EARLY RESULTS OF A RANGE-WIDE PROVENANCE TRIAL OF <u>ALNUS GLUTINOSA</u> (L.) GAERTN.

By C. A. Maynard and R. B. Hall, Research Associate, School of Forestry, SUNY College of Environmental Science and Forestry, formerly Research Assistant, Forestry Department, Iowa State University; and Associate Professor, Forestry Department, Iowa State University. (Journal Paper No. J-9962 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. The study was supported by Station Project 2210 (NC-99 Regional Tree Improvement) and Cooperative Aid Agreement 13-721 with the U. S. Forest Service Intensive Fiber Yield Project.)

ABSTRACT .-- First-year growth, dormancy onset, winter dieback, and spring budbreak were observed for 48 provenances of <u>Alnus</u> glutinosa (L.) Gaertn. planted at two locations: central Iowa and northern Wisconsin. First-year growth was best for provenances from north central and southern Europe and poorest for northern Scandinavian sources. Budset was strongly correlated with latitude of origin, the Scandinavian sources set bud in mid-summer and some southern-latitude sources continued growth until late October in Iowa. Winter dieback was insignificant for most provenances, but severe for the extreme southern sources. Even those provenances, however, produced some hardy individuals. Budbreak occurred first in Scandinavian and Yugoslavian sources and last in the other southern provenances and in those sources from the British Isles. There was also considerable variation in morphological traits that might be economically exploited.

In a companion greenhouse study, it was determined that the source of <u>Frankia</u> used to initiate nodules on the alder roots had a substantial impact on initial plant growth. This finding should be considered in evaluating past work with <u>Alnus</u> and in planning future studies.

INTRODUCTION

The European black alder, <u>Alnus glutinosa</u> (L.) Gaertn., was first introduced to North America during colonial times for charcoal production (Harlow and Harrar 1969). More recent interest in the species has centered on its use for spoil reclamation (Dale 1963; Funk 1973; Plass 1977), rapid production of fiber and sawlogs (Phares et al. 1975), and as a nitrogen-fixing tree crop grown in pure plantations or in mixture with other species (Tarrant and Trappe 1971; Plass 1977).

The natural range of A. glutinosa covers an extremely wide area in Europe, western Asia, Asia Minor, and northern Africa (Robison et al. 1978). Little is known about the source of seeds for the early introductions of black alder on this continent, but the material was well enough suited to our climates that the species escaped cultivation and became naturalized in a large area of the eastern United States (Furlow 1979). The first U. S. provenance test for the species was initiated in 1963 with the planting of 15 north-central European sources on a coal strip mine area in Ohio (Funk 1973). In that study, the best combinations of performance in terms of survival, height and diameter growth were found in German provenances. Danish and Belgian sources gave intermediate performance, and the one source tested from central Scandinavia gave very poor performance (Funk 1973, 1979). No provenances from the eastern, western or southern portions of the species range were included in the study.

A somewhat unique feature of alder that should be considered when studying its field performance and variation is its symbiotic association with a nitrogen-fixing actinomycete of the genus <u>Frankia</u>. Although information on genetic variation in <u>Frankia</u> is presently limited (van Dijk 1978), we had earlier found reason to expect the strain that of <u>Frankia</u> used on a root system did significantly affect growth and that endophyte strains endemic to Iowa soils might not give good levels of symbiosis and tree growth (Robison et al. 1978; Hall et al. 1979). To date, no survey of variation in alder performance has taken the source of root inoculation into account.

With interest in the use of alder increasing rapidly, we believed that a much expanded germplasm collection and evaluation program was needed (Robison et al. 1978; Hall et al. 1979; Hall and Maynard 1979). This paper reports the early results of two aspects of that program, first-year performance in a range-wide provenance test planted at two locations, and the interaction between strains of <u>Frankia</u> and the host plant in initial plant growth.

MATERIALS AND METHODS

Beginning in the fall of 1976, seed collections were obtained through cooperators from more than 150 natural stands of A. <u>gluti-</u><u>nosa</u> (a complete listing of seed available for testing by other researchers is available from R. B. Hall). Forty-eight seed sources (Table 1) were chosen from this large group for evaluation in this study. An attempt was made to choose provenances for study as evenly distributed across the entire species range as possible. However, no sources were available from the eastern portion of the range in the U.S.S.R. and the disjunct populations

Provenance Type		Geographic Origin	Latitude			Longitude			Elev. (meters)	
118			54		1	7	1.			
127	F	Coote Hill, Ire.	54	5*		8	5*		122	
	P	Bandon, Tre.		461		6	40 *		53	
131	P	Golspie, Scot.	58					H	90	
172	P	Innerleithen, Scot.	55	38*		3		8	145	
	T T	Wrexham, N. Wales U.K.	52	57*	N	10	21	H E		
211		Oslo, Worway	59				47.		80	
213	в	Burgen, Norway	60	164		5	27.	E	55	
216	F	Steinkjer, Norway	64	121	N	12	14"	E	50	
221	В	Humleback, Den.	56	0.		12	30 '		10	
222	E	Sakskobing, Den.	54	451		11	30 *		25	
261	P	Turku, Pin.	60		N	22	15*		5	
201	F	Tartu, Estonia, USSR	5B	101		27	36 *		50	
291	P	Riga, Latvia, USSR	56	444		24	91	E	20	
131	B	Uetze, W. Ger.	52	25*		10			140	
151	В	Kinzig River, W. Ger.	50		u	9	0.4	В	470	
172	F	Offenburg, W. Ger.	48	304	N	8	0 *	E	140	
181	B	Ingolstadt, W. Ger-	49		Ħ	11	0 *	E	370	
511	P	Naklo, Pol.	53	84	н	17	24 "	E.	60	
41	F	Brzeziny, Pol.	51	48"		19		B	168	
42	B	Bialovieza, Pol.	52	30*	н	23	50*		150	
561	P	Lezajsk, Pol	50		刮	20	30 .		200	
562	P	Brezesko, Pol	49	50*		20	10+		220	
571	F	Rosteler, Czech.	50	1*		14	51*		240	
582	2	Zvolen, Czech.	48		N	19	18,		280	
591	В	Gyor, Hungary	47	40"		17	00 *		114	
592	E	Sormend, Hungary	46	551		16	40 *		170	
594	B	Scherpenzeel, Neth.	52		10	5	50 *	Ξ	- 1	
533	P	Zurich, Switz.	47	16!		8	20'	B	455	
638	Ŧ		46	32'	N	6	46+		681	
53	F	Nancy, France	48	48*		б	16*		240	
82	F	St. Julien En Born, France		4. "	•	1	161	¥.	5	
122	P	Novoselec, Tugos.	45	35'	N	16	31!		95	
124	P	Durdevac, Tugos,	46	51	м	17	51	E	113	
792	B	Sofia, W. Bulgaria	43	0'	H	23	0 *	E	700	
195	B	Klisura, Bulgaria	42	45+	N 1	24	30 *	E	700	
104	F	Kosti, Bulgaria	42			27	451	E	180	
1.01	F -	Sochii, USSB	113	36*		39	46*	E	10	
143	P	Yalta, USSB	a a	40*	N	34	20 *		50	
101	F	Hashtpar, Iran	37	30'		49	0 *	E	5	
103	¥	Woor, Iran	36		м	52	1.		15	
111	p	Zamora, Spain	41	11!	М	6	081		800	
12	P	Pontewedra, Spain	49	521		7	30.		0	
62	F	Pordenone, Italy	46	2"		12	29"		40	
73	P	Pinaura D. Lucca, Italy	43	491		10	74 .		12	
75	P	Villa Basilica, Italy	43	0.8.	ы	10		E	670	
81	F	Cosenza, Italy	39	10*	N	16	211	E	650	
85	P	Forli Del Sannio Is, Italy		391	W	14	12"		400	
01	P	Lanja, Greece	38	540	H	22	251	Ē	100	
		planting i.e., two offspri								

Table 1. Provenances of A. glutinosa included in study.

9 = Bulk seed collected from several trees at one location.

in northern Africa. Some of the seedlots used were bulked collections from several parent trees in a stand, but, whenever possible, equal representation of single-tree seedlots from four parent trees per provenance were used (see Table 1). The seeds were sown on the surface of a 1:1:1 peat:pearlite:vermiculite mix in Tinus-size Roottrainers, and then covered with 1/8" of silica sand.

Since the source of Frankia inoculum is likely to affect performance, we tried to standardize this portion of our experiment. After the germinating seedlings had produced their first few true leaves (about four weeks), they were inoculated with a crushed nodule inoculum consisting of a mixture of the spore(+) and spore(-) <u>Frankia</u> strains provided by M. Lalonde of the Kettering Research Institute (Table 2). These two strains are natural associates of A. <u>glutinosa</u> derived from field-collected nodules in the Netherlands. These strains should give more effective symbioses than the native strains of <u>Frankia</u> found in the soils of the two planting sites (Robison et al. 1978; Hall et al. 1979), but no claim is made that they are the optimal symbionts for any of the seed sources.

Table 2. Frankia inocula used in symbiont evaluations and for inoculating containerized seedlings.

Inoculum code no.	Other designa- tions	Spore type	Inoculum type	Original host spp.	Geographic source	Cooperators
1	control	N.A.a	sterile buffer	N.A.	N.A.	N.A
2	control	N.A.	sterile buffer	N.A.	N.A.	N.A.
3	AcN1	(-)	pure culture	Alnus crispa	United States	M. Lalonde, Kettering Research Laboratory
4	ArI3	(-)	pure culture	A. rubra	United States	A. Berry, Harvard Univ.
5	CpII	(-)	pure culture	Comptonia	United States	J. Torrey, Harvard Univ
6	ø21 ^b	(-)	crushed nodules	A. glutinosa	Amance, France	M. Aubert, Station D'Amelioration des Arbres
7	037 ^b	(-)	crushed nodules	A. glutinosa	Bad Soden, Cermany	H. Weisgerber, Institute fuer Forstpflanzenz- zuchtung
В	#33 ^b	(+)	crushed nodules	A. glutinosa	Gohrenberg, Germany	
9	N.L. ^C (+)	(+)	crushed nodules	A. glutinosa	The Netherlands	M. Lalonde, Kettering Research Laboratory
10	N.L.(-)	(-)	crushed nodules	A. glutinosa	The Netherlands	

^aNot applicable.

^bThe original code number from collection records.

^CThe Netherlands.

After three months of growth in the greenhouse and at least one week of hardening-off in a shade frame, the trees were planted on June 5-6, 1979 at the Rhodes experimental farm in central Iowa (441° 55' N lat., 93^{deg} 15' W long.) and on June 21-22, 1979, at the U.S.F.S. Harshaw forestry research farm near Rhinelander in northern Wisconsin (45 40' N lat., 89 31' W. long.). At the time of planting most trees were top pruned to reduce the top to root ratio. (There were no substantial size differences among seedlots at the end of the greenhouse growth period.) The weather during the week following the planting in Iowa was unseasonably cool and moist. The Wisconsin plantings received overhead irrigation whenever soil moisture tension reached -0.5 bar. Still, the relatively late planting dates should be kept in mind in assessing the results.

The Rhodes planting site is a gently undulating bottomland area with predominantly a Colo silty clay loam soil type. Previous use was as a hay field and pasture. The Harshaw site is a flat siltloam, Padus soil type previously used as a potato farm. Both sites would be considered prime agricultural land in their respective areas. At both locations the trees were planted on 1.5 x 1.5 m spacing with 8-tree plots (two 4-tree rows side by side) for each provenance/ block. There are four randomized blocks of the experiment at each location. Two border rows of alder were planted around the outside edge of each set of blocks. At Harshaw the planting is inside a woven wire deer exclosure fence. At Rhodes the trees were exposed to heavy contact with deer as judged by sightings and tracks in the plantation. At both sites the winter of 1979/80 was considered less severe than normal. Both sites were plowed and disked before planting. Subsequent weed control was achieved by the use of shielded Roundup® spray at Harshaw and by cultivation with a tiller at Rhodes.

Beginning on August 26, all trees at Rhodes were observed at 5-to 9-day intervals to determine when growth cessation and bud set occurred. If the stem leader or lateral branches exhibited succulent (to touch), unfolding leaves, a tree was considered as still active. When closed, hard buds were present throughout the plant, or no enlargement of immature leaves was noted between observations, a plant was considered to have ceased growth. Growth cessation was recorded as the number of days past the summer solstice, June 21. No attempt was made to interpolate growth cessation between observation dates, rather data were analyzed as the mean of the eighttree plot. A single observation of the stage of growth cessation was made in the Wisconsin plantation on Sept. 27, 1979.

Total height to the nearest cm for all trees was recorded in Nov., 1979, after all growth had ceased at Rhodes, and on Sept. 27, 1979, when most trees had ceased growth at Harshaw. All trees were remeasured in June, 1980, to the height at which successful flushing of buds occurred. For those trees that did not flush from the terminal bud, a notation was made as to probable cause of dieback-overwinter damage, animal browsing, or mechanical damage.

Budbreak was also observed on the Rhodes plantation in the spring of 1980. The first observation was made on April 5, and subsequent examinations were made at 3-to 10-day intervals depending on weather conditions in the intervening periods. Budbreak was recorded as the number of days past March 1 when the terminal and (or) a significant number of lateral buds had split open to release expanding leaves. The 1980 spring weather was very unusual for central Iowa with a ten-day period of 32-38 C daytime temperatures in mid-April. This probably was responsible for considerably compacting the budbreak season in comparison with a "normal" spring.

The analysis of variance, correlation, and plotting procedures available through the 1979 Statistical Analysis System (SAS79) package were used in analyzing the data at the Iowa State University computer facilities. Variables analyzed were height at the end of the first season, days to fall growth cessation, days to spring budbreak, dieback over the first winter, and height at the beginning of the second growing season. Analyses were done on the means of the surviving trees on each 8-tree plot. Correlations and graphs were based on provenance means for both planting sites. Diebackback between the fall and spring measurements was calculated by subtraction of spring from fall heights. Trees that had been damaged by animals, or other nonweather related causes, were excluded from the analysis so that only winter injury and measurement error would influence the results for dieback.

To study the effects of endophyte strain on plant growth, eight sources of Frankia symbionts (Table 2) were collected through cooperation with other researchers and a germplasm collection trip in Europe. These were tested on two seed sources of A. glutinosa: AG-0X04, collected from a selected tree in a central Iowa plantation (original provenance unknown) and AG-4X08, from a selected parent in a German seed orchard. All 16 possible host X symbiont combinations were tested. Seedlings were inoculated at approximately six weeks of age while growing in Dispo Growth Pouches in a growth chamber. Ten weeks later seedlings of each host x endophyte combination with approximately equal numbers of nodules were transplanted into Leonard jars (Leonard 1943) filled with oil dry, a fired dolomitic clay potting medium. All jars received an initial treatment with a nitrogen-free nutrient solution. To prevent dieback, the transplanted seedlings were left in a high humidity growth chamber for approximately one month, then moved to a greenhouse for an additional nine weeks of growth. Plants were then divided into leaves, stems, roots and nodules, dried and weighed. Additional details of the experiment are given elsewhere (Maynard 1980).

RESULTS AND DISCUSSION

First-growing-season survival and growth was good for most provenances. Survival was 96% and 90% at Rhodes and Rhinelander, respectively, and little or no transplant shock was noted. The good start these seedlings had was probably due to the unusually cool, wet weather at Rhodes and the irrigation at Rhinelander, because the plantings were set out later in the season than would normally be recommended. The containerized seedling system worked well for the organization, handling, and planting of the provenance study.

Total height at the end of the first growing season averaged 63 cm over all plots at Rhodes and 33 cm at Rhinelander. Because the difference in planting dates is probably responsible for a large, but unknown, portion of this difference between sites, separate analysis of the data was carried out for each site. This also avoided the problem of nonhomogeneity in the variances at the two sites. Table 3 gives the summary of the analysis of variance statistics for all the variables studied; Table 4 lists the means and ranks for all provenances; and Table 5 is a correlation matrix for the studies.

In first-year height growth at Rhodes, Iowa, a northern Italy source (962) ranked first (Table 4). Other top provenances were predominantly from north central Europe (221, 614, 481, 472, 653, 431). Southern latitude sources from the Mediterranean, Black, and Caspian Sea regions were also in the top third of the first-year height rankings. At Rhinelander, Wisconsin, this last group provided many of the tallest provenances; two Iranian sources (803, 801), a Black Sea provenance (841), and a southern Italy source (975) held the top four rankings. Most of the other top performers at RhineTable 3. Analysis of variance summary for <u>A</u>. <u>glutinosa</u> provenance tests.

-cara.							
HODES, TOWA			1111	Bod	lset	Budh	reak
	Fall H	eight					
Source of Variation	đĒ	85	F	RS	F	85	r
Provenance	07	834.02	10.05**	797.59	13.97**	22.36	12.69**
	3	2993.03	36.05**	91.30	1.60MS	1.23	0.7015
Block		83.02	2014211	57.11		1,76	
Ereor	141	83,02		311.11			
		Die	back	Spring	Height		
the second second	35	#S	F	MS	P		
Source of Variation	ar	65		a.u			
Provenance	47	564.68	14.76**	1459,34			
Block	3	64.77	1.69%5	3582,57	24.58**		
Preor	140	38.26		145.74			
FILOU	194	200.97					
RHINBLANDER, WISCON	SIN						
		Fall P	eight	Di	eback	Spring	Height
Source of Variation	đź	HS	F	MS	P	#S	P
source of variation						2.36.67	# 12455
Provenance	47	96.29	3.310#	215.04		175.41	5.25**
	3	132.94	4.57**	61.97	4,45**	93.47	2.80*
		1.4.6.8.9.7				33.43	
Block	137	29,10		13.94			

** Indicates significance at the 0.01 level of probability
Indicates significance at the 0.05 level of probability
NS Indicates non-significance at the 0.05 level of probability

Table 4. Summary of provenance means and ranks.

Th each column the mean performance of each provenance is given followed by its rank in parentheses. Banks are arranged 1 = highest to 4B = lowest mean value for each variable. Reams are rounded to the mearest whole unit. 8 = 4 for most means.

8

			-RHODES	*****		********	RRIBELANDES	
Prov.	Pall Height	Spring Height	Winter Dieback	Budset	Budbreak (Days from	Fall Height	Spring	Winter Dieback
PEGV.	(CW)	(CB)	(cm)	June 21)	March 1)	(ca)	(ca)	(CB)
118	66 (23)	67 (15)	2 (21)	98 (41)	50 (28)	34 (18)	32 (14)	2 (29)
127	62 (30)	56 (32)	5 (12)	102 (31)	50 (27)	36 (9)	32 (12)	a (14)
131	43 (44)	44 (39)	0 (37)	91 (44)	56 (2)	29 (40)	27 (31)	2 (40)
151	63 (32)	70 (12)	0 (40)	98 (40)	56 (3)	27 (43)	26 (34)	1 (45)
172	76 1 51	81 (2)	1 (30)	102 (32)	52 (12)	35 (15)	34 (5)	2 (34)
211	30 (45)	29 (42)	-1 (47)	72 (45)	49 (40)	38 (6)	35 (2)	3 (22)
213	24 (47)	20 (43)	3 (19)	64 (46)	49 (37)	19 (48)	18 (43)	2 (32)
216	18 (48)	11 (48)	5 (11)	40 (48)	49 (35)	22 (47)	19 (42)	3 (25) 0 (48)
221	80 (2)	77 (3)	2 (24)	104 (22)	50 (30)	25 (46)	25 (37) 27 (29)	1 (46)
222	54 (41)	48 (35)	4 (14)	101 (37)	49 (38)	28 (42) 29 (39)	25 (36)	5 (11)
261	25 (46)	16 (45)	8 (8)	51 (47)	47 (48)	29 (39) 25 (45)	25 (36)	1 (47)
281	46 (42)	47 (37)	1 (36)	92 (43) 93 (42)	48 (41)	31 (35)	28 (25)	3 (27)
291	46 [43]	47 (36)	0 (42)	104 (20)	51 (22)	35 (17)	32 (11)	2 (30)
431	74 (10)	76 (5) 60 (27)	2 (23)	100 (38)	51 (23)	34 (19)	32 (16)	3 (26)
451	65 (27)	73 (9)	3 (18)	105 (19)	49 (34)	39 (5)	34 (3)	5 (12)
472	74 (8)	76 (6)	0 (94)	101 (35)	50 (26)	36 (11)	33 (9)	3 (18)
481	68 (18)	58 (29)	4 (13)	103 (28)	51 (18)	30 (38)	27 (32)	3 (23)
541	55 (39)	56 (33)	1 (27)	103 (25)	51 (20)	32 (29)	31 (19)	1 (42)
542	57 (38)	46 (38)	13 (7)	100 (39)	51 (19)	31 (31)	30 (21)	1 (43)
561	66 (24)	57 (30)	-1 (48)	101 (34)	51 (16)	34 (20)	33 (8)	1 (44)
562	64 (30)	64 (22)	0 (46)	103 (27)	51 (14)	33 (26)	32 (15)	1 (91)
571	66 (25)	64 (21)	1 (33)	102 (33)	52 (10)	34 (22)	32 (13)	2 (38)
582	64 (31)	60 (28)	1 (35)	103 (30)	50 (32)	31 (33)	30 (22)	2 (39)
581	75 (6)	71 (11)	1 (32)	103 (29)	50 (29)	33 (27)	31 (17)	2 (33)
592	69 (15)	70 (13)	0 (38)	103 (24)	49 (36)	35 (13)	33 (7)	2 (35)
614	79 (3)	75 (7)	3 (17)	104 (21)	53 (8)	34 (21) 35 (16)	30 (23) 32 (10)	2 (28)
633	67 (22)	65 (17)	0 (45)	101 (36) 104 (23)	50 (31)	31 (34)	27 (30)	3 (19)
E38	69 (17)	61 (26)	1 (26)	103 (25)	52 (13)	33 (24)	31 (20)	2 (31)
653	74 (9)	73 (8) 68 (14)	0 (93)	106 (12)	51 (17)	28 (41)	24 (38)	4 (13)
682 722	74 (12) 64 (29)	65 (20)	0 (39)	106 (11)	49 (33)	31 (30)	29 (24)	3 (20)
724	69 (16)	72 (10)	0 (41)	106 (13)	48 (42)	37 (7)	34 (6)	4 (17)
792	68 (20)	65 (19)	2 (25)	105 (16)	49 (39)	37 (8)	35 (1)	2 (36)
795	65 (28)	62 (25)	3 (15)	106 (14)	47 (47)	36 (12)	34 (4)	2 (37)
704	63 (33)	62 (24)	1 (28)	105 (18)	47 (46)	30 (36)	27 (33)	3 (21)
841	71 (13)	57 (31)	14 (6)	119 (9)	51 (24)	44 (2)	24 (39)	20 (4)
843	70 (14)	66 (16)	2 (22)	105 (17)	48 (03)	31 (32)	28 (28)	4 (16)
801	68 (19)	14 (47)	53 (1)	116 (2)	53 (7)	41 (3)	10 (46)	31 (2)
803	59 (37)	16 (46)	42 (3)	116 (1)	55 (5)	48 (1)	11 (45)	36 (1)
911	55 (40)	36 (41)	19 (5)	113 (5)	57 (1)	35 (14)	17 (44)	18 (6)
912	62 (36)	19 (44)	43 (2)	114 (4)	55 (4)	34 (23)	8 (48)	26 (3) 3 (24)
962	84 (1)	83 (1)	1 (31)	105 (15)	48 (44)	33 (28)	31 (18) 21 (41)	9 (10)
973	62 (35)	54 (34)	3 (16)	108 (10)	51 (21) 52 (11)	30 (37)	28 (26)	13 (7)
975	76 (4)	77 (6)	3 (20)	111 (7)	53 (6)	26 (44)	8 (47)	18 (5)
981	74 (11)	42 (40)	30 (4) 6 (10)		53 (9)	33 (25)	22 (40)	12 (8)
985	67 (21)	63 (23)	6 (9)			36 (10)	28 (27)	9 (9)
901	66 (26)	65 (18)	e (9)	111 [0)	21 (12)	30 (10)	20 15.1	
H. S.	83.02	145.74	38.26	57.11	1.76	29.10	33.43	13.94
đE	141	140	140	141	141	137	137	137
		0.077						

lander were from north central and south central Europe. At both test locations most of the Scandinavian sources were ranked at the bottom of the total height list. Two of these sources behaved erratically when the results of both plantations are compared. The Oslo, Norway source ranked sixth at Rhinelander, but fell to a rank of 45 at Rhodes. Conversely, a Danish source (221) was ranked second at Rhodes, but a lowly 46th at Rhinelander. Two other portions of the species range deserve mention. In the British Isles, two Scottish sources (131, 151) were relatively poor performers at both test sites while a Wales provenance (172) was consistently good. Secondly, in light of the earlier findings of superior growth from north central Europe (Funk 1973, 1979) and considering the well developed distribution of A. glutinosa in Poland, we had expected to find some superiority in our samples from that region. However, the highest ranking achieved by this group was an 18 at Rhodes for source number 511. Overall, there was a correlation of about -0.5to -0.6 between first-year growth and latitude of origin (Table 5), but the exceptions to this general trend as just discussed seem more important to any future selection and breeding efforts.

Table 5.	Correlation c	oefficients	between	provenance	characteris-
	tics and perf	ormance vari	ables.		

			BODES, ION	A		BHI	ELANDER,		
	Pall Beight	Spring Height	Winter Dieback	Budset	Budbreak	Fall Height	5pring Beight	Winter Dieback	
Provenance									
Characteristics	1.2.2.1	1. 6974		81##	240	57**	.31*	62**	
Latitude	-, 51**	-, ORNS	51**					47**	
Longitude	.0685	. 33*	46**	19NS	. 24 MS	-,35*	. 29*		
Elevation	. 17NS	. 17HS	06NS	.2685	. 12NS	. 1685	. 08%5	.0385	
Rhodes									
Variables							a second		
Fall Height		.78**	-, 02NS	.774#	.1685	. 4444	.27WS	.0485	
Spring Height			- 64**	.3900	1785	, 13#S	. 72**	55**	
Winter Dieback	~~~			, 33*	.4700	.34*	82**	.9300	
					. 37 **	.49**	-, 1185	.40**	
Budset				**		. 1785	46++	.5144	
Budbreak				**					
Rhinelander									
Variables									
Pall Height		**					.15115	+50**	
Spring Height								78**	
Winter Dietack									

** Correlation significant at the 0.01 level of probability * Correlation significant at the 0.05 level of probability NS Correlation non-significant at the 0.05 level of probability

Detailed observations and analysis of growth cessation and budset were taken only at the Iowa plantation. Alders are noted for their late growth in the season. In this study, most of the trees did not set bud until the first or second week of October. The exceptions to this were many of the Norwegian and Finish trees that set bud soon after being planted and some of the southern latitude trees, especially Spanish and Iranian sources, that never did fully set bud before winter started. The single observation of growth activity in the Wisconsin planting late in September suggested the same pattern was followed there, but approximately three weeks earlier. One of the strongest correlations found in this study was a -0.81 r-value for the relationship between budset and latitude (Table 5 and Figure 1). In a study limited to British Isle provenances, McVean (1953) observed that the loss of leaf function occurred 2-3 weeks earlier in the fall for each $_{40}$ of movement north in latitude of origin.

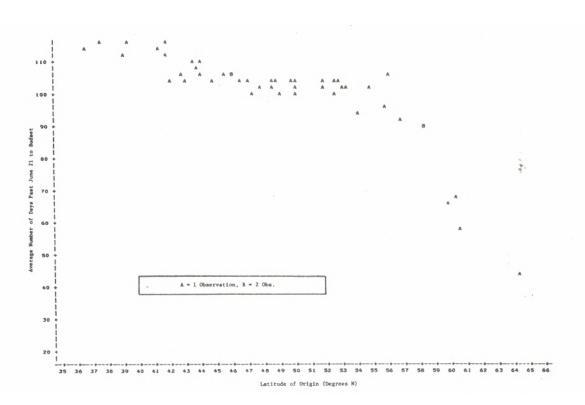


Figure 1. Average number of days to budset plotted as a function of latitude of origin.

Spring budbreak was also studied in the Rhodes, Iowa planting. Because of the unusually hot spring weather experienced at this site in 1980, it is likely that the budbreak patterns observed are compressed and perhaps modified from the normal schedule. The first groups of provenances to flush were Scandinavian and Yugoslavian; they broke bud over the period 17-19 April, and two of the southern sources (843, 962) also broke bud then. With these two exceptions, the southern and British Isles sources were the last to flush, breaking bud on about April 25-27. A plot of mean number of days to budbreak versus latitude of origin has an unusual "W" shape (Figure 2). On a more localized scale, McVean (1953) reported that southern sources within the British Isles were the first to leaf out in the spring, and a similar pattern was noted among Swedish provenances. Of the five British Isles sources included in this study, four followed the pattern suggested by McVean while one northern Ireland source flushed relatively early. Anomalous patterns of budbreak have been observed in other species (e.g. see Kriebel et al. 1976; Steiner 1979) and are probably due to a complex of local environmental selection pressures acting on several physiological processes

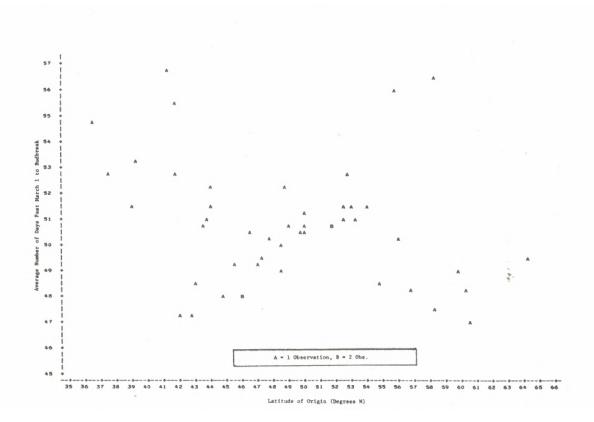


Figure 2. Average number of days to budbreak plotted as a function of latitude of origin.

that control re-initiation of growth in the spring (Steiner 1979).

In spite of the late budset dates for the alder trees studied, there was relatively little winter dieback. Averaged over entire plantations, the top dieback was 10% at Rhodes and 16% at Rhinelander. However, some trees from southern provenances were killed back to ground level, and provenance means ranged as high as 78% dieback. There was a strong negative correlation (Table 5) between dieback and latitude of origin at both test sites. When the Rhodes data for budset and winter dieback are plotted (Figure 3), there is an abrupt cut-off for safe growth cessation at about 110 days, or October 9, in this particular year. If the relationship between photoperiod, growth cessation, and dieback in more severe winters can be defined, eventually this abrupt transition offers hope for indirect selection for hardiness under controlled environment conditions. Not withstanding the latitudinal trend in dieback, there was considerable within-provenance variance in winter dieback that might be exploited through clonal propagation or breeding.

At Rhodes the winter dieback was severe in only six provenances: the two Iranian sources (801, 802), the two Spanish sources (911, 912), the southernmost Italian source (981), and the Sochii source (841) from the eastern Black Sea region. In contrast, provenance number 843 from the north rim of the Black Sea and the northern Italian source that ranked number one in fall height at Rhodes both

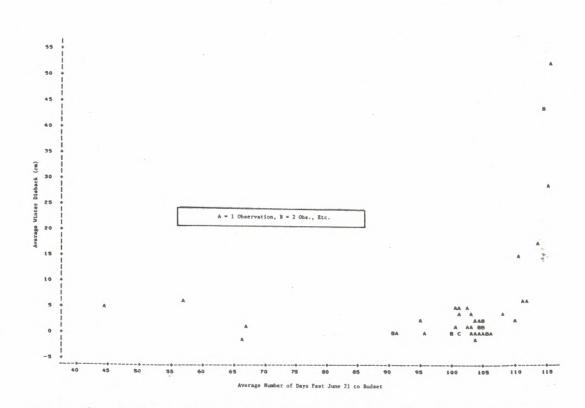


Figure 3. Average winter dieback plotted as a function of average number of days to budset.

came through the winter with no significant damage. One puzzling observation was the overwinter dieback and in some cases death of some trees from Scandinavian sources. This may have been due to their small size and subsequent difficulty in storing enough food reserves to compete successfully. It was noted during the budset observations in the fall, however, that some of these trees that had set bud in midsummer seemed to have some bud swelling late in the fall. It is possible that fluctuating fall temperatures partially satisfied the dormancy requirement and the mechanisms of renewed growth were started. With their cold-hardiness thus diminished they experienced overwinter dieback just as did those trees from the south that had never set bud. As a net result of winter damage at Rhodes, there was little change in the top 10 rankings for height in the spring of 1980, most of shifts in ranking came in the second and lower echelons of provenances.

At the Wisconsin test site, winter dieback seriously affected more provenances, eleven of them losing more than 20% of their fall heights, and many more suffering lesser damage. The worst losses came in the same six sources that suffered the most in Iowa, plus all the central Italy and Greek sources (975, 985, 973, 201). Again, as at Rhodes, the northern Italy (963) and northern Black Sea (843) sources suffered little damage and two Scandinavian sources (261, 213) suffered greater than average dieback in percentage of their total height lost. The net overwinter result at Rhinelander was a substantial shakeup in the height rankings. Many of the fall leaders fell to rankings near the bottom of the list, while provenances from northern Italy, Yugoslavia, Hungary, Poland, and Germany moved up substantially in the rankings.

Although most of the data were analyzed separately for the two sites, correlations were run on provenance means to evaluate the consistancy of results at Rhinelander and Rhodes (Table 5). The correlations for fall and spring heights at the two sites were moderate to strong (r = 0.44 and 0.72, respectively), but the exceptions already discussed are numerous enough to warrant separate evaluation programs for overall growth. However, the correlation between winter dieback at the two sites is very high (r= If this relationship continues to hold in future winters, then winter dieback in northern test sites could be used as a good, conservative basis for predicting dieback in more southern plantings.

During the first year, several other observations were made that may be important to follow. In addition to the winter dieback at the Iowa planting, two other damaging agents caused significant growth loss. The first agent has yet to be identified. It was first noted during the budset observations in early September. The tops of a few trees were turning yellow and a few eventually died back to a canker-like lesion on the main stem. A detailed survey was then made of the entire plantation, and the symptom was found on many other trees often on a side branch and (or) in a less seriously developed status. The problem was most serious in three "pockets" dispersed in two blocks. It also seemed to be concentrated on and localized around the two Iranian (801, 803) and two Black Sea sources (841, 843) where they occurred in these two blocks. An attempt to culture fungal organisms from the affected tissues found no organisms that could be considered primary invaders. Abiotic agents such as heat or frost do not seem plausible considering the provenances involved, the portion of the trees that was damaged, and the more protected nature of both blocks. A similar appearing injury on Populus has been noticed the last two years at Rhinelander, and is thought to be caused by insect feeding. Because of the potential seriousness of such a stem and branch dieback problem, we are continuing to monitor this situation closely. Indications during the first half of the second growing season are that healthy sprouts have taken over below dead stem areas. Less seriously affected stems and branches seem to have healed over the lesion and continued growth.

The second problem did not occur until the trees had gone dormant, and it occurred only in the Iowa plantation that was not protected by deer fencing. During the growing season there were few signs of deer browsing in the plantation in spite of the fact that there was always an abundance of fresh deer tracks. However, starting just before leaf fall and continuing on into the winter, there was a high incidence of deer nipping off the upper 10 cm or so of the main stem. This appeared to be either a "curiosity/nuisance" behavior or selective feeding on the main terminal bud rather than the heavy browsing to which many other broad-leaf trees are subjected. It did not seem to have serious impact on tree growth or form. In an earlier study with alder there was a high frequency of stem girdling and death because of rodent damage. With better control of herbaceous ground cover in these plantations, no rodent problems were encountered.

Several morphological characteristics are so distinctive between regions of origin that some provenances can be recognized on sight. Mediterranean provenances typically have more anthocyanin pigmentation in twigs and petioles and small, numerous, upright branches that give them a more pyramidal form. This was especially true of the Spanish sources, which also have small wavy leaves that would make them attractive ornamentals where they are cold hardy. The Iranian and Black Sea provenances have leaves that are much larger and more oblong than the typical A. <u>glutinosa</u> shape. Because of their early growth cessation, many of the northern Scandinavian trees had very short internodes and sometimes developed rosettes of leaves. Variation in branchiness and form was substantial throughout all provenances, indicating there will be ample material for future selection and improvement work.

Influence of Frankia Symbiont Strain

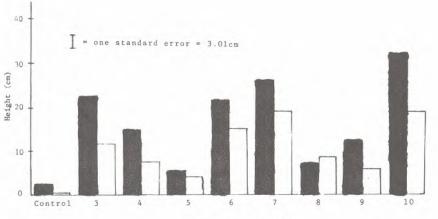
In the greenhouse experiment with different combinations of sumbionts, both the host seed source and the endophyte strain had highly significant effects upon height growth and dry weight production (Table 6 and Figures 4 and 5). The intereaction between host seed source and inoculum strain was not significant at the 0.05 level for either height or dry weight (Table 6).

Table 6. Analysis of variance summary for tests of Frankia inocula.

	He	ight Gr	owth	To	tal Dry	Weight
Source of Variation	đf	HS	F	đ£	HS.	F
Rost Seed Source	1	968	21. 24**	1	50	14.43**
Inocula	8	736	16.15**	7	31	8.77**
Rost X Inocula	8	49	1.0785	7	3	0.93W5
Frror	72	46		43	3,5	

** Indicates significance at the 0.01 level of probability * Indicates significance at the 0.05 level of probability NS Indicates non-significance at the 0.05 level of probability

After the three-month growth period, the AG-0X04 seedlings averaged 15.9 cm in height as compared to 10.4 cm for the AG-4X08 seedlings. Dry weights showed a similar difference between seed sources. But the important observations in this study were the differences among inocula in the seedling growth they promote. The best growth resulted from the use of two native European A. <u>glutinosa</u> endophytes (inocula 7 and 10 in Table 2). These two inocula gave



Inoculum code number

Figure 4. Three-month height growth for two <u>A. glutinosa</u> seed sources, as affected by <u>Frankia</u> inocula. Seed source AG-0X04 is indicated by shading, seed source AG-4X08 is indicated by open bars.

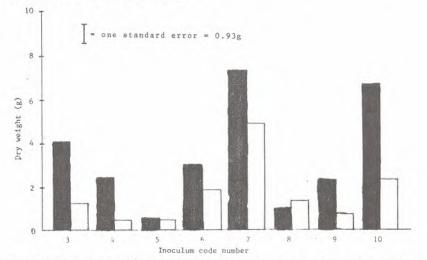


Figure 5. Whole plant dry weight values for each inoculum-host combination. Seed source AG-0X04 is indicated by shading, seed source AG-4X08 is indicated by open bars.

over sixfold better growth of seedlings than did an inoculum isolated from <u>Comptonia peregrine</u>, sweetfern. The later type of endophyte is native to many Lake States and New England soils where it might give rise to inefficient nodules on alders planted there. Inocula 3 and 4, which were derived from North American species of alder, contributed to intermediate growth on the plants inoculated with them. Again, these are the types of inocula that might be expected to be endemic to the planting sites where we wish to establish European black alder.

The large differences in plant growth rate attributed to the strains of <u>Frankia</u> indicate the desirability of testing soil populations of <u>Frankia</u> in areas where A. <u>glutinosa</u> is to be grown.

Direct manipulation of the <u>Frankia</u> population in large plantations may be difficult, but in the nursery it may be feasible to replace an existing soil population with superior strain through the use of soil fumigants. Furthermore, actinorhizal nodules are perennial structures often remaining active on the plant roots for a number of years (Akkermans and van Dijk 1976). Nodules established by an improved <u>Frankia</u> strain in nursery beds or in containers should, therefore, be able to benefit the plant for several seasons after planting in the field. Whether or not the improved strains of <u>Frankia</u> might also be able to displace poorer native strains as the nodules decompose in the soil remains to be studied.

In selecting strains of <u>Frankia</u> to achieve improved alder growth, our results indicate that it will be important to evaluate different strains naturally found on alder roots. Of the five A. <u>glutinosa-</u> derived inocula studied, two gave high levels of growth, one was intermediate and two were definitely poor for use on the two seed sources tested (Figures 4 and 5). Unfortunately, we did not have these results at the time we started our field studies because a mixture of inocula 9 (poor) and 10 (good) was used on our seedlings. Since we also have evidence (Maynard 1930) that inoculum 9 is more aggressive in colonizing root systems than inoculum 10, we may not he obtaining as much initial growth as is possible in our provenance test.

Another consideration for future studies is provenance/strain interaction, i.e. some alder genotypes may grow better in symbiosis with strain A while other alder genotypes would grow better in association with strain R. The interaction term in this study was nonsignificant, but only two seed sources were studied, and there were trends in the data that suggest this question is not yet adequately resolved.

CONCLUSION

Since these results are based on only one year's observations, it would be unwise to try to draw too many conclusions. The risks of early selection are even greater in black alder than in most other tree species because of the randomey for some promising trees to decline drastically when they reach flowering age (Mall and Maynard 1979; Funk 1979). However, by comparing these early results with Funk's (1973, 1979) longer tara evaluations, there is consistancy for the portions of the species range included in both sets of studies. Sources from morth of the Baltic Sea perform poorly, at least in part owing to their early growth cessation each year. North central European sources exhibit superiority, but not uniformly. In both studies provenance material from pear Netze, W. Germany (431) is among the top performers, while some other German sources are mediocre. One short cut to selection and breeding of A. glutinosa in North America may be to utilize parants already selected for superiority in Europe and fit their use ou this continent to the locational/climatic trends delineated by our provenance tests.

Indeed, the tallest tree in the Rhodes plantation was a border tree offspring from a German seed orchard. It had a height of 1.4 m after the first growing season and winter.

Although field tests and other studies are still needed to confirm the importance of the strain of <u>Frankia</u> that first associates with our trees, accumulating laboratory results indicate we should definitely not ignore this source of variation in our tree improvement efforts. In light of the poor effectiveness and strong infectivity of the Netherlands spore(*) strain that is reported in this paper, we have stopped using it for routine inoculation and have switched to the use of the AcN1 strain (Table 2) that is more effective and is available in a pure culture form. Additional studies are needed to determine the significance of using particular symbiont strains under field conditions.

Footnote 1. Although not reported on in this paper, duplicates of this study have been planted by cooperators in Maine, Pennsylvania, and southern Illinois. Subsamples of the provenances listed here plus many of the other ones available in our seed collection are being tested by other researchers throughout the eastern United States.

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