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Effects of *Frankia* on field performance of *Alnus* clones and seedlings

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Abstract

Field performance of tissue cultured clones and seedlings of *Alnus viridis* ssp. *crispa*, *A. glutinosa*, *A. incana*, and *A. japonica* was assessed five years after outplanting in central Ontario. Half the individuals were inoculated with a mixture of four *Frankia* isolates prior to planting. Inoculation produced significant increases (25% to 33%) in biomass production of two clones of *A. glutinosa* and one of *A. incana*. Woody biomass increments for the first five years, averaged across all clones and seedlings, were highest in *A. japonica* and *A. incana* (4.3 and 3.7 Mg ha^{-1} yr^{-1}, respectively). Individual tree growth improved markedly in lower slope positions, but total plot biomass did not show similar gains in downslope positions owing to higher mortality and aphid (*Paraprociphilus tessellatus*) infestation. Aphids occurred in 22% of *Frankia*-inoculated individuals, and 15% of non-inoculated individuals. The fastest growing species, *A. incana* and *A. japonica*, were most susceptible to aphid attack. Growth of the best clones of *A. glutinosa* and *A. incana* exceeded seedling growth by 51% and 76%, respectively. The high growth variation in clones of the same species with similar geographic origins and the excellent performance of tissue cultured stock suggest that rapid genetic gains in an *Alnus* breeding program might be obtained by clonal propagation.

Introduction

An ability of alders (*Alnus* sp.) to fix atmospheric nitrogen and grow rapidly has prompted interest in their use in biomass energy plantations, either in single-species stands (Pregent and Camire, 1985) or in mixed plantings with other fast-growing hardwoods such as *Populus* spp. (Cote and Camire, 1987). There is a large potential, mostly untapped, for genetic gains via selection and hybridization of *Alnus* sp. (Hall and Maynard, 1979). Furthermore, the development of tissue culture techniques for production of clonal alder stock (Tremblay and Lalonde, 1984) should make it possible to capture these gains in the field.

Concomitant with interest in the genus *Alnus* has been an explosive growth of research on *Frankia*, the N_2-fixing symbiont of alders and other actinorhizal plants. Since the first well-documented isolation of *Frankia* in pure culture (Callaham et al., 1978), many additional isolates have been obtained, and their mode of infection, host specificity, genetic variation, physiological characteristics, etc. have been studied in great detail (Schwintzer and Tjepkema, 1990). Many studies have revealed significant variation in response of different alder clones to a particular

Frankia isolate, or in response of a particular alder clone to different *Frankia* isolates (Carpenter et al., 1984, Dawson and Sun, 1981, Dillon and Baker, 1982; Huss-Danell, 1980; Normand and Lalonde, 1982; Sellstedt et al., 1986; Simon et al., 1985; Steele et al., 1989; Weber et al., 1989). These studies were all performed under controlled conditions. Thus, although the potential for improved yields of selected *Alnus–Frankia* combinations under field conditions is widely recognized, there is limited evidence that these yields can be obtained.

There are risks associated with clonal forestry of fast-growing hardwoods such as *Alnus* sp. A desire for product uniformity and reduced processing costs may encourage managers to plant a limited range of clones, despite recommendations of scientists to maintain a broad genetic base. Resistance to insects and fungal diseases is a major concern for fast-growing trees. Alder provenances that perform well under normal weather conditions may be damaged by unusually low late spring or early fall temperatures (Cannell et al., 1987; Tremblay and Lalonde, 1987), or by prolonged drought. There may be additional risks in introducing a clonal host plant – N_2-fixing symbiont combination selected for maximum growth under controlled conditions, if higher nitrogen levels cause increased insect or frost damage.

As more field trials are established and evaluated, and results are made available to a larger audience, the benefits and risks associated with clonal forestry will be more precisely defined. Here we present results of a study comparing the performance of alders grown from seeds or from tissue culture, both with and without *Frankia* inoculation prior to outplanting. Our aim was to show that theoretical gains in biomass yield obtained with clonal propagation and *Frankia* inoculation could be realized in the field.

Methods

The alders were planted at the Petawawa National Forestry Institute (PNFI), in Chalk River, Ontario (45° 58'N, 77° 23'W). Mean temperatures are −13°C in January and 20°C in July, and annual precipitation is 80 cm. The soil at the field site is a well-drained, slightly acid (pH 5.8 in water) sandy loam formed on glacial till, and is relatively fertile for this region. It was farmed in the early 1900s, abandoned, cleared around 1960, and kept in grass until being tilled in 1984 and planted with alders in 1985.

At the time of the study, the plantation contained 5-year-old individuals of four *Alnus* species (Table 1). Nomenclature follows Furlow (1979). It included three clones and seedlings of *A. glutinosa* (black alder, a Eurasian tree species). Two clones and seedlings of *A. incana* spp. *incana* (grey alder, a Eurasian tree) were planted. Two clones of *A. japonica* (an east-Asian tree species) were planted, but seedlings were not available. For *A. viridis* ssp. *crispa* (green alder, a small shrub of North American boreal forests), clone AC-4 was selected for lack of nodule formation (Tremblay et al., 1984). However, subsequent investigations revealed that it had formed numerous effective *Frankia* nodules in the field. For convenience, we will refer to the subspecies name *crispa* as a "species" throughout the text.

The clonal alder stock was micropropagated in winter and spring 1985 according to Perinet and

Table 1. Source of *Alnus* clones and seedlings planted at the field site

Species	Clone(s)	Origin	Reference
A. glutinosa	AG-1	Germany	1
A. glutinosa	AG-3, AG-8	Russia (53°N, 43°E)	2
A. glutinosa	seedlings ('AG-0')	Russia (53°N, 43°E)	
A. incana	AI-1, AI-2	Ontario (Swedish ancestry)	2
A. incana	seedlings ('AI-0')	Ontario (Swedish ancestry)	
A. japonica	AJ-6, AJ-7	Scotland (ancestry unknown)	2
A. crispa	AC-4	Quebec	3
A. crispa	seedlings ('AC-0')	Northern Ontario	

References: 1, Perinet and Lalonde (1983); 2, Tremblay and Lalonde (1984); 3, Tremblay et al. (1984).

Tremblay (1987) at Rhizotec Inc. (St.-Jean Chrysostome, Quebec). One or two weeks after the rooted plantlets were transferred to a peat-vermiculite medium, half were inoculated (Perinet et al., 1985) with a mixture of *Frankia* isolates ACN1a, AGN1g, TN18^{AC}b, and ARgN22d. The purportedly non-nodulating clone AC-4 was not inoculated. Half of the seedling stock, grown at PNFI, was also inoculated three weeks after germination with the same mixture of *Frankia* isolates.

In June 1985, the alders were taken directly from their containers and planted at the field site in a split plot, randomized block design at a spacing of 0.5 m by 1.0 m. Each of five blocks (aligned perpendicular to the slope) contained one plot per species (Fig. 1). Three buffer rows of unmeasured trees were used to split each plot into areas containing inoculated and uninoculated individuals. The entire plot was surrounded by two buffer rows of unmeasured trees. Each split plot contained 26–32 individuals for each individual clone (seedlot) available for measurements. The two to four clones (seedlots) of each species were randomly mixed within each split plot. Thus, the total number of uninoculated individuals measured per plot was 104–128 for *A. glutinosa*, 78–96 for *A. incana*, and 52–64 for *A. crispa* and *A. japonica*. Numbers of inoculated individuals were the same except for *A. crispa*, at 26–32 per plot (AC-4 was not included).

During the winter of 1989–90, all trees were harvested by cutting the stem(s) at 10 cm above the ground surface (this stump height was chosen to encourage coppicing). Height, diameter at 1.3 m (dbh) and fresh weight were recorded. When multiple stems were present, height and diameter were recorded only for the largest stem, but fresh weights included all stems. If fruits were present, this was noted. Many trees were infested by woolly aphids (*Paraprociphilus tessellatus*), and this was noted as well. Within each split plot, all individuals of each clone or seedlot were pooled and passed through a chipper, and three samples were randomly taken and measured for fresh and dry (at 80°C) weights. If substantial numbers of aphid-infested trees were present, these were chipped and processed separately. Dead trees were also processed separately. Samples were analyzed for total Kjeldahl-N using a K_2SO_4-$CuSO_4$-H_2SO_4 digest and selenium catalyst on an aluminum block heater; an automated system (Tecator AB, Hoganas, Sweden) was used for distillations and titrations.

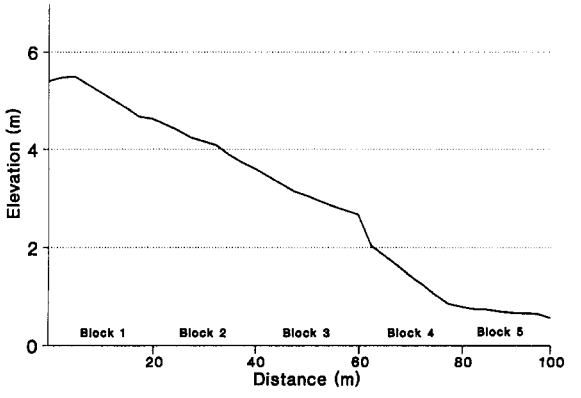

Fig. 1. Vertical gradient across the planting site, showing the relative positions of blocks 1–5. The slope faces east.

Effects of the *Frankia* treatment upon individual clones (or seedlots) were examined using a two-way analysis of variance (ANOVA) that included block and inoculation treatment as main effects. The interaction term was included in the analysis of individual tree biomass values, but not for total biomass or nitrogen accumulation within plots. For the overall analysis, clone (or seedlot) mean values were calculated for each of the block – *Frankia* treatment combinations. These values were used in an analysis of variance that included block, clone (or seedlot), and *Frankia* treatment effects, and the respective interactions. Overall mean values were separated using Duncan's multiple range test.

Results

Highly significant clonal (seedlot) variation in mortality, aphid infestation, fruiting, and average tree biomass was evident in the ANOVA (Table 2). After five years in the field, overall survival exceeded 97% except for the two *A. japonica* clones and the *A. glutinosa* clone AG-1 (Table 3). Aphid infestation varied by species, with *A. incana* most severely affected, followed by *A. japonica*. Nearly all individuals of *A.*

Table 2. F-values and significance levels for ANOVA of mortality, aphid infestation, fruiting, and average tree biomass

Source of variation	dF	Mortality		Aphids		Fruiting		Biomass	
		F	P	F	P	F	P	F	P
Clone	10	26.36	0.001	29.46	0.001	285.92	0.001	30.03	0.001
Block	4	19.72	0.001	41.29	0.001	9.29	0.001	7.15	0.002
Treatment	1	3.55	0.068	5.30	0.027	0.51	0.479	3.44	0.072
C × B	40	5.24	0.001	6.62	0.001	2.73	0.001	1.48	0.117
C × T	9	0.54	0.832	2.15	0.050	1.67	0.132	1.18	0.340
B × T	4	1.40	0.253	6.21	0.001	0.51	0.732	1.19	0.331

Table 3. Clonal variation in mortality, aphid infestation, fruiting, and growth properties

Clone	Mortality (%)	Aphids (%)	Fruiting (%)	Height (m)	Dbh (cm)	Biomass (g/tree)	Total-N (mg g^{-1})
AC-0	0.4 c	3.0 c	96.5 a	1.71 h	0.58 g	342.9 f	10.09 a
AC-4	0.0 c	0.0 c	92.7 a	1.91 g	0.80 f	557.3 e	9.24 b
AG-0	1.1 c	2.5 c	1.2 e	3.10 f	1.89 e	621.9 de	7.52 de
AG-1	10.0 b	7.6 c	75.1 b	3.03 f	1.91 e	790.3 cd	8.50 c
AG-3	0.3 c	1.3 c	0.6 e	3.52 e	2.32 cd	938.8 c	7.68 d
AG-8	1.1 c	0.0 c	0.7 e	3.40 e	2.18 d	672.6 de	7.03 e
AI-0	2.9 c	40.6 a	46.7 c	3.99 c	2.26 d	787.4 cd	5.70 f
AI-1	1.6 c	46.2 a	20.3 d	4.07 bc	2.09 de	691.7 de	5.61 f
AI-2	0.3 c	43.7 a	42.4 c	4.60 a	2.63 b	1386.5 ab	5.90 f
AJ-6	14.3 b	26.0 b	0.0 e	4.20 b	2.85 a	1502.5 a	5.61 f
AJ-7	22.7 a	23.0 b	0.0 e	3.78 d	2.53 bc	1200.0 b	5.72 f

Note: Means in columns followed by the same letter do not differ significantly ($p < 0.05$, Duncan's multiple range test).

crispa were sexually mature, as were 70% of the individuals in clone AG-1, and 20–47% of individuals in the two clones and one seedlot of *A. incana*. *Alnus japonica* clone AJ-6 and *A. incana* clone AI-2 had the highest average tree biomass, while *A. crispa* seedlings (AC-0) had the lowest. Total nitrogen concentrations generally varied inversely with tree size, with highest levels in *A. crispa* and lowest levels in *A. incana* and *A. japonica*. However, total-N varied significantly among clones of *A. glutinosa* independently of their size differences.

Variation among blocks was also highly significant in the ANOVA (Table 2). In *A. glutinosa* and *A. japonica*, mortality increased downslope, and was particularly high in block 5 at the base of the slope (Table 4). Fruiting of *A. glutinosa* and *A. incana* declined in the lower slope positions. Aphid infestation was lowest in midslope positions, and increased greatly at the bottom of the slope. Excluding aphid-infested trees, there was a strong trend toward higher individual tree biomass of *A. incana* and *A. japonica* in the lower slope positions (Fig. 2). Aphid infestation

Table 4. Species and block variation in mortality, aphid infestation, and fruiting

Block	Mortality (%)				Aphid infestation (%)				Fruiting (%)			
	cri[a]	*glu*	*inc*	*jap*	*cri*	*glu*	*inc*	*jap*	*cri*	*glu*	*inc*	*jap*
1	0.0	0.0	0.0	2.7	8.0	2.6	87.5	43.5	93.3	26.8	39.9	0.0
2	0.0	2.0	1.0	4.6	1.1	1.2	0.5	8.0	96.7	23.0	42.6	0.0
3	1.4	1.3	2.4	20.4	0.0	1.3	0.6	31.1	95.8	21.8	44.8	0.0
4	0.0	2.0	4.2	21.5	0.0	6.0	44.8	16.8	94.5	16.5	22.4	0.0
5	0.0	11.4	0.0	41.0	0.0	3.9	86.1	57.6	95.9	9.4	28.3	0.0

[a] *cri*, *A. crispa*; *glu*, *A. glutinosa*; *inc*, *A. incana*; *jap.*, *A. japonica*.

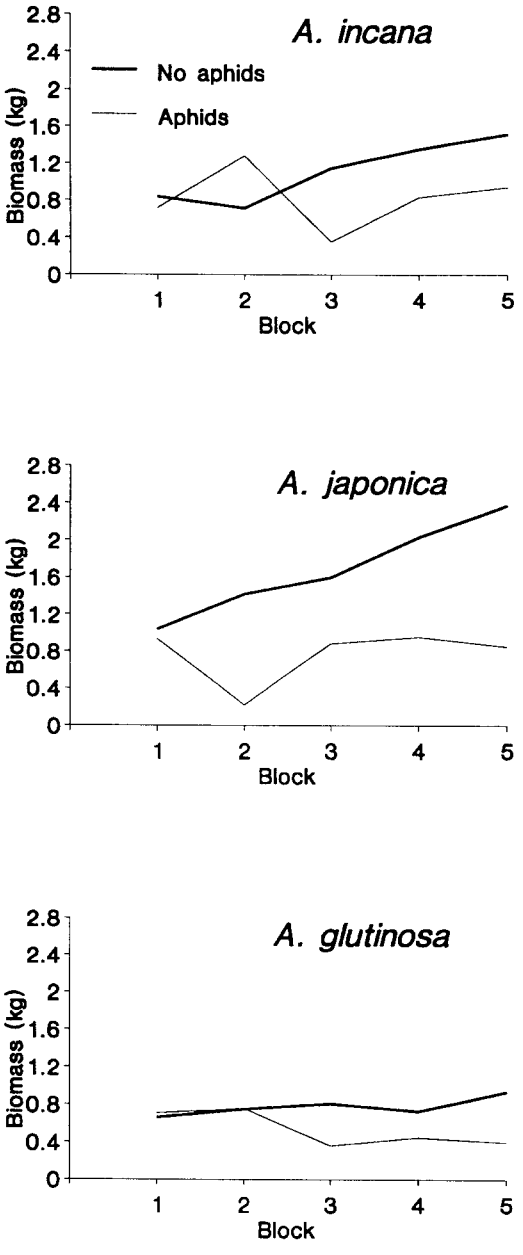

Fig. 2. Effect of aphid infestation on individual tree biomass of *A. incana*, *A. japonica*, and *A. glutinosa* at different slope positions.

was detrimental to growth, and the magnitude of this growth loss increased downslope.

The *Frankia* inoculation treatment had significant ($p < 0.05$) effects on aphid infestation, and weakly significant ($p < 0.10$) effects on mortality and average tree biomass across all clones (Table 2). Of those trees inoculated as greenhouse seedlings with the mixed *Frankia* isolates, 22% were infested by aphids, compared to 15% of those that received no inoculation treatment. The inoculation treatment was also associated with a slight reduction in overall mortality, from 5.8% to 4.6%.

Frankia inoculation had positive effects on *A. glutinosa* clones AG-8 and AG-1, with gains in total biomass production of 33% and 25%, respectively, for the inoculated individuals (Table 5). Clone AI-1 also showed a significant ($p < 0.05$) biomass increase of 27% with the inoculation treatment. *Frankia* inoculation had a weakly significant ($p < 0.10$) negative effect on biomass production of *A. incana* seedlings (AI-0), and significantly ($p < 0.05$) reduced their individual tree biomass and total-N concentrations. Most clones showed a non-significant trend toward higher total-N accumulations in subplots with *Frankia*-inoculated individuals. Clone AI-2 had the highest rates of biomass and N accumulation, at 5.5 Mg ha^{-1} yr^{-1} and 31.7 kg N ha^{-1} yr^{-1}, respectively.

Total plot biomass values were relatively low at the top of the slope (block 1) and increased in the midslope positions (Table 6). Total biomass of *A. japonica* and *A. crispa* declined again at base of the slope. Averaging across blocks, woody biomass accumulation during the first five years of growth was 1.5 Mg ha^{-1} yr^{-1} for *A. crispa*, 3.0 Mg ha^{-1} yr^{-1} for *A. glutinosa*, 3.7 Mg ha^{-1} yr^{-1} for *A. incana*, and 4.4 Mg ha^{-1} yr^{-1} for *A. japonica*.

Although nearly all individuals of *A. crispa*

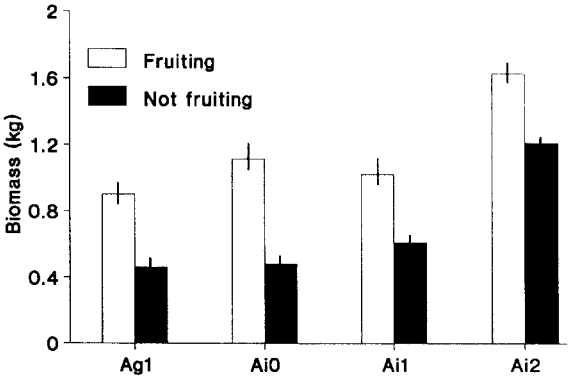

Fig. 3. Average individual tree biomass (mean ± SE) for sexually mature and immature individuals of different clones and seedlings.

Table 5. Effects of *Frankia* inoculation (+ = inoculated, − = uninoculated) on alder growth and N accumulation

Clone	Biomass (g/tree)		Biomass (Mg ha^{-1})		Total-N (mg g^{-1})		Total-N (kg ha^{-1})	
	+	−	+	−	+	−	+	−
AG-0	599	624	12.0	12.2	7.11	7.69	85.0	94.3
AG-1	887*	719	16.0*	12.8	8.47	8.19	136.1	104.2
AG-3	999*	879	19.8	17.5	7.32	8.01	144.8	138.8
AG-8	769*	576	15.0*	11.3	6.65	7.44	99.3	84.1
AI-0	677*	865	13.4	16.7	5.45*	6.18	72.3*	100.2
AI-1	759*	625	15.2*	12.0	5.43	5.71	88.2	69.4
AI-2	1355	1420	27.1	28.0	6.00	5.66	161.4	155.8
AJ-6	1562	1428	26.8	23.0	5.46	5.80	145.5	134.4
AJ-7	1379*	999	19.4	13.6	5.42	5.65	101.5	76.3

* Inoculation treatments differ significantly ($p < 0.05$).

Table 6. Total biomass (Mg ha^{-1}) by species and block

Block	*A. crispa*	*A. glutinosa*	*A. incana*	*A. japonica*
1	7.89	13.18	14.64	18.57
2	8.25	14.55	14.17	23.66
3	8.99	15.86	22.23	22.38
4	6.40	13.87	21.45	26.94
5	5.94	16.49	20.18	17.14
Mean	7.49	14.79	18.53	21.74

were sexually mature, only some individuals of *A. incana* and of *A. glutinosa* clone AG-1 had produced fruits by year 5. Sexually mature individuals tended to be larger, with greater average tree biomass (Fig. 3).

Discussion

Clonal variation

Stock derived from tissue culture was generally superior to seedling stock, although there were marked differences in growth among clones within a species. Variation was high even between clones of similar geographic origins. For example, individual tree biomass of clone AI-2 averaged twice that of AI-1, and clone AG-3 exceeded AG-8 by 40%. Also note that most individuals of the German clone of *A. glutinosa* (AG-1) were sexually mature at age 5, while the Russian clones (AG-3 and AG-8) were not. These results provide encouraging evidence that the high degree of natural variation within and among alder species (Hall and Maynard, 1979) can be captured using clonal propagation. Clonal stock might also be expected to show more uniform growth in plantations. Compared to the *A. incana* seedlings (AI-0), clones AI-1 and AI-2 both showed less variance in heights and diameters at the field site ($p < 0.001$, F-test of variances). However, variances for the *A. glutinosa* clones did not differ from seedlings of this species.

Frankia inoculation

Inoculation with a mixed culture of *Frankia* isolates yielded significant increases in biomass production of 25% to 33% in two *A. glutinosa* clones and one *A. incana* clone. These gains were similar to those previously observed for these species in a nearby study in which a single *Frankia* isolate (ACN1g) was used to inoculate non-clonal stock (Burgess et al., 1986, Hendrickson et al., 1991). Gains from *Frankia* inoculation were not as dramatic as those for different clonal lines, suggesting that selection of *Alnus* could be given higher priority than selection of actinorhizal symbionts in yield improvement programs.

Apart from our earlier work, we are aware of

only one other field study of *Alnus – Frankia* interactions, that of Teissier du Cros et al. (1984). These authors observed both growth increases and decreases for different alder species following inoculation of nursery soil with an *A. rubra* isolate. Thus, available field data agree with greenhouse experiments indicating that no single *Frankia* isolate, or even a mixture of isolates, will be optimal for all *Alnus* clones or species.

Field-planted alders will normally become nodulated. Most soils contain abundant *Frankia* populations even in the absence of suitable host plants (Huss-Danell and Frej, 1986; Smolander, 1990; Smolander and Sundman, 1987). Native *Frankia* populations show considerable variation within small areas, and even a single nodule may contain two recognizably different strains (Benson and Hanna, 1983; Simonet et al., 1989).

We observed an overall poor growth and lack of response to inoculation at the top of the slope, probably associated with the limiting effects of low water availability on growth and nitrogenase activity in *Alnus* spp. (Hennessey et al., 1989; Sundstrom and Huss-Danell, 1987). *Frankia* inoculation also appeared to provide less benefit in plots at the bottom of the slope. We noted that greater frost damage occurred there, and inoculated individuals may have been more strongly affected. Frost hardiness problems led to abandonment of an alder breeding program in Japan (Hall and Maynard, 1979) and seriously limit the use of *A. rubra* in Britain (Cannell et al., 1987). Greater mortality at the bottom of the slope could also have resulted from increased self-thinning associated with higher individual tree growth rates.

Aphid attack

In an earlier study, we found that the faster growing species *A. japonica* and *A. incana* were resistant to attack of an alder leaf miner, *Fenusa dohrnii* (Hendrickson et al., 1991). However, these two species were most often attacked by aphids in the present study, and infested individuals showed highly significant growth losses. *Alnus japonica* was also preferentially attacked by yellow bellied sapsuckers (*Sphyrapicus varius*) in our earlier study. The higher susceptibility of these species to herbivory conforms with the notion of a tradeoff between growth and defense (e.g., Coley et al., 1985).

Slower-growing individuals at the top of the slope were not significantly affected by aphids, while the magnitude of the growth loss increased downslope. This suggests that a growth-defense tradeoff may also occur within species, as observed for *A. glutinosa* in our previous study (Hendrickson et al., 1991). Under improved site conditions, individuals within a given species may allocate a lower proportion of energy resources to defense. This strategy succeeds only if increased growth compensates for increased herbivory.

Conclusions

The large clonal variation observed in the present study suggests that rapid gains could be made in growth of *Alnus* sp. by combining selection of promising individuals with clonal propagation. *Alnus incana*, *A. glutinosa*, and *A. japonica* all appear to warrant additional study under the climatic conditions of central Ontario. The former two species have shown some coppicing ability following the harvest in our study area (D Burgess, pers. commun.), which may be a valuable trait in biomass plantations. Although further gains might be achieved by introducing selected *Frankia–Alnus* combinations, plantation failure owing to lack of soil inoculum is unlikely at most sites. However, growth losses due to herbivory, disease, drought, or lack of frost tolerance are serious concerns and a continual selection program would likely be required for successful use of *Alnus* in biomass plantations.

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References

Benson D R and Hanna D 1983 *Frankia* diversity in an alder stand as estimated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis of whole cell proteins. Can. J. Bot. 61, 2919–2923.

Burgess D, Chatarpaul L and Hendrickson O 1986 Field performance of *Frankia*-inoculated and non-inoculated alders (*Alnus* spp.): Preliminary results. Petawawa Nat. For. Inst. Info. Rep. PI-X-67. Chalk River, Ontario.

Callaham D, Torrey J G and del Tredici P 1978 Isolation and cultivation in vitro of the actinomycete causing root nodulation in *Comptonia*. Science 199, 899–902.

Cannell M G R, Murray M B and Sheppard L J 1987 Frost hardiness in red alder (*Alnus rubra*) provenances in Britain. Forestry 60, 57–68.

Carpenter C V, Robertson L R, Gordon J C and Perry D A 1984 The effect of four new *Frankia* isolates on growth and nitrogenase activity in clones of *Alnus rubra* and *Alnus sinuata*. Can. J. For. Res. 14, 701–706.

Coley P D, Bryant J P and Chapin F S III 1985 Resource availability and plant antiherbivore defense. Science 230, 895–899.

Cote B and Camire C 1987 Tree growth and nutrient cycling in dense plantings of hybrid poplar and black alder. Can. J. For. Res. 17, 515–523.

Dawson J O and Sun S-H 1981 The effect of *Frankia* isolates from *Comptonia peregrina* and *Alnus crispa* on the growth of *Alnus glutinosa*, *Alnus cordata*, and *Alnus incana* clones. Can. J. For. Res. 11, 758–762.

Dillon J T and Baker D 1982 Variations in nitrogenase activity among pure-cultured *Frankia* strains tested on actinorhizal plants as an indication of symbiotic compatability. New Phytol. 92, 215–220.

Furlow J J 1979 The systematics of the American species of *Alnus* (Betulaceae). Part I. Rhodora 81, 1–21.

Hall R B and Maynard C A 1979 Considerations in the genetic improvement of alder. *In* Symbiotic Nitrogen Fixation in the Management of Temperate Forests. Eds. J C Gordon, C T Wheeler and D A Perry. pp 322–344. Oregon State University, Corvallis.

Hendrickson O Q, Fogal W H and Burgess D 1991 Growth and resistance to herbivory in N_2-fixing alders. Can. J. Bot. 69, 1919–1926.

Hennessey T C, Vishniac H S, Lorenzi E M and Williams J C 1989 Dinitrogen fixation in a water-stressed *Alnus* clone is limited by host xerotolerance. Plant and Soil 188, 89–96.

Huss-Danell K 1980 Nitrogen fixation and biomass production in clones of *Alnus incana*. New Phytol. 85, 503–511.

Huss-Danell K and Frej A-K 1986 Distribution of *Frankia* in soils from forest and afforestation sites in northern Sweden. Plant and Soil 90, 407–418.

Normand P and Lalonde M 1982 Evaluation of *Frankia* strains isolated from provenances of two *Alnus* species. Can. J. Microbiol. 28, 1133–1142.

Perinet P, Brouillette J G, Fortin J A and Lalonde M 1985 Large-scale inoculation of actinorhizal plants with *Frankia*. Plant and Soil 87, 175–183.

Perinet P and Lalonde M 1983 In vitro propagation and nodulation of the actinorhizal host plant *Alnus glutinosa* (L.) Gaertn. Plant Sci. Lett. 29, 9–17.

Perinet P and Tremblay F M 1987 Commercial micropropagation of five *Alnus* species. New Forests 3, 225–230.

Pregent G and Camire C 1985 Biomass production by alders on four abandoned agricultural soils in Quebec. Plant and Soil 87, 185–194.

Schwintzer C R and Tjepkema J D 1990 The Biology of *Frankia* and Actinorhizal Plants. Academic Press, San Diego, CA.

Sellstedt A, Huss-Danell K and Ahlqvist A S 1986 Nitrogen fixation and biomass production in symbioses between *Alnus incana* and *Frankia* strains with different hydrogen metabolism. Physiol. Plant. 66, 99–107.

Simon L, Stein A, Cote S and Lalonde M 1985 Performance of in vitro propagated *Alnus glutinosa* (L.) Gaertn. clones inoculated with *Frankiae*. Plant and Soil 87, 125–133.

Simonet P, Le N T, Moiroud A and Bardin R 1989 Diversity of *Frankia* strains isolated from a single alder stand. Plant and Soil 118, 13–22.

Smolander A 1990 *Frankia* populations in soils under different tree species – with special emphasis on soils under *Betula pendula*. Plant and Soil 121, 1–10.

Smolander A and Sundman V 1987 *Frankia* in acid soils of forests devoid of actinorhizal plants. Physiol. Plant 70, 297–303.

Steele D B, Ramirez K and Stowers M D 1989 Host plant growth response to inoculation with *Frankia*. Plant and Soil 118, 139–144.

Sundstrom K R and Huss-Danell K 1987 Effects of water stress on nitrogenase activity in *Alnus incana*. Physiol. Plant. 70, 342–348.

Teissier du Cros E, Jung G and Bariteau M 1984 Alder-*Frankia* interaction and alder-poplar association for biomass production. Plant and Soil 78, 235–243.

Tremblay F M and Lalonde M 1984 Requirements for in vitro propagation of seven nitrogen-fixing *Alnus* species. Plant Cell Tissue Organ Culture 3, 189–199.

Tremblay F M, Nesme X and Lalonde M 1984 Selection and micropropagation of nodulating and non-nodulating clones of *Alnus crispa* (Ait.) Pursh. Plant and Soil 78, 171–179.

Tremblay F M and Lalonde M 1987 Effect of photoperiod and temperature on the development of frost hardiness in three *Alnus* species. Physiol. Plant. 70, 327–332.

Weber A, Sarsa M-L and Sundman V 1989 *Frankia-Alnus* symbiosis: Effect of endophyte on nitrogen fixation and biomass production. Plant and Soil 120, 291–298.

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