitivity to ozone. In our study KL2M and V5818 were well performing clones, but V5834 was a poor one (Fig. 4, 5 and 6).

Moose and the voles are the most important biotic damage agents in young birch cultivations in Finland. Jia et al. (1997) studied the browsing of free-ranging moose on nine different silver birch clones in a field cafeteria experiment. The clones V5818, KL2M (=39), JR1/1, V5834 and V5832 in our study were included in their study. Clone V5818 and KL2M were found to be least palatable to moose, JR1/1 and V5834 were intermediate and V5832 the most palatable clone. In our study significant differences in the frequency of moose damage were observed only in trial series 1444, in which clone V5834 was the most severely damaged and V5818 intermediately.

Very high frequencies of plants damaged by voles were recorded in trial series 1443 and trial 1444/1. This was because the vole population had been very high during the previous winter in that area (Kaikusalo and Henttonen 1992). The clones K2674 and E9702 were the most severely browsed, the clones V5832 and V5279 the least browsed and JR1/1 and V5818 intermediate. Interestingly, clone K2674, which was multiplied from a phenotypically selected plus tree from Eno, was significantly more damaged than the Eno stand seed origin. In the cafeteria feeding experiments of Rousi et al. (1997) clones KL2M (39) and K2674 were reported to be among the least palatable to voles, which is quite contradictory with respect to the clone K2674. The clones JR1/1, V5818, V5832 and V5279 showed similar performance with their related families in our study. Genetic differences between families of silver birch with respect to browsing by voles have been shown earlier by Rousi (1990) and in the field by Henttonen et al. (1995).

Damage caused by hares was low in the whole material, the frequency of damaged trees varying from 0 to 3%. No further analysis of the data was made.

Stem lesions and cankers are among the most serious disease problems in the cultivation of birch (Lilja et al. 1996). The frequency of plants suffering from stem lesions can be particularly high in cultivations on former agricultural land (Hytönen 1995). Stem lesions are caused by several fungi, e.g. *Godronia multispora*, *Fusarium avenaceum*, *Alternaria alternata*, *Botrytis cinerea* and *Phytopthora cactorum* (Lilja et al. 1996). The occurrence of these fungi depend on several factors, including ground vegetation and weather conditions. Infection may occur already in the nursery or later in the field. Very often the plants are infected through a mechanical wound caused by frost damage, insects or voles for instance. The frequency of plants with stem lesions was high in this study, about the same level as reported by Hytönen (1995) from field plantations of silver birch (49%). The highest frequencies were observed on sites with good fertility, dense ground vegetation, low slope position and sheltered conditions, e.g. trials 1336/3 at Imatra and 1444/1 at Ruokolahti. The lowest frequencies were observed in trials 1336/2 at Liperi and 1444/2 at Juankoski, which are both situated on a drier site, more exposed hill slope with more windy conditions. Clones V5834, KL1M and V5832 had the highest frequencies of stem lesions. We were unable to find published results on suspectibility to fungi causing stem lesions in relation to clonal variation. Poteri and Rousi (1996) showed significant variation among seven silver birch clones as regards susceptibility to birch rust (*Melampsoridium betulinum*). Clone V5832 was the most resistant one, whereas K2674 and V5818 the most susceptible ones.

In conclusion, micropropagation can be used to provide genetically uniform material from desired genotypes with equal growth characteristics as the seedlings. The risks included in the genetic uniformity of the planting stock as well as the occurrence of clones with exceptionally poor performance should, however, be taken into consideration. We emphasize the careful selection of clones and recommend the long-term field testing of the selected material before any wide-scale commercial micropropagation and practical forest cultivation takes place.

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