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NITROGEN FIXATION AND BIOMASS PRODUCTION IN SOME ALDER CLONES

A greenhouse experiment

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Seloste

ERÄIDEN LEPPÄKLOONIEN TYPENSIDONTA JA BIOMASSAN TUOTOS
Kasvihuonekoe

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In a greenhouse experiment that lasted for two years, nitrogenase activity (C_2H_2-reduction), height growth and biomass production was compared in six clones of *Alnus* of which four were known clones of *A. incana* and two *A. incana* × *A. glutinosa* hybrids. In addition the effect of a fertilizer nitrogen gradient was tested on one of the clones. The alder cuttings were grown in a mixture of gravel, wood ash and field soil rich in the nitrogen fixing *Frankia* actinomycete.

Clonal differences in height growth and nitrogenase activity were recorded already 8 weeks after replanting. The *A. incana* × *A. incana* clones survived the winter outdoors better than that of the *A. incana* × *A. glutinosa* hybrid. During the second growing season the growth rhythm of some of the clones were markedly different compared to the previous year, but towards the end of the second growing season the differences in height growth between clones disappeared. The alders were 55–70 cm at the end of the experiment. There were significant differences in nitrogenase activity between clones during the active growing period but which also later levelled out. The total biomass of the grey alder clone with the fastest growth rate was significantly larger than that of the hybrid clone with the slowest growth rate.

Nitrogen fertilization suppressed nodulation during the first growing season. At the beginning of the second growing season nodulation occurred at 2,5 and 5 g nitrogen/m^2. Nitrogenase activity was, however, significantly higher in alders grown without nitrogen supplement. At the end of the second growing season nodulation and nitrogenase activity also occurred in alders given 10 and 15 g nitrogen/m^2. However, nitrogen supplemented at $\geq$ 15 g/m^2 level significantly depressed growth and total biomass. The height of the alders in the fertilization experiment ranged between 45–80 cm.

The results of this study show that there are clonal differences concerning rooting capacity, winter survival, growth rhythm, nitrogenase activity and biomass production and that alder transplants apparently do not benefit from a surplus of nitrogen, at least not given in a single dose.

1. INTRODUCTION

In temperate forests alders (*Alnus* spp.) are among the most important nitrogen fixing tree species. The fixation of nitrogen is based on the symbiosis between the alder and certain soil actinomycetes. After infection by the actinomycete, nitrogen fixing nodules are formed on the alder roots. According to Becking (1970), the endophyte of such actinorhizal plants belongs to the genus *Frankia*. *Frankia* possesses the nitrogenase enzyme system, that reduces inert atmospheric nitrogen to plant available ammonium nitrogen.

The value of alder in improving forest soil conditions is well known. Tarrant and Trappe (1971) have estimated that the annual accretion of nitrogen to the soil in alder stands ranges between 40–300 kg N/ha depending on site, stand age and density. Investigations carried out by Virtanen (1957) and Mikola (1966) have shown that even in such northern latitudes as in Finland, alders may fix significant quantities of nitrogen.

A small amount of inorganic nitrogen is known to stimulate the nitrogen fixation (e.g. MacConnell and Bond 1957, Stewart and Bond 1961, Zavitkovski and Newton 1968), but upper limits for a positive response by alder are not known. The successful cultivation of alder in coniferous plantations can be expected to reduce or even eliminate the need for nitrogen fertilizer. By making use of wood ash, which can provide all other nutrients, especially phosphorus, the need for supplementary fertilization may be further reduced in both alder monocultures and mixed stands.

Data on the potential capacity of alder for nitrogen fixation and biomass production has been presented by Gordon and Dawson (1979). Grey alder (*A. incana*), which is widely spread in northern Europe and adapted to various sites, is an efficient nitrogen fixing and fast growing alder species. Thus it may be suitable for short rotation energy forestry in northern latitudes.

The genetic variation within *Alnus* species is considerable (Bajuk et al. 1978, Gordon and Wheeler 1978), and differences in both nitrogenase activity and biomass production have been shown by Huss-Danell (1980), who also found a clear positive correlation between these characteristics. The potential use of alder for either biomass production or as a soil ameliorator in forest plantations makes the selection of alder clones possessing both high nitrogen fixation efficiency and such characteristics as vigorous growth, drought and cold hardiness important.

The aim of this study was to compare the nitrogenase activity, height growth and biomass production in six alder clones grown from greenwood cuttings, and to assess the effect of increasing amounts of nitrogen fertilizer on the nodulation, nitrogenase activity and biomass production of the clones.

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2. MATERIAL AND METHODS

21. Production of cuttings and experimental design

Clonal material of alder was available when this experiment was being planned. The clones used in the experiment together with information on rooting and survival are listed in Table 1. Greenwood cuttings were prepared from 4-year-old ortets at the Haapastensyrjä Forest Tree Breeding Centre of the Foundation for Forest Tree Breeding in May, 1982. Each internode cutting was about 4 cm long and had one leaf, the area of which was reduced by 50 % to limit evapotranspiration. After rooting in a mixture of peat and gravel for 7 weeks in natural light at +20°C and 90 % humidity the cuttings were replanted in a growth substrate consisting of a mixture of field soil (fine sand) known to be rich in the alder endophyte (Saarsalmi et al. 1985) and gravel (1:1, w/w) to which wood ash had been added to supply mineral nutrients, especially phosphorus (Table 2).

One of the clones, clone 4, (*A. incana* × *A. incana*) was arbitrarily selected for the nitrogen fertilizer gradient experiment. The nitrogen was given as ammonium nitrate with lime as a single dose at planting (Table 3). The alders were irrigated with tap water but in the second year with a nitrogen free nutrient solution (Huss-Danell 1978) 1/10 dilution once a week. At the end of the first growing season, the greenhouse temperature was lowered stepwise so as to prepare the alders for overwintering outdoors.

Table 1. The origin, rooting and survival of the clones.
Taulukko 1. Eri kloonien alkuperä, pistokkaiden juurtuminen ja eloonjäämis-%.

Clone *Klooni*	Origin *Alkuperä*	Rooting [a], % *Juurtuminen,* 1980	1982	Survival in spring 1983 *Elossa keväällä* %
1. *Alnus incana* × *Alnus incana*	Loppi × Sääksmäki	86	67	88
2. *A. inc.* × *A. inc.*	"	56	51	88
3. *A. inc.* × *A. inc.*	Loppi × Lohja	44	47	76
4. *A. inc.* × *A. inc.*	Loppi × Loppi	no observation ei havaintoa		100
5. *A. inc.* × hybr.[b]	Loppi × Helsinki	49	36	47
6. *A. inc.* × *Alnus glutinosa*	Loppi × Tammisaari	50	33	24

[a] According to experiments carried out at the Haapastensyrjä Forest Tree Breeding Centre of the Foundation for Forest Tree Breeding. Metsänjalostussäätiöllä tehtyjen kokeiden perusteella.
[b] *A. inc.* × *A. glut.*, Helsinki × Helsinki

Table 2. Content and amount of total nutrients in the wood ash supplied to the alders.
Taulukko 2. Puutuhkan ravinnepitoisuudet ja lepille tuhkalannoituksessa annetut ravinnemäärät.

Nutrient *Ravinne*	Content *Pitoisuus* %	ppm	Amount [a] *Määrä* g/m²
P	1,3		5
K	4,2		16
Ca	25,0		95
Mg	2,3		9
Mn	1,6		6
Zn		380	1
B		54	0,2
Co		39	0,1
Cu		23	0,1
Mo		5	0,02

[a] surface area of 2-litre pot = 0,0165 m²
2-l astian pinta-ala

Table 3. Experimental design (10 replications in each treatment).
Taulukko 3. Koejärjestely (10 koejäsentä käsittelyä kohti).

Clone[a] *Klooni*	Treatment – *Käsittely* Wood ash[b] *Puutuhka*	Nitrogen[c], g/m² *Typpi,*
1–6	+	–
4_{00} (control) (*kontrolli*)	–	–
4_0	+	–
$4_{2,5}$	+	2,5
4_5	+	5,0
4_{10}	+	10,0
4_{15}	+	15,0
4_{20}	+	20,0
4_{50}	+	50,0
4_{75}	+	75,0

[a] See Table 1 – *Ks. taulukko 1.*
[b] Amount equalling 5 g P/m². *Määrä vastaa*
[c] Ammonium-nitrate with lime. *Oulunsalpietari*

22. Height growth and biomass production

The growth of each alder transplant was recorded at the end of the first growing season and weekly during the second growing season as the increase in height (±0,1 cm) of the single shoot emerging from the axillary bud on the cutting. At the end of the experiment the alders were carefully extracted from the substrate and the biomass (dry weight after 24 h at +70°C) determined separately for stems, leaves, winter buds, roots and nodules.

23. Nitrogenase activity

The nitrogenase activity of each alder transplant was estimated using the acetylene reduction method (Hardy et al. 1968). Nitrogenase, which reduces atmospheric nitrogen to ammonium also reduces acetylene (C_2H_2) to ethylene (C_2H_4). The amount of ethylene produced, determined by gas chromatography, therefore reflects the rate of nitrogen fixation. Since this assay has shown to provide a simple and accurate method with many applications for assessing nitrogenase activity, it has been used with increasing frequency over the last decades (e.g. Hardy et al. 1973; Turner and Gibbson 1980, Beringer 1984). Due to difficulties with the conversion of measured amounts of ethylene to amounts of nitrogen fixed by the plant, it is not well suited for estimates of actual nitrogen inputs, but for comparative purposes, however, the method is handy and adequate (Minchin et al. 1983).

Nitrogenase activity was measured when the alders were 2, 12, 13 (Fig. 1a), and 14 months old. The pots were enclosed in 35×60 cm air tight polyethene bags (Fig. 1b)

Fig. 1. The alders at 13 months (a), and one of the alders enclosed in the polyethene bag for nitrogenase activity measurement (b).

Kuva 1. Lepät 13 kk ikäisinä (a) ja nitrogenaasiaktiivisuusmittausta varten polyeteenipussiin ilmatiiviisti suljettu lepän taimi (b).

(Wakuking, Wipak Oy) and 400 ml of acetylene was injected, giving an acetylene concentration of 5–10 %. Propene (2 ml) was used as the tracer gas for volume determination. Duplicate gas samples were taken after 1, 2, 3, and 4 hours of incubation with acetylene and injected into evacuated tubes (Venoject, Terumo Corp. Oriola Oy). The gas samples were analysed with a Carlo Erba gas chromatograph equipped with a flame ionization detector and a 2 m porapak R column (80 mesh, Ø 3,2 mm) using an oven temperature of +50°C. Checks for traces of ethylene in the acetylene used, endogenous ethylene formation and background nitrogenase activity in the soil *per se* by heterotrophic organisms were included. Measurements were performed just before noon, at which time there is known to be a peak in nitrogenase activity in alders (Wheeler 1969). Due to low nitrogenase activity at the end of the first growing season, the mean values given per hours are based on the 4 hour acetylene incubation period. During sampling in August in the second growing season, the temperature in the greenhouse was high (+30 – +34°C). Since high temperature influence the nitrogenase activity (Wheeler 1971, Johnsrud 1978), the measurements were consequently interrupted after 2 hours incubation. The results are therefore given as mean values for the difference between one hour and two hour incubation with acetylene.

24. Nutrient analyses

At the end of the experiment the growth substrate from replicate pots were combined and composite samples analysed for pH (H_2O) total N, extractable contents of P, K, Ca and Mg. Leaves were analysed for total N, P, K, Ca and Mg separately for each alder except in the nitrogen fertilizer part of the experiment, where leaf samples were composited according to treatment. The methods used are described by Halonen et al. (1983).

The data was statistically treated using analysis of variance and Tukey's test. Correlations were calculated using multiple linear regression analysis.

3. RESULTS

31. Height growth and biomass production

Height growth was small during the first growing season, the mean height in September being about 8 cm. Shoot development was best in clone 1, but also satisfactory in both hybrid clones 5 and 6 (Table 4).

The survival of clones 1, 2, 3 and 4 was good (Table 1). Because of high mortality (76 %) during the winter, the hybrid clone 6 had to be excluded from the experiment. Increasing amounts of nitrogen increased mortality, as shown by the decreasing number of replicates in Figure 5. Nitrogen at a level of 50 and 75 g/m^2 killed all the alders during the first growing season.

During the second growing season, the increase in height was the fastest between mid-

Table 4. The nitrogenase activity and height of the alder clones at the end of the first growing season.

Taulukko 4. Leppäkloonien nitrogenaasiaktiivisuus ja pituus ensimmäisen kasvukauden jälkeen.

Clone[a] *Klooni*	µ mol C_2H_4/h Per alder transplant – *Lepän tainta kohti*	Height, *Pituus,* cm
1	1,4	9,1
2	0,6	4,5
3	0,6	4,5
4	0,2	4,3
5	0,1	7,7
6	0,9	6,1
	r = 0,39***	

[a] See Table 1 – *Ks. taulukko 1.*

*** Correlation (r) significant at 0,001 level

*** *Korrelaatio (r) merkitsevä*

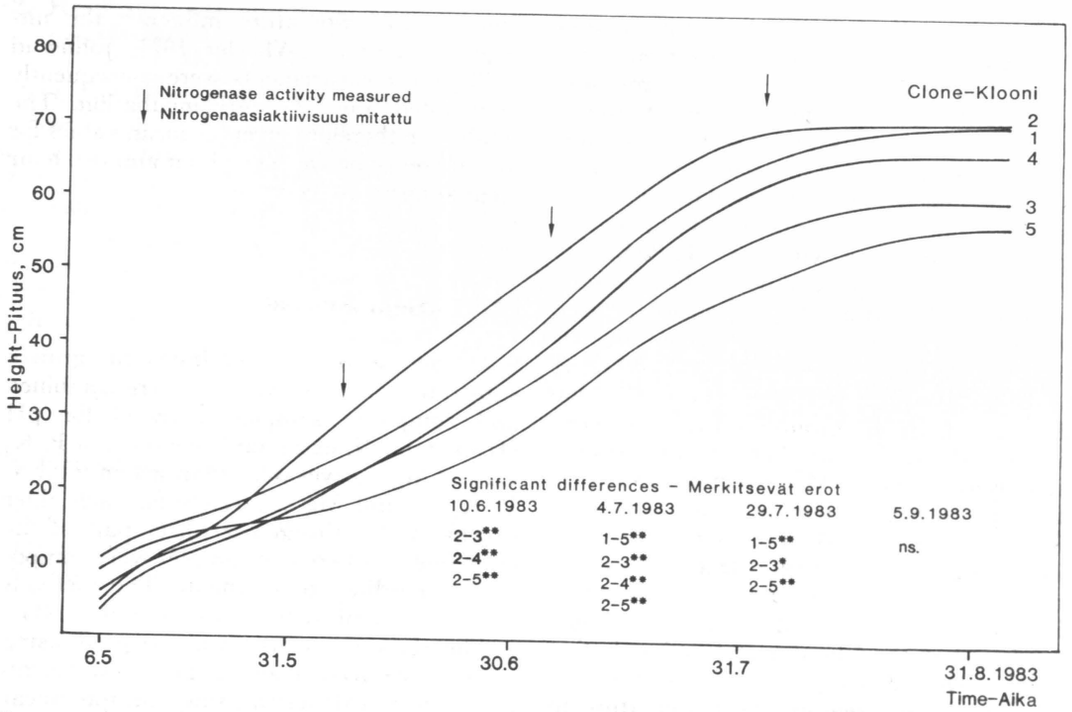

Fig. 2. The height growth of the alder clones during the second growing season (clones see Table 1). Level of significance: * and ** = P<0,05, 0,01 respectively.

*Kuva 2. Leppäkloonien pituuskasvu toisena kasvukautena (kloonit ks. taulukko 1). Merkitsevyystaso: * ja ** = P<0,05, 0,01 vastaavasti.*

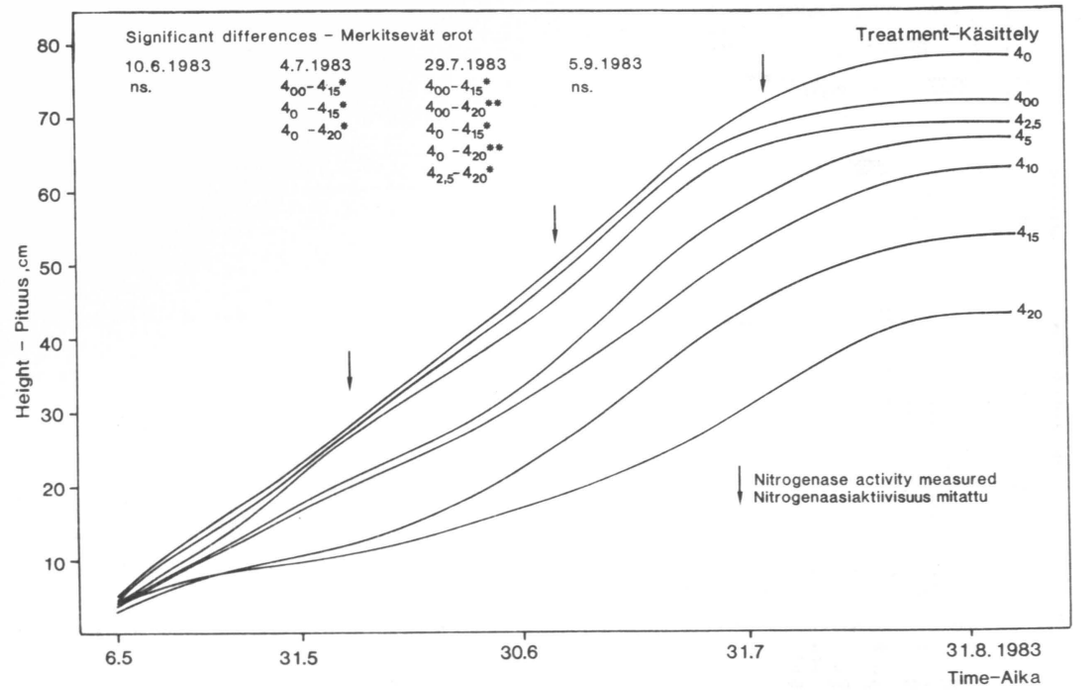

Fig. 3. The effect of nitrogen fertilizer treatment on the height growth of alder clone 4 during the second growing season (nitrogen gradient see Table 3).

Kuva 3. Typpilannoituksen vaikutus leppäkloonin 4 pituuskasvuun toisena kasvukautena (typpigradientti ks. taulukko 3).

June and the end of July and the vigour of some of the clones was markedly altered compared to the previous year (Figure 2). The difference in height between clone 2 and clones 3, 4 and 5, respectively, was significant through most of the growing season. The height growth of the clones ceased by about August 20, and differences levelled out. By the end of the experiment, when the plantlets were 58 weeks old, the tallest alders, about 70 cm, were those of clone 1 and 2. The height of the alders of clone 3 and 5 was about 55 cm. In the nitrogen fertilizer part of the experiment the tallest alders, about 75 cm, were found among those grown without ash and nitrogen fertilizer or those treated with ash only or ash and 2,5 g nitrogen/m^2 (Figure 3). Higher levels of nitrogen retarded growth. The effect became significant when ⩾ 15 g nitrogen /m^2 was added: the height growth was depressed by almost one third compared to that of the alders given only ash.

The average total biomass was highest for clone 2 and lowest for clone 5 (Figure 4). The control alders as well as those receiving only ash or ash and 2,5 g nitrogen/m^2 produced most biomass (Figure 5). With higher amounts of nitrogen fertilizer, biomass production was reduced, significantly when ⩾ 15 g nitrogen/m^2 was given. Roots were clearly the major component of the biomass, about 40 %. The proportion of roots to total biomass decreased, as expected, with increasing levels of nitrogen, being about 30 % when ⩾ 15 g nitrogen/m^2 was given. The proportion of nodules in clone 1 and 2 was larger compared to that of the other clones. For all clones, the proportion of nodules to total alder biomass ranged between 4–6 %. The nitrogen fertilizer treatments did not alter this proportion or affect the nodule biomass.

32. Nitrogenase activity

Root nodules began to appear three weeks after replanting. Those alders which had received nitrogen fertilizer did not nodulate during the first growing season. When the nitrogenase activity was measured eight weeks after replanting (1. 9. 1982), height growth had ceased and formation of winter buds had started. The nitrogenase activity was rather low, but differences between clones were obvious (Table 4). Clone 1 had the highest nitrogenase activity, while that of the back crossing hybrid, clone 5, was negligible.

During the second growing season the nitrogenase activity of the clones showed generally a clear rising trend from June to August (Figure 6). In June and July, during the active growing period, significant differences between the clones were found. The lowest activity was still shown by clone 5. The difference in nitrogenase activity between the most efficient grey alder clones 1 and 2 and the back crossing hybrid, clone 5, was highly significant. In August the growth rate had decreased (Figure 2) and, the differences in nitrogenase activity between clones levelled out and were no longer significant.

Alders that had received low amounts of nitrogen fertilizer (2,5 and 5 g/m^2) began to nodulate in early June during the second growing season. With amounts of 10 and 15 g/m^2 nodulation commenced one month later. With larger nitrogen dozes nodulation was either inhibited or the alders were killed. In June and July nitrogenase activity was highest in the control (Figure 7). The differences between the control and treatment 5 g nitrogen/m^2 or more were significant. The control nitrogenase activity in June and July was about five fold that of the nitrogen treatments which did not inhibit nodulation. By August the situation had changed and nit-

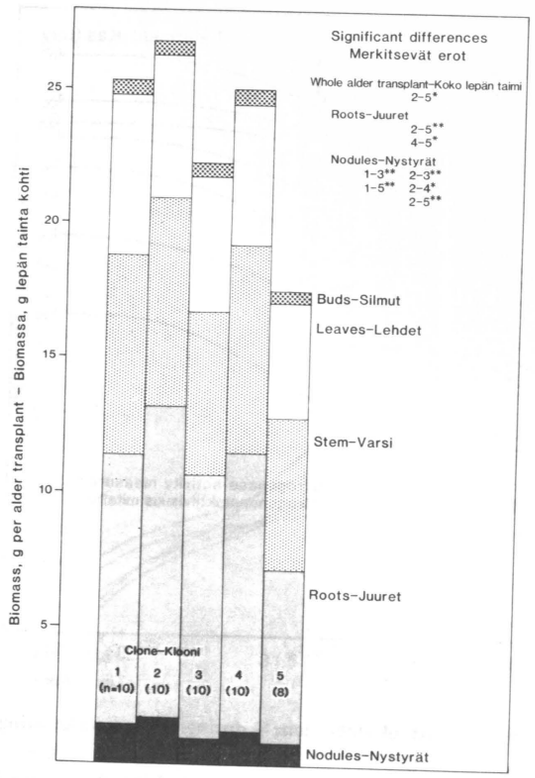

Fig. 4. The biomass (dw) of the different compartments of the alder clones after two growing seasons (n = the number of replicates; clones see Table 1).

Kuva 4. Leppäkloonien eri kasvinosien kuivamassa kahden kasvukauden jälkeen (n = koejäsenten lukumäärä; kloonit ks. taulukko 1).

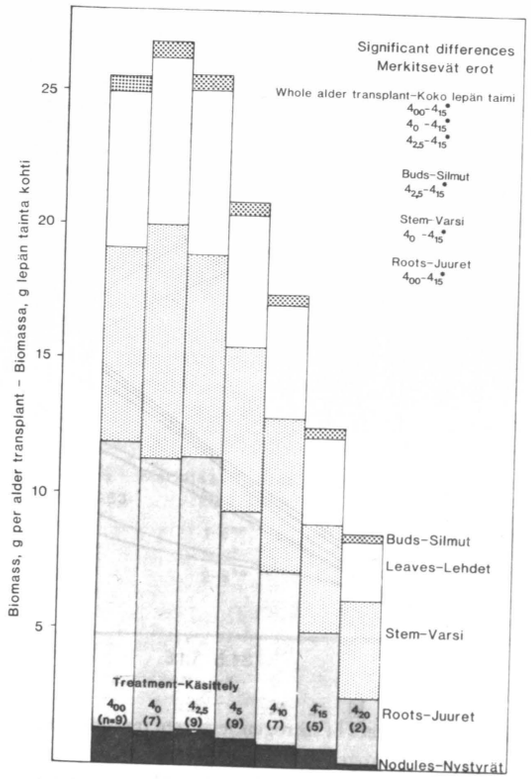

Fig. 5. The effect of nitrogen fertilizer treatment on the biomass (dw) of the different compartments of alder clone 4 after two growing seasons (nitrogen gradient see Table 3).

Kuva 5. Typpilannoituksen vaikutus leppäkloonin 4 eri kasvinosien kuivamassaan kahden kasvukauden jälkeen (typpigradientti ks. taulukko 3).

rogenase activity was highest in those alders given the lowest dose of nitrogen (2,5 g/m^2). However, differences between nitrogen treatments concerning nitrogenase activity were no longer significant at the end of the growing season.

For all the alders of the different clones, as well as for the alders in the nitrogen fertilizer gradient experiment, the correlation (r) between nitrogenase activity and height growth was highly significant throughout the growing season as shown below:

	June 7–8.	July 5–6.	August 3–4. 1983.
Clones, r =	0.77***	0.65***	0.51***
N-gradient, r =	0.75***	0.69***	0.38**

33. pH and nutrient content of the growth substrate and the leaves

As was expected, the addition of ash raised the pH (from 5,8 to 6,8) and increased mineral nutrient contents. Independent of clone or nitrogen treatment, the contents of P, K, Ca and Mg was similiar in all the substrates at the end of the experiment. The content of calcium and magnesium was nevertheless significantly higher, and the content of potassium lower in the leaves of clone 2 as compared to some of the other clones (Table 5). When compared to the nitrogen status at the beginning of the experiment, no clear changes in the total nitrogen pool of the growth subs-

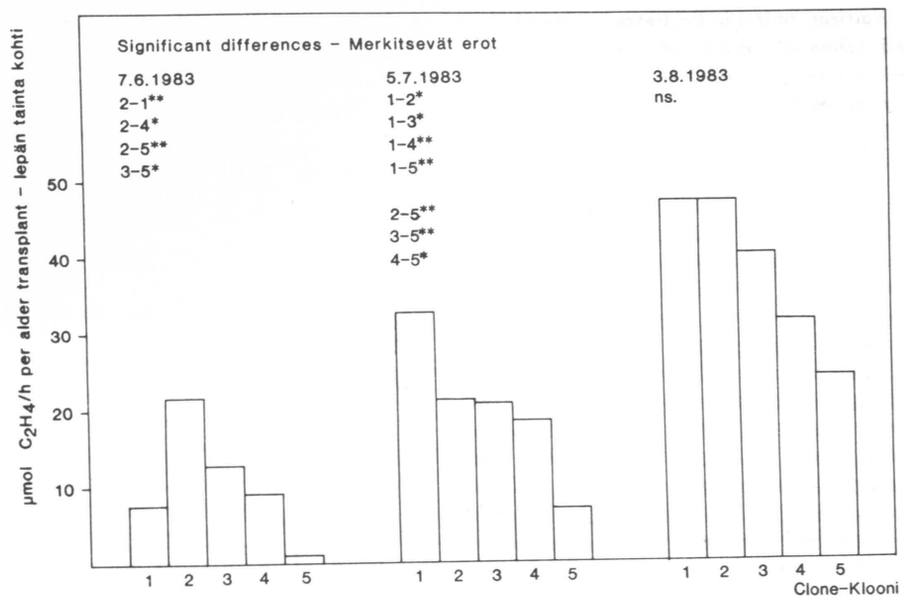

Fig. 6. Nitrogenase activity in the alder clones during the second growing season (clones see Table 1).

Kuva 6. Leppäkloonien nitrogenaasiaktiivisuus toisena kasvukautena (kloonit ks. taulukko 1).

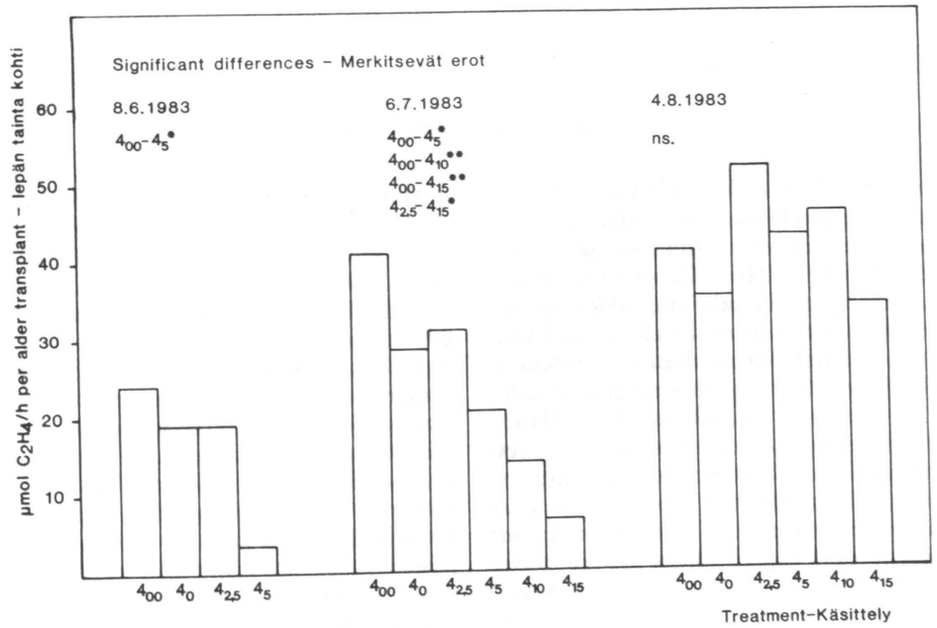

Fig. 7. The effect of nitrogen fertilizer treatment on the nitrogenase activity of alder clone 4 during the second growing season (nitrogen gradient see Table 3).

Kuva 7. Typpilannoituksen vaikutus leppäkloonin 4 nitrogenaasiaktiivisuuteen toisena kasvukautena (typpigradientti ks. taulukko 3).

Table 5. Nutrient content in the leaves at the end of the experiment.
Taulukko 5. Lehtien ravinnepitoisuudet kokeen lopussa.

Clone and treatment[a] *Klooni ja käsittely*	N	P	K	Ca	Mg	Signific. diff. between clones *Kloonien väliset merk.erot*	
	% dw – % *ka*						
1	3,4	0,17	1,9	1,8	0,21	K	Mg
2	3,2	0,16	1,6	2,4	0,35	2–5**	1–2**
3	3,4	0,18	1,9	2,1	0,31		1–3**
4	3,4	0,17	1,8	2,0	0,29	Ca	1–4*
5	3,3	0,19	2,0	1,9	0,31	2–1**	1–5**
						2–3*	
4_{00}	3,5	0,16	1,3	1,6	0,31	2–4**	
4_{0}	3,5	0,18	1,7	1,8	0,31	2–5**	
$4_{2,5}$	3,4	0,18	1,8	1,5	0,26		
4_{5}	3,4	0,18	1,8	1,8	0,29		
4_{10}	3,6	0,18	1,8	1,8	0,31		
4_{15}	3,4	0,20	1,9	1,6	0,28		
4_{20}	3,5	0,22	1,7	1,9	0,42		

[a] See Tables 1, 3 – *Ks. taulukot 1, 3.*

trates were recorded at the end of the experiment. Nitrogen fertilizer treatments of 15 and 20 g/m^2 raised the phosphorus content and that of 20 g/m^2 the magnesium content of leaves. Due to composite samples, the significance of this increase was not statistically asserted.

4. DISCUSSION

Although alders are classified as "difficult-to-root" species (Blake and Roderick 1983), reports on successful rooting are also available (Lepistö 1970, Huss-Danell et al. 1980). Roots developed in all the alder clones studied. However, one of the clones had a far better rooting percentage than all the others. Clonal differences in the rooting of grey alder cuttings was also found by Huss-Danell (1981), who suggested that rooting is a genetically controlled characteristic. According to Ljunger (1959), the cold hardiness of grey alder is better than that of black alder. In our experiment more than 80 % of the grey alder ramets, but less than 30 % of the grey and black alder hybrid ramets survived the winter. Frost damage and poor early development by black alder in field experiments has been shown also by Mikola (1975) and Korpelainen (1982).

During the first growing season the above ground growth of the alders was generally small and a considerable part of the growth went into developing roots and, in non-fertilized alders, nodules. Differences in the growth rate between clones were, however, already observed. During the second growing season the growth rhythm varied between the clones. Clone 2, with the highest growth rate, had ceased growing already by the end of July while clone 5, with the slowest growth rate, continued growing until September. Although the differences in height growth diminished towards the end of the growing season, significant differences in the total biomass were found between these clones. This result is in good agreement with the finding of others (e.g. Bajuk et al. 1978, Gordon and Wheeler 1978, Dawson and Gordon 1979, Huss-Danell 1980).

Those alders given the highest dozes of nitrogen (50 and 75 g/m^2) died during the first year of the experiment. The growth retarding effect of nitrogen fertilization became evident at lower levels in July, the following year. The total biomass was also reduced by as much as 50 % with a dose of 15 g nitrogen/m^2 compared to that of the alders given only ash.

Mainly due to cessation of growth and formation of winterbuds, the nitrogenase activity at the end of the first growing season was low. Notable differences between the clones were, however, already recorded. Nitrogenase activity during the second growing season reached its highest rate in the beginning of August, but significant differences between the clones were found only during the active growing period in June and in July. In this study, the correlation between nitrogenase activity and height growth was positive and highly significant as has been shown also by Huss-Danell (1980). It is noted that clone 2 showed a high nitrogenase activity in August (Fig. 6) although its growth had slowed up (Fig. 2). In comparison, clone 1 with an equally high nitrogenase activity continued its growth until the end of August. As pointed out by Wheeler et al. (1981), there is a close parallel between the pattern of nitrogen demand for growth and the rate of nitrogen fixation throughout most of the growing season, but substantial nitrogenase activity may still occur after the main period of active growth, presumably contributing to the nitrogenous reserves built up by the alder for overwintering.

Due to high energy requirements of the nitrogen fixation process, mineral nitrogen, if available, is always taken up in preference to molecular nitrogen. Small amounts of ammonium and nitrate have, however, been found to both stimulate nodulation and symbiotic nitrogen fixation, (e.g. MacConnell and Bond 1957, Stewart and Bond 1961, Zavitkovski and Newton 1968). In this study even the lowest doses of nitrogen inhibited nodulation during the first growing season and still hampered nitrogenase activity during the second, indicating that the plants were taking substitute nitrogen from the substrate. This inhibitory effect on the nodulation was expected, but the retarding effect on growth and biomass production was not. Ingestad (1980) pointed out that nitrogen in the rooting medium in such concentrations as used in practical forestry, may kill the nodules and lead to damage of the alder plant and therefore should not be used in alder plantations. The results from our study seem to support this conclusion.

An important factor controlling nitrogen fixation is the content of total soil nitrogen. Zavitkovski and Newton (1968) showed an accretion of nitrogen to a red alder ecosystem when the content of total soil nitrogen ranged between 0,03–0,05 %, when it exceeded 0,2 % the efficiency of nitrogen fixation decreased. The content of total soil nitrogen in the growth substrate used in this study was 0,15 %. Nitrogen mineralised therefore probably acted as a starter dose, making nitrogen fertilization at higher levels not only unnecessary but even harmful since even 5,0 g nitrogen/m^2 depressed nitrogenase activity, retarded growth and reduced the total biomass.

Nitrogen fertilization did not seem to have affected the nutrient uptake in general and no increase in substrate nitrogen content was observed. This indicates a probable loss of nitrogen from the growth substrate through leaching and leakage during outdoor storage of the pots in late autumn and early spring, volatilization of ammonia or through denitrification, the conditions for which were rather favourable; i.e. available nitrate, pH near neutral, high water status (Müller et al. 1980, Jaakkola 1985, Weber et al. 1985).

In this study the leaf nitrogen content of the different clones varied little and within the values given also by Mikola (1975). Despite the addition of rather high amounts of nitrogen, leaf nitrogen content in this study remained about the same, 3,4–3,6 %. Results by Mikola (1966) and Näsi and Pohjonen (1981) imply that the nitrogen content of alder leaves is a stable characteristic regardless soil nitrogen status. Concerning the leaf content of Ca, K, and Mg some differences between the clones were recorded. Rodriguez-Barrueco et al. (1984) has shown that during the growing season there are significant differences in the nutrient uptake in black alder. Information on clonal differences in nutrient uptake in alders is lacking. For herbaceous plant species, however, diversity among genotypes concerning mineral uptake

has been pointed out by, for example, Clark (1983).

The clone which ranked lowest on the basis of height growth and nitrogenase activity after 8 weeks, was superior at the end of the second growing season, ie. after 58 weeks, when height growth, nitrogenase activity, and biomass are considered. In other studies where nitrogenase activity and biomass production have been evaluated, the plant material has been only 5–13 weeks old (Huss-Danell 1980, Granhall et al. 1983). The dangers of interpreting the results obtained from short term experiments and young plant material are obvious as is also shown by Verweij (1977). This study also showed that neither the nodulation nor the growth of young alders was promoted by nitrogen fertilization given in a single dose.

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SELOSTE

ERÄIDEN LEPPÄKLOONIEN TYPENSIDONTA JA BIOMASSAN TUOTOS

Astiakokeessa, joka kesti kaksi vuotta, tutkittiin kuuden leppäkloonin (taulukko 1) typensidonnan tehokkuutta, pituuskasvua ja biomassan tuotosta sekä typpilannoituksen vaikutusta yhden, satunnaisesti valitun kloonin vastaaviin ominaisuuksiin.

Pistokkaat juurrutettiin rahkasammaleen ja soran seoksessa, jonka jälkeen ne istutettiin lepän endofyyttejä sisältävän, karkeahietaisen peltomaan, soran ja puutuhkan (taulukko 2) seokseen. Lannoituskokeessa typpi annettiin oulunsalpietarina (taulukko 3). Leppiä kasvatettiin kasvihuoneessa, talveksi ne kuitenkin siirrettiin ulos. Typensidontaa kuvaavan nitrogenaasientsyymin aktiiviisuus määritettiin asetyleeni-pelkistysmenetelmällä kaasukromatografisesti, ensimmäisenä kasvukautena kerran, toisena kolmesti. Taimien pituus mitattiin ensimmäisen kasvukauden lopussa sekä toisena kasvukautena viikottain. Syyskuun puolivälissä määritettiin talvisilmujen, lehtien, varsien, juurien ja nystyröiden kuivamassa. Lehtien ja kasvualustan ravinnepitoisuus sekä pH määritettiin lannoituskokeen osalta käsittelyittäin, kloonien osalta astioittain ja klooneittain.

Typpeä saaneita pistokastaimia lukuunottamatta juuriin kehittyi nystyröitä istutusta seuraavan kolmen viikon aikana. Typpilannoitus 50 ja 75 g/m^2 oli lepille liian suuri, ja ne kuolivat ensimmäisen kasvukauden aikana. Ensimmäisen kasvukauden päättyessä leppien nitrogenaasiaktiivisuuden ja pituuden välinen korrelaatio oli selvästi positiivinen ja klooneista erottui kaksi muita parempaa kloonia (taulukko 4). Harmaalepän risteytykset talvehtivat hyvin, mutta harmaa- ja tervalepän risteytyksistä oli talven jälkeen elossa vain 24 %, mistä syystä ko. klooni hylättiin (taulukko 1). Toisena vuonna nitrogenaasiaktiivisuus oli tehokkaimmillaan elokuussa, mutta alkukesästä esiintyvät kloonien väliset erot, kuten myös erot kasvurytmissä, olivat jo silloin tasaantuneet (kuvat 2 ja 6). Toisen kasvukauden alussa oli nystyröitä alkanut muodostua myös typellä lannoitettujen leppien juuriin. Nitrogenaasiaktiivisuus oli alkukesästä kuitenkin suurin lepillä, joille ei oltu annettu tuhkaa eikä typpeä. Vasta kasvukauden lopussa pienin typpilisäys (2,5 g/m^2), edisti typensidontaa.

Typpilannotus ei vaikuttanut merkitsevästi pituuskas-

vuun (kuva 3). Paras klooni tuotti keskimäärin 27 g, heikoin 17 g biomassaa/leppä (kuva 4). Lepät tuottivat keskimäärin yhtä paljon biomassaa, 26 g/leppä, ilman tuhkaa kuten myös annettaessa tuhkan lisäksi 2,5 g typpeä/m^2. Jo typpilisäys 5 g/m^2 pienensi biomassan tuotosta. Juurten osuus kokonaisbiomassasta pieneni lannoitetypen määrän kasvaessa (kuva 5). Niin kloonikuin lannoituskokeen osalta nitrogenaasiaktiivisuus korreloi selvästi pituuskasvun kanssa. Eri kloonien lehtien ravinnepitoisuuksissa oli selviä eroja. Typpilannoitus kohotti lehtien fosfori- ja magnesiumpitoisuutta (taulukko 5). Kasvualustan ravinnepitoisuudessa ei ollut eroja eri kloonien eikä eri lannoituskäsittelyjen välillä.

Yhteenveto tuloksista:

1. *Eri leppäkloonit eroavat juurtumisen, talvehtimisen, nitrogenaasiaktiivisuuden, pituuskasvun ja biomassan tuotoksen suhteen.*
2. *Suuret typpilannoitemäärät, ainakin yhdellä kertaa annettuna, heikentävät nuorten leppien nitrogenaasiaktiivisuutta, pituuskasvua ja biomassan tuotosta.*