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Effects of inoculation with *Frankia* on the growth and nutrition of alder species and interplanted Japanese larch on restored mineral workings

A.J. MOFFAT

Forest Research, Forest Research Station, Alice Holt Lodge, Farnham, Surrey GU10 4LH, England

Summary

The value of interplanting various alder species as a nitrogen-supplying nurse crop to Japanese larch on restored opencast coal spoil was investigated experimentally. Six alder species were inoculated with two strains of *Frankia* in a nursery experiment, and their performance tested in the field over a 6-year period. Their effect on the growth and foliar nutrition of Japanese larch was also studied. The experiments demonstrate that alders can significantly improve growth, although there is a period of about 5 years before this nursing effect is detectable, corresponding to the time necessary to accumulate nitrogen in the growth substrate. However, compared with other methods for improving spoil nutrition, such as application of sewage sludge, or mixing in organic soil-forming materials, the growth of larch using the alder nursing method is poor.

Introduction

Land restored after mineral working often suffers from infertility, especially if soil materials have been lost and overburden materials have to be used for vegetation establishment. Nitrogen is the nutrient most commonly lacking because mine-spoils are usually deficient in organic components. A number of strategies have been tried to deal with this, including choosing low-demanding species, using nitrogen-fixing species either pure or in mixture, or amending the spoils with organic materials such as sewage sludge.

In the UK, sites lacking sufficient soil for reclamation to intensive uses such as agriculture have often been restored to forestry. For example,

approximately 1300 ha of land have been planted with trees following opencast coal extraction in South Wales. Nevertheless, infertility has remained a major cause of poor forest performance on these restored lands (Bending *et al.*, 1991; Bending and Moffat, 1999).

Alders have been used for many years in woodland establishment on restored land in the UK (Moffat *et al.*, 1989; Moffat and McNeill, 1992). Alders are actinorhizal plants which fix atmospheric nitrogen in a symbiotic relationship with the *Frankia* micro-organism (Akkermans and Van Dijk, 1975). In natural forest stands, the estimated contribution of alder to soil nitrogen ranges from 60–320 kg N ha⁻¹ a⁻¹ (Newton *et al.*, 1968; Tarrant and Trappe, 1971). In South

Wales, many hundreds of hectares of opencast coal spoils have been planted since the 1980s with mixtures of Japanese larch (*Larix kaempferi* (Lamb.) Carr.) and either common alder (*Alnus glutinosa* (L.) Gaertner) or red alder (*A. rubra* Bong.). There is some evidence from the South Wales coalfield that alders can increase the nitrogen capital of a restored site so that other tree species can benefit (Fourt, 1984). However, there has been little experimental work to verify this and quantify the effects. In addition, the benefits that alders can bring about are dependent on the effectiveness of the *Frankia* symbiosis. Research has shown that *Frankia* populations are low in restored minespoils, and that nitrogen-fixation may depend on suitable, effective strains of *Frankia* being attached to alder seedlings before they are planted out on the restored site (McNeill *et al.*, 1989; Wheeler *et al.*, 1991).

This paper describes a nursery experiment to test the effectiveness of two strains of *Frankia* on the nodulation and growth of six species of alder, and their consequent growth when planted out at a restored opencast coal site in South Wales. In addition, the effect of these alders on interplanted Japanese larch has been studied over a 10-year period.

Materials and methods

Nursery experiment

The Forestry Commission research nursery at Headley, Hampshire was used for the initial experiment. The nursery is located on a sandy cultivated humo-ferric podzol soil developed in Lower Cretaceous Lower Greensand beds. Important soil chemical characteristics from the area used for the experiment are shown in Table 1.

Six alder species employed in the reclamation of disturbed land (Fourt, 1984) were chosen for study. These were *Alnus glutinosa*, *A. incana* (L.) Moench, *A. cordata* (Lois.) Duby, *A. rubra*, *A. sinuata* (Regel) Rydberg and *A. viridis* (Chaix) DC. Seeds were obtained from the Forestry Commission Seed Section and sown in nursery beds previously sterilized with Dazomet (Basamid) in April 1986. Each plot was 1 m², separated by unplanted buffers of 1 m². Three treatments were

Table 1: Soil properties at the Headley experiment

Property	Value
pH (1 : 2.5 water)	5.35
ADAS extractable P (mg l ⁻¹)	73
ADAS extractable K (mg l ⁻¹)	54
ADAS extractable Mg (mg l ⁻¹)	127
Bray P (µg g ⁻¹)	74
Mineralizable N (µg g ⁻¹)	2.47

The values are means based on six replicates. Analyses were performed by the Department of Soil Science, Reading University.

applied to the six species in two replicate blocks. These were (1) an uninoculated control treatment, (2) treatment with a suspension of CPI3 *Frankia* inoculum, and (3) treatment with a suspension of ARI7 *Frankia* inoculum. The inocula were prepared by the Department of Microbiology, University of Surrey, using static culture of pure strains derived from *Comptonia peregrina* (L.) Coult. and *Alnus rubra* respectively (Collins, 1988). Inoculation took place in June 1986, after the seedlings had reached the two-leaf stage. Suspensions were applied evenly over the treatment areas using spreading equipment developed by Rothamsted Experimental Station (Woodcock *et al.*, 1982).

To assess the effect of inoculation and development of nodulation, approximately 50 seedlings were excavated from each assessment plot (the inner 0.64 m² of the treatment plot) in July, August and September 1986, using random number tables to select the sites of excavation within the plot. On each occasion, tree roots were carefully removed and remaining soil sieved to retrieve broken root fragments. The roots were washed free of soil, stem heights were measured and nodule sites counted. Shoots were detached from roots and each dried at 80°C to constant weight. Concentrations of N, P, K, Ca and Mg were determined for both leaf and root samples sampled in July 1986 using standard laboratory procedures.

At the end of the first growing season, all remaining seedlings were transplanted into unsterilized line-out beds to allow growth for a further growing season before transplanting out. In September 1987, at the end of the second growing season, mean height for each treatment was assessed by measuring the heights of 80

seedlings, and the degree of nodulation was assessed by sampling 25 seedlings.

Analysis of variance was used to study effects of inoculation on nodulation, growth and tissue macronutrient concentrations using GENSTAT 5 (Genstat 5 Committee, 1993).

Field experiment

The field experiment was located at the restored Maesgwyn opencast coal site in Mid-Glamorgan-shire. Here, overburden materials composed mainly of Carboniferous shales were used as final cover on which to establish vegetation (Bending *et al.*, 1994). The shales were alkaline in reaction and infertile (Table 2), coarse and stony. The experimental site was cultivated in the summer before tree planting using the 'Neath plough', an armoured tine 750 mm in length.

An experiment using a replicated block design was set up using unit plots of eight rows of larch in line mixture (2 : 1) with four rows of the alder species under test. In each of the two blocks, two plots of pure larch were included, to act as controls of a pure, non-nitrogen-fixing species. Treatments were randomized across each of the blocks. Trees of all species were planted at 2 m spacing in the rows. Spacing between rows varied from 1.4 to 2.7 m depending on the ground configuration created by the Neath ploughing. The 1 + 1 alder transplants from the Headley nursery experiment together with 1 + 1 Japanese larch transplants from the same nursery were planted in the field experiment in January 1988. Triple superphosphate was applied at 100 g per tree in July 1990. Missing larch were replaced in March 1991, with 1 + 1 stock from a commercial nursery.

The survival and heights of all larch in assessment plots were measured at planting in 1988, and at the end of the growing season in 1988, 1989, 1992 and 1994. Alder survival and height were recorded in 1988 and 1992. Foliar samples from some alder treatments and all larch treatments were taken during the mid-growing season in 1991 and 1993. Soil samples were taken from beneath central rows of certain alder species and the larch control plots in 1995.

Foliar samples were analysed for macronutrients (N, P, K, Mg, Ca) in the Forestry Commission Research Laboratories, using standard analytical methods. Soil samples were sent to Natural Resources Management Ltd for analysis of soil pH, total Kjeldahl nitrogen, ammonia-N and nitrate-N.

Analysis of variance was used to study tree survival, height and foliar chemistry data using GENSTAT 5 (Genstat 5 Committee, 1993).

Results

Nursery experiment

Table 3 shows the effects of inoculation treatments on indices of inoculation and seedling growth. Unfortunately, only one block was sampled for percentage nodulation and number of nodule sites in July, so these results could not be analysed using ANOVA. However, in August and September, treatment had a clear significant effect on these two indices. On each occasion, AR17 treatment had a larger effect than CPI3 on percentage seedling inoculation and number of nodule sites per tree irrespective of alder species, although in August a significant species \times

Table 2: Soil properties of selected treatments at Maesgwyn

Property	Control (larch)	<i>A. cordata</i>	<i>A. rubra</i>	<i>A. viridis</i>
pH	7.3 ^a	7.6 ^a	7.3	7.4
Total N (%)	0.16	0.17	0.17	0.17
NH ₄ -N (mg l ⁻¹)	3.5 ^{ab}	5.1 ^a	5.9 ^{bc}	4.4 ^c
NO ₃ -N (mg l ⁻¹)	0.1 ^{ab}	1.4 ^{ac}	0.9 ^{bd}	0.2 ^{cd}

The values are based on six replicates. Analyses were performed by Natural Resources Management Ltd, Bracknell, Berks. For each property, same letter subscripts denote significant ($P < 0.05$) differences when tested using the *t* test.

Table 3: The effect of inoculation on the growth of alders in the nursery experiment

Variable	Species			Treatment			Species \times treatment		
	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.
Mean % nodulation	n/a		*	n/a	***	***	n/a	*	
Nodule sites per tree	n/a			n/a	***	*	n/a		
Mean shoot weight	***	**	***						
Mean root weight	*	**	***						
mean shoot/root ratio	***		**						

Analysis of variance: * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

treatment percentage nodulation interaction was caused by the preference of *A. glutinosa* for CPI3 rather than ARI7. Untreated controls showed a low level of nodulation (5–7 per cent) on each sampling occasion.

Indices of growth (mean shoot and root weights, shoot/root ratio) were unaffected by inoculation treatment but were significantly different between species. Similarly, foliar and root macronutrient concentrations were mainly affected by species. No significant effects of inoculation were obtained, although species \times treatment interactions were found for foliar phosphorus and potassium.

At the end of the second growing season, in unsterilized line-out beds, there were no significant differences due to inoculation treatment in mean seedling height, percentage tree nodulation or number of nodule sites per tree. There was a large increase in nodulation, with all species exceeding 79 per cent. In contrast, height and number of nodule sites differed significantly between alder species ($P < 0.001$).

Field experiment

Alder In 1988 after only one growing season, no significant differences due to inoculation treatment in the nursery were found. Instead, significant differences in survival ($P = 0.05$) and growth ($P = 0.001$) were found between alder species, with *Alnus cordata* showing poor survival, and *A. rubra* making the most rapid growth in the early years. In 1992, survival of *A. glutinosa* was significantly ($P = 0.003$) poorer than the other species, and there was a significant species \times inoculation treatment interaction ($P < 0.001$)

with *A. glutinosa* survival considerably poorer in CPI3 plots, whilst other species showed poorest survival in the other two treatments. Across all species and nursery treatments, survival was correlated with degree of nodulation at the end of the second year in the nursery ($r = 0.49$, $P = 0.038$). Height growth was also significantly related to species ($P < 0.001$), with tree species such as *A. cordata* and *A. rubra* growing taller than the shrub species *A. sinuata* and *A. viridis* (Figure 1). There were no significant differences due to inoculation treatment.

Foliar analyses (Table 4) showed some expected differences in concentration of macronutrients between alder species. However, compared with published norms (van den Burg, 1985; Taylor, 1991), there was no suggestion of nutrient deficiency in any species.

Larch Larch failed to respond to treatment during the first four growing seasons. However, in 1994, by the end of the fifth season, statistically significant and relatively important differences were obtained (Figure 2), notably if alder survival was used as a covariate in the ANOVA. However, the only effect was due to alder species. Alder inoculation treatment in the nursery had no effect on larch performance.

Foliar analyses in 1993 showed the beneficial effects of interplanting with alder species (Table 5). Pure larch was acutely nitrogen deficient (mean N concentration = 1.035 per cent). The presence of *A. cordata* produced the largest increase in nitrogen concentration in the larch (1.745 per cent), though this level is still considered deficient (Taylor, 1991). Previous inoculation treatment had no significant effect on foliar

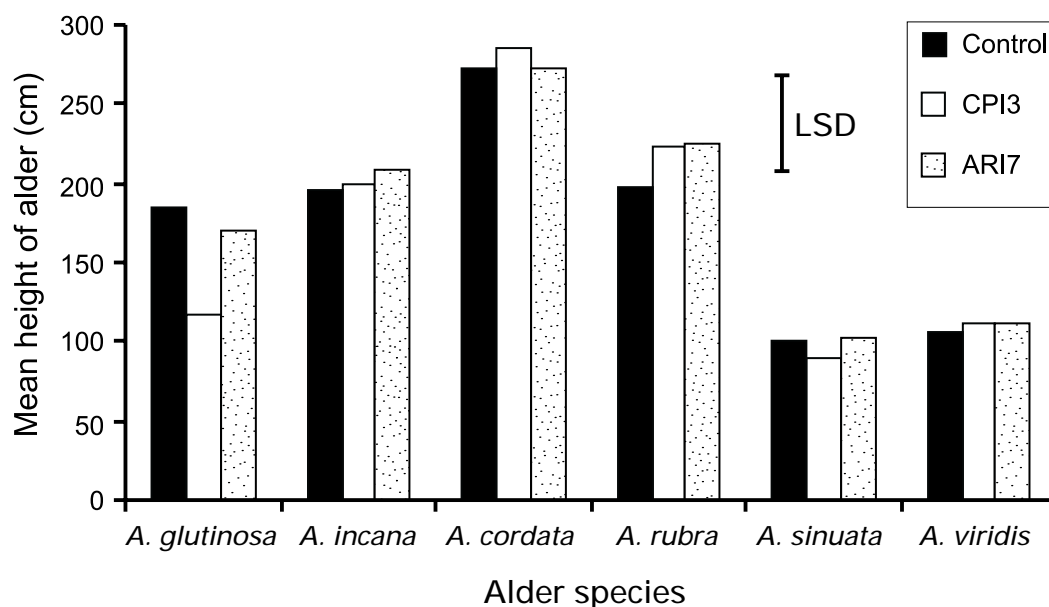


Figure 1. Growth of alders after five growing seasons at Maesgwyn (1988–92). The key refers to inoculation treatment in the nursery (control = uninoculated in first year, CPI3 and ARI7 represent inoculation with elite *Frankia* strains; all seedlings were transplanted out into unsterilized beds in the second year).

Table 4: Mean foliar concentrations (standard deviations) for alder species in 1991 (mean of three samples)

Species	Nitrogen	Phosphorus	Potassium	Magnesium	Calcium
<i>A. glutinosa</i>	3.42 (0.10)	0.26 (0.010)	1.01 (0.02)	0.42 (0.01)	0.57 (0.01)
<i>A. incana</i>	3.58 (0.06)	0.25 (0.004)	0.97 (0.02)	0.44 (0.01)	0.52 (0.01)
<i>A. cordata</i>	2.36 (0.04)	0.24 (0.004)	1.02 (0.02)	0.29 (0.01)	0.31 (0.01)
<i>A. rubra</i>	2.73 (0.05)	0.19 (0.003)	0.74 (0.01)	0.29 (0.01)	0.46 (0.01)
<i>A. sinuata</i>	2.75 (0.05)	0.17 (0.002)	0.65 (0.01)	0.45 (0.01)	0.62 (0.01)
<i>A. viridis</i>	3.16 (0.06)	0.21 (0.004)	0.70 (0.01)	0.37 (0.01)	0.46 (0.01)

The units are expressed as percentage oven dry weight.

Table 5: Mean foliar concentrations (percentage oven dry weight) for *L. kaempferi* in 1993

Mixture species	Nitrogen	Phosphorus	Potassium	Magnesium	Calcium
Control	1.035	0.19	1.03	0.27	0.22
<i>A. glutinosa</i>	1.340	0.20	1.06	0.30	0.23
<i>A. incana</i>	1.410	0.20	1.12	0.28	0.23
<i>A. cordata</i>	1.745	0.23	1.11	0.30	0.25
<i>A. rubra</i>	1.585	0.23	1.21	0.30	0.24
<i>A. sinuata</i>	1.110	0.18	0.99	0.27	0.24
<i>A. viridis</i>	1.340	0.22	1.13	0.29	0.25

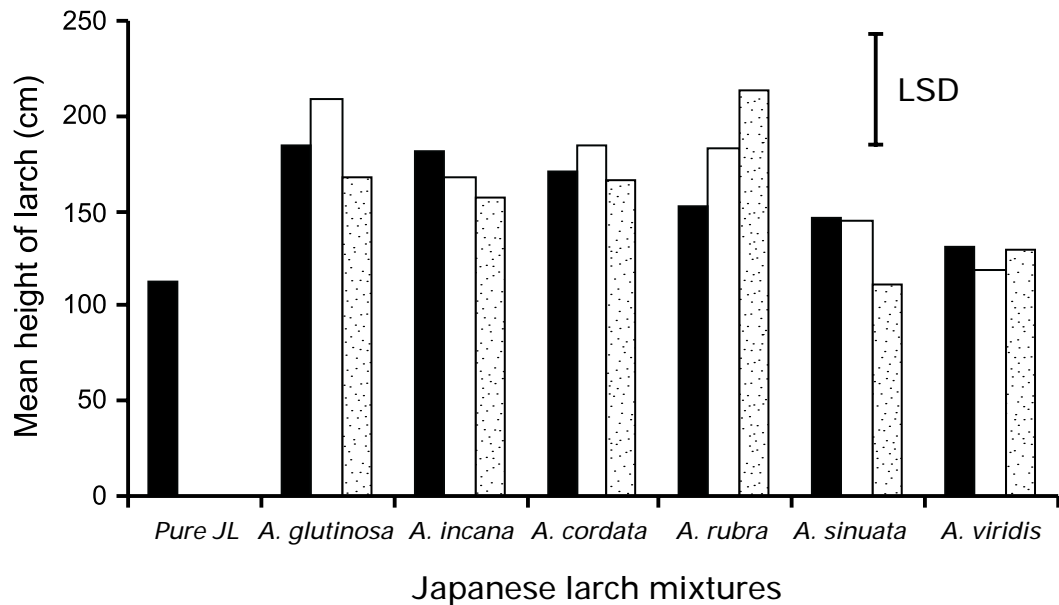


Figure 2. Growth of Japanese larch after five growing seasons at Maesgwyn (1988–92). For explanation of key, see Figure 1.

nitrogen concentrations. In contrast, larch phosphorus and potassium concentrations were only significantly elevated in *A. rubra*, *A. cordata* and *A. viridis* plots, though in no plots were levels raised above deficiency. Larch in plots containing alder treated with ARI7 inoculum had significantly higher foliar phosphorus and potassium concentrations than control and CPI3 treatments. No significant differences in calcium and magnesium concentrations were found.

Soil analysis The soil results (Table 2) show some significant differences between treatments. Both ammonia-N and nitrate-N were slightly elevated in some alder plots compared with the larch controls, and pH was also increased under *A. cordata* compared with pure larch. Total nitrogen was unaffected by treatment.

Discussion

Nursery experiment

Nursery inoculation with elite strains of *Frankia* has proven effective in increasing alder seedling growth in several published experiments

(Wheeler *et al.*, 1991). In contrast, this study failed to achieve similar results despite evidence for improved nodulation and nitrogen fixation. *Frankia* derived from the group of actinorhizal plants including *Alnus rubra* and *Comptonia peregrina* usually nodulate across the host genera (Collins, 1988). No attempt was made to assess whether these strains had inoculated alder seedlings microbiologically, but percentage nodulation and number of nodule sites per seedling was low for all alder species in the CPI3 treatment, and there were many unnodulated plants at the end of the first year. In addition, lining out in unsterilized beds produced a large increase in the percentage of nodulated trees presumably due to inoculation by native *Frankia* strains in the nursery soil. Alder performance was thus very dependent upon the interaction between the sterilization regime in the nursery and the type and behaviour of the inoculum used, supporting results from other experiments by Moffat (1994). In this experiment, there was no evidence that the alder seedlings grown to a standard 1+1 nursery regime (Aldhous and Mason, 1994) benefited from inoculation because part of the growing cycle was performed on unsterilized beds.

Field experiment

The effect of lining out to produce 1+1 planting stock at Headley nursery resulted in nodulation ranging from 80 to 100 per cent depending on species. The lack of nursery inoculation treatment effect on the height or foliar nitrogen concentrations of trees grown in the field was therefore expected, in contrast to the results of McNeill *et al.* (1989). Nevertheless, the nursery experiment and nodulation evaluation gave confidence that the large majority of plants (of all alder species) used in the field experiment were well nodulated, and capable of nitrogen fixation.

It is clear that nodulated alders can grow well on comparatively infertile opencast coal spoils. Hood and Moffat (1995) showed that *Alnus glutinosa* can 'fix' up to 75 per cent of its total plant needs from the atmosphere. Other alder species may fix up to 90 per cent of their needs if grown on nitrogen-poor substrates (Ekblad and Huss-Danell, 1995). Nevertheless, there was no evidence of deficiency from foliar samples taken during the field experiment. Although containing low total nitrogen concentrations, there appeared to be sufficient in the coal spoil, or from atmospheric sources as NO_x to supply the remaining needs of each of the alder species.

In contrast, Japanese larch foliar concentrations revealed widespread nitrogen deficiency across all treatments, despite elevated levels in alder plots. Concentrations of all other macronutrients were satisfactory, except calcium which was low across all treatments compared with published norms (van den Burg, 1985). Poor plant-available nitrogen was shown by Bending *et al.* (1991) to be an important factor reducing the potential growth of larch on South Wales coal spoils. In this experiment, larch growth was very poor on the control treatments (Figure 1), but the most successful alder treatment (*A. rubra*) resulted in a 63 per cent increase in mean height increment over the pure larch control. Comparison with information from Everard (1974) on early larch growth suggests that this represents a difference of approximately two yield classes.

A number of different alders were tested in these experiments because each has particular preferences for site and soil characteristics, and they differ in their form and growth habit. The results suggest that the shrubby alders (*A.*

sinuata, *A. viridis*) were markedly inferior in promoting larch growth than alders with a tree-like habit. This contrasts with experience on other reclaimed substrates, where *A. viridis*, in particular, has performed well (Vann *et al.*, 1988; Moffat and Roberts, 1989). Of the tree alders, it is interesting that the only native alder (*A. glutinosa*) performed poorly in comparison with non-native equivalents. *A. glutinosa* has a reputation for preferring well-watered locations, often in locations which experience a high water-table (Matthews, 1987). Measurement of soil moisture deficit on similar spoils in the district have shown that despite a high average rainfall, sites like Maesgwyn can suffer from comparatively severe summer drought (Bending *et al.*, 1991). The field experiment demonstrates that non-native alders, notably *A. cordata*, are probably better suited to establishment on coarse-textured coal spoils which are prone to drought (Borghetti *et al.*, 1989; Hall, 1990).

The use of nitrogen-fixing species is well established in land reclamation (Bradshaw and Chadwick, 1980), partly because such plants are well adapted to nitrogen-deficient substrates, and because they may serve to increase the nitrogen capital of a site, to the advantage of non-nitrogen-fixing vegetation. The field experiment shows that the strategy of planting nitrogen-fixing species with nitrogen-demanding ones has led to increased height growth in the latter after about 5 years. This is in accord with the modelling of nitrogen availability by Hood and Moffat (1995). They predicted that a period of 5–10 years was necessary before the spoil nitrogen status would be raised sufficiently to benefit other trees. However, the delay in accelerated larch growth means that there is usually a severe difference in mean height between the larch and the alder component. This was obviously most apparent in the plots containing tree alders, and competition for space and light was evident, leading to some physical abrasion.

In addition, larch growth in alder plots remained slow in comparison with that obtained using other reclamation strategies, also tested at Maesgwyn. Table 6 shows that replacement of suitable soil-forming materials, or the use of thermally dried sewage sludge to increase site nitrogen capital, are far more effective, as monitored over a 4- or 5-year period. This suggests that if

Table 6: Comparison of growth of *L. kaempferi* in three experiments at Maesgwyn using different reclamation strategies

Strategy	Mean height (cm)	No. of growing seasons	Approximate yield class ¹
Interplanting with <i>A. rubra</i> (this experiment)	183	6	6
Pure plantation on peat and weathered shale soil-forming material (A.J. Moffat, unpublished)	336	5	12
Pure plantation on coal spoil treated with 300 tds ha ⁻¹ thermally dried sewage sludge (Bending and Moffat, 1997)	241	4	10

¹Using information on early growth from Everard (1974).

such materials can be found for reclamation, they should be used in preference to strategies that rely on the planting of a large component of alders to supply plant-available nitrogen.

Conclusions

The experiments reported here confirm the importance of using nodulated alder stock when planting out on reclaimed land (Moffat and McNeill, 1992). However, the use of supposed elite strains of *Frankia* may not always serve to increase field performance, suggesting that further research is required to identify suitable strains for individual alder species. Nitrogen-demanding species interplanted with alders grow better than as pure stands, although there is a delay of about 5 years before this occurs. On severely infertile sites, this can lead to silvicultural management problems such as unequal height growth and competition for space.

Alders will clearly continue to have an important role in land reclamation to woodland. However, suitable soil-forming materials amended with nitrogen-rich organic materials such as peat or sewage sludge appear to offer more promise for the growth of other tree species such as larch.

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