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Forest-Restoration Effects at *Alnus hirsuta* (Spach) Rupr. Plantations on Two Small Islands in Northern Japan.

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Summary

Plantations of *Alnus hirsuta*, which were protected by earthworks, earthwork-stockades or hurdle-fences, were established in Yagishiri (3 plantations) and Teuri (2 plantations) Islands. Soil samples from the taproot system of *A. hirsuta* and from 5 cm below the ground surface at about 5 m distance from the targeted tree were taken at each plot as well as from 2 neighboring plots of autochthonous *A. crispa* and *A. hirsuta* as a control. The soil samples under the taproot system on Teuri island contained several forms of nitrogen in high concentrations except for 1 plot from one plantation.

A. hirsuta trees formed large masses of root nodules about 2-3 cm in diameter and they contained endophytic vesicles of *Frankia* as observed by electron microscopy.

The natural abundance of ^{15}N in the leaves of *A. hirsuta* and non-nodulated (reference) plants from 1 plot on each island was also measured. The N value derived from atmospheric N_2 (%Ndfa) measured was 94% and 84% in *Alnus* leaves from 5-year old and 26-year old plantations respectively. The effect of forest and soil restoration by plantations of *A. hirsuta* is discussed.

Key words: *Alnus hirsuta* - *Frankia* - natural abundance of ^{15}N - plantations - small islands - analysis of soils

Introduction

Two small islands, Yagishiri (Lat. $44^\circ.25'.30''$ - $44^\circ.26'.40''$, N.; Long. $141^\circ.23'.00''$ - $141^\circ.$

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26'.00", E.) and Teuri (Lat. 44°.24'.30"-44°.26'.20", N.; Long. 141°.18'.00"-141°.20'.20", E.), consist primarily of volcanic soil and measure 530.0 and 540.0 ha in area, respectively. The annual average temperature is 7.9°C. The forests on these islands had been felled to supply fuel woods since the 1800's, and had been harmed several times by forest fires or by clearing of forest land for cultivation. Consequently, the stands of trees on the islands had become poor. Also, the drying up of headwaters on these islands caused by decreased water-holding capacity has created a serious problem.

Generally, it is difficult to restore demolished stands of this type in northern areas. The young trees in plantations are often damaged in winter by frozen soil or by soil lifting by frost, factors which result from gales from the cold sea. Therefore, there are undesirable conditions for planting stocks or plantlets on these islands. At the two plots on Teuri island, *Alnus hirsuta* was planted for forest restoration using the earthworks in 1966 and using both earthworks and stockades in 1981. These structures were constructed by Higashi *et al.* as a way to protect planting stock of *A. hirsuta*. Also, Higashi *et al.* (1991) developed a method called a hurdle fence, which is a woody triangular fence (1.4 m in height, 1.8 m in width at the base of a triangle and 3.0 m in length) for protection of young trees at the experimental plots. The hurdle fences were established directly on wastelands in plots about 20 m² in size. Planting stock of *A. hirsuta* (2 years seedlings) was planted in this square and the young trees were protected by the piled snow in setting hurdle fence square in winter (Fig. 1 a and b).

Alnus plants can fix atmospheric nitrogen by associating with *Frankia* as symbiotic microorganisms in the nodules formed on roots (Sellstedt *et al.* 1986, Newcomb and Wood 1987, Vikman *et al.* 1990). The increase in soil fertility as a result of *A. hirsuta* trees was examined at established plots in Yagishiri and Teuri islands.

In this paper, the natural abundances of ¹⁵N (δ¹⁵N) in the leaves of both *A. hirsuta* and of non-N₂ fixing plants as the reference were analyzed to estimate the contribution of N₂ fixation. The effects of forest-restoration by *A. hirsuta* plantation were also discussed.



Fig. 1a Plantation of *Alnus hirsuta*, which was protected by installation of a hurdle fence at plot 2 on Yagishiri island (p2Y). Picture was taken in September 1988.
1b Same plantation of *Alnus hirsuta* at p2Y in July 1991.
These photographs show the growth of the plantation of *Alnus hirsuta* for three years.

Materials and Methods

Experimental plots

The experimental plots were established at three areas on Yagishiri island; the 1st plot (p1Y) at Nogoshinosawa, the 2nd plot (p2Y) at Moriyanosawa and the 3rd plot (p3Y) at Gyokoninosawa. On Teuri island, two plots were established; the 1st plot (p1T) at Tsurudanosawa and the 2nd plot (p2T) at Bententakinosawa. Soils from native *Alnus* communities at Nukkakushi Furano Riverside, Sanpohyamazawa (*A. crispa*) (p1S) and at Furano Riverside, Asahino (*A. hirsuta*) (p1A) were also examined as controls.

Soil analysis from the experimental plots

Soil specimens were taken directly under the taproot system of a targeted *Alnus* and about 5 cm below the ground surface at 5 m distance from the targeted tree as the control. These specimens were sent to Tokachi Federation Agricultural Cooperatives for computerized measurement of 21 soil characteristics as summarized in Fig. 2 and Table 1.

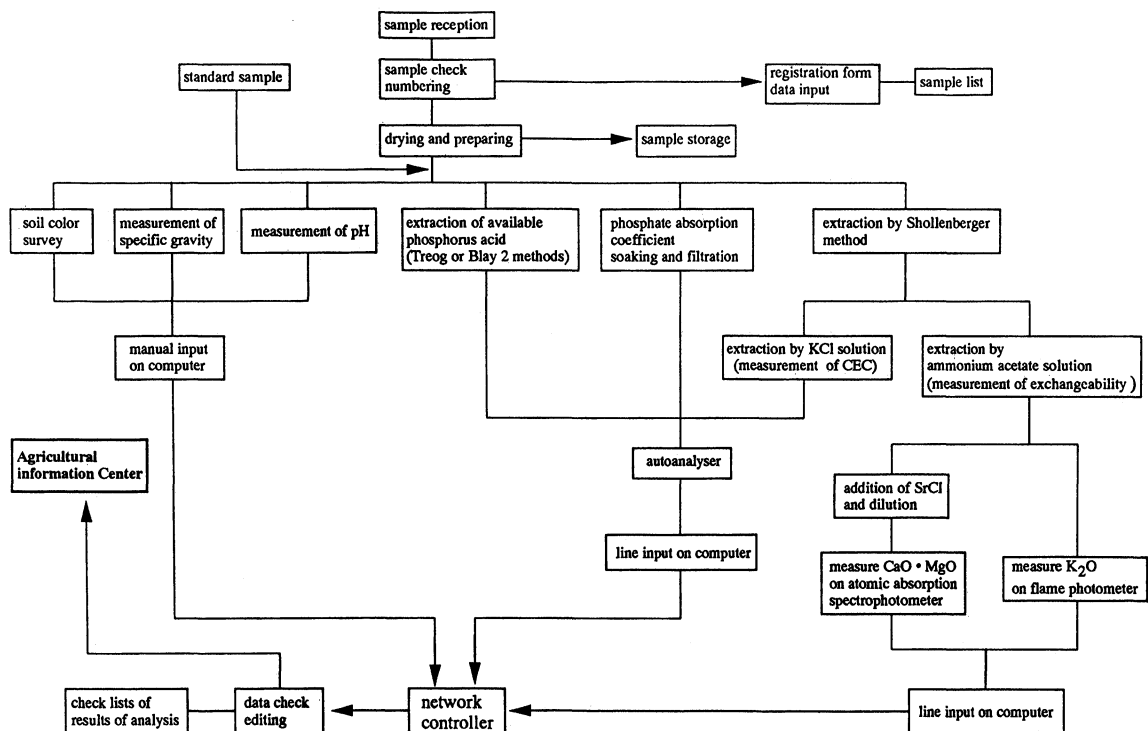


Fig. 2 Flowchart of soil analysis system.

Table 1 Plot information, tree characteristics, and analysis of soils from *Alnus hirsuta* plantations on Yagishiri and Teuri Islands (including data from autochthonous *A. crispa* at Nukkakushi Furano Riverside and from *A. hirsuta* at Asahino Furano Riverside).

Plantation	Sampling place	Place abbr.	Place name	Planting age (year)	Tree height (m)	Basal diameter (cm)	R.Nodule diameter (mm)	Sampling position	pH	Available phosphor (mg/100g)	Exchangeable K (mg)	Exchangeable Mg (mg)	Exchangeable Ca (mg/100g)	Hg/K (molar ratio)	Ca/Mg (molar ratio)	Ca Saturation (%)	Base Saturation (%)	Phosphate absorbing constant	Base exchange capacity (mg/100g)	Apparent specific gravity	Humic content	Cu (ppm)	Zn (ppm)	Hn (ppm)	B (ppm)	Hot water extractable N (mg/100g)	NO ₃ -N (mg/100g)	NH ₄ -N (mg/100g)	Inorganic N (mg/100g)	Soil type (color)
PLANTATION	Yagishiri Is.	p1Y	Nogoshinosawa	1987 (5)	2.5~4.0 M=2.5	8	5~10	tapr. ^{a)}	5.0	4.5	14.4	13.2	32.8	2.1	1.8	6.8	12.4	971	17.2	0.94 inc. ^{c)}	0.31	1.47	64.1	0.70	4.0	0.2	1.9	2.1	Alluvial	
		p2Y	Moriyanosawa	1989 (3)	2.5~3.0 M=2.5	5	10~25	tapr.	5.2	2.9	20.0	18.8	43.1	2.2	1.6	9.5	17.9	932	16.2	0.78 inc.	0.41	2.36	171.5	1.49	6.0	0.2	1.8	2.0	Volcanic (ocher)	
		p3Y	Gyokohinosawa	1990 (2)	0.6~1.0 M=0.7	2	5~10	cont. ^{b)}	5.2	1.0	17.7	15.1	28.7	2.0	1.4	5.8	12.1	1099	17.7	0.95 inc.	0.37	1.66	29.3	0.66	3.7	0.1	2.1	2.2	Volcanic (ocher)	
	Teuri Is.	p1T	Tsurudanosawa	1981 (1)	4.3~5.5 M=4.0	10	20	tepr.	5.5	0.6	32.4	48.5	46.8	3.5	0.7	7.5	21.5	1201	22.2	0.89 inc.	0.22	1.69	195.9	0.91	6.8	0.2	3.9	4.1	Volcanic (ocher)	
		p2T	Bententakinosawa	1966 (2)	8.0 M=8.0	18	15~35	tapr.	4.7	1.9	32.2	41.9	115.1	3.0	2.0	10.9	18.3	1688	37.6	0.71 rich	0.30	4.77	266.2	1.74	19.5	5.5	3.7	9.2	Volcanic (black)	
	AUTOCHTHON	Sampo-Yamazawa	p1S	Nukkakushi Furano Riverside	-	1.3~1.5 M=1.5	5~20	5~20	tapr.	4.7	1.2	6.7	3.6	36.3	1.3	7.2	8.4	10.5	733	15.4	1.00 inc.	1.78	0.85	9.7	1.05	2.2	0.0	1.3	1.3	Alluvial
p1A			Furano Riverside	-	1.0 M=1.0	2	10~20	tapr.	4.6	0.3	11.8	3.9	19.1	0.8	3.5	5.3	8.7	774	12.9	0.89 inc.	1.38	1.05	1.5	0.52	1.2	0.0	3.0	3.0	Alluvial	

a) tapr.; Under taproot system of the target tree.
 b) cont.; About 5 cm below ground surface at about 5 m distance far from the target tree.
 c) +, 2~5%; ++, 5~10%; +++, 10~20%.
 * M; Height of the target tree. (As of 1991, the data were arranged).

Measurements of leaves for $\delta^{15}\text{N}$

Plant leaves for $\delta^{15}\text{N}$ measurement were collected at Yagishiri and Teuri islands (July 22, 1991). The leaves were oven-dried at 70°C for three days and all subsequent procedures were done according to Yoneyama (1987). Briefly, the samples were digested in hot sulfuric acid with occasional addition of hydrogen peroxide so to convert the nitrogenous compounds to ammonium sulfate. The ammonium sulfate thus produced was converted into N_2 *in vacuo* by reaction with NaOBr, and the ^{15}N abundance in the resulting N_2 was measured by a Finnigan MAT 251 mass spectrometer (Yoneyama 1987). The natural ^{15}N abundance ($\delta^{15}\text{N}$) in a sample is expressed as follows:

$$\delta^{15}\text{N} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 (\text{‰})$$

where $R = ^{15}\text{N}/^{14}\text{N}$. Atmospheric N_2 was used as the standard. The precision of analysis was $\pm 0.2\text{‰}$.

Electron microscopy

Nodule specimens from *A. hirsuta* were prepared for scanning electron microscope (SEM) and transmission electron microscope (TEM) as previously outlined (Higashi *et al.* 1986). SEM specimens and thin sections of nodules were viewed with a Hitachi S-450 SEM or Hitachi H-600S TEM, respectively.

Results and Discussion

Growth of *A. hirsuta* in the hurdle fence square

Two- and five-year-old *A. hirsuta* plantations growing at plot p1Y are shown in Figs. 1a and b. Plot p1Y was established in 1987 with planting stock. Two-year-old *A. hirsuta* seedlings (stem was about 8 mm in diameter and 40 cm in high), were used for the plantation. The *Alnus* was inter-planted with young *Quercus dentata* Thunb. and *Picea glehnii* (Fr. Schm.) Mast. The photographs (Fig. 1) indicate that these trees have been surviving well under severe weather conditions in the northern area.

Root nodule morphology of *A. hirsuta*

An *A. hirsuta* root bearing several root nodules is shown in Fig. 3. Masses of large root nodules (about 2 to 3 cm in diameter) are formed on the taproot. Small nodules are formed on the lateral roots. The fine structure of *A. hirsuta* root nodules was observed by using scanning (SEM) and transmission electron microscopy (TEM). A longitudinal section through a small root nodule was examined by SEM shown in Fig. 4. Its organization resembles the structure of leguminous root nodules except for the location of the vascular bundle system. In general, vascular bundles in legume root nodules formed by *Rhizobia* are arranged in a spherical pattern under the epidermal layer of the nodule. However, *Alnus* nodules have branched vascular bundles located at the center of nodule. Newcomb and Wood



Fig. 3 Intact large root nodule of *Alnus hirsuta*.

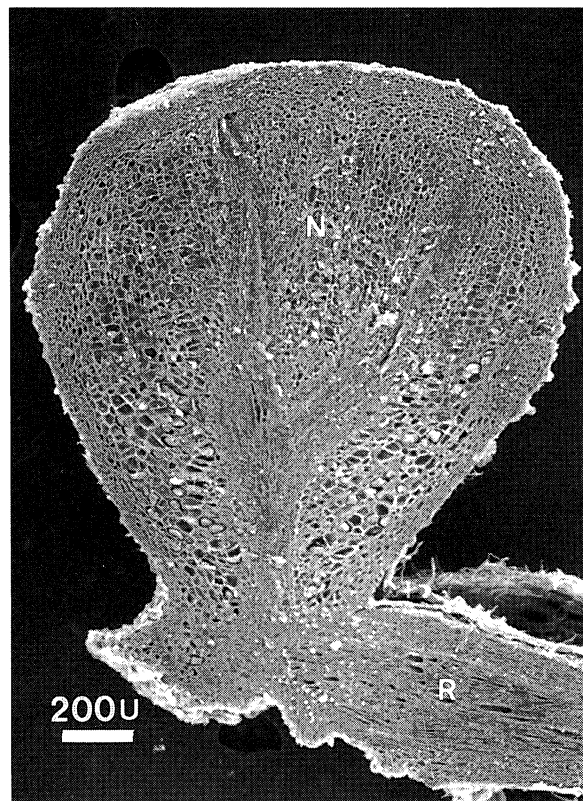


Fig. 4 Scanning electron micrograph of root nodules of *Alnus hirsuta*.

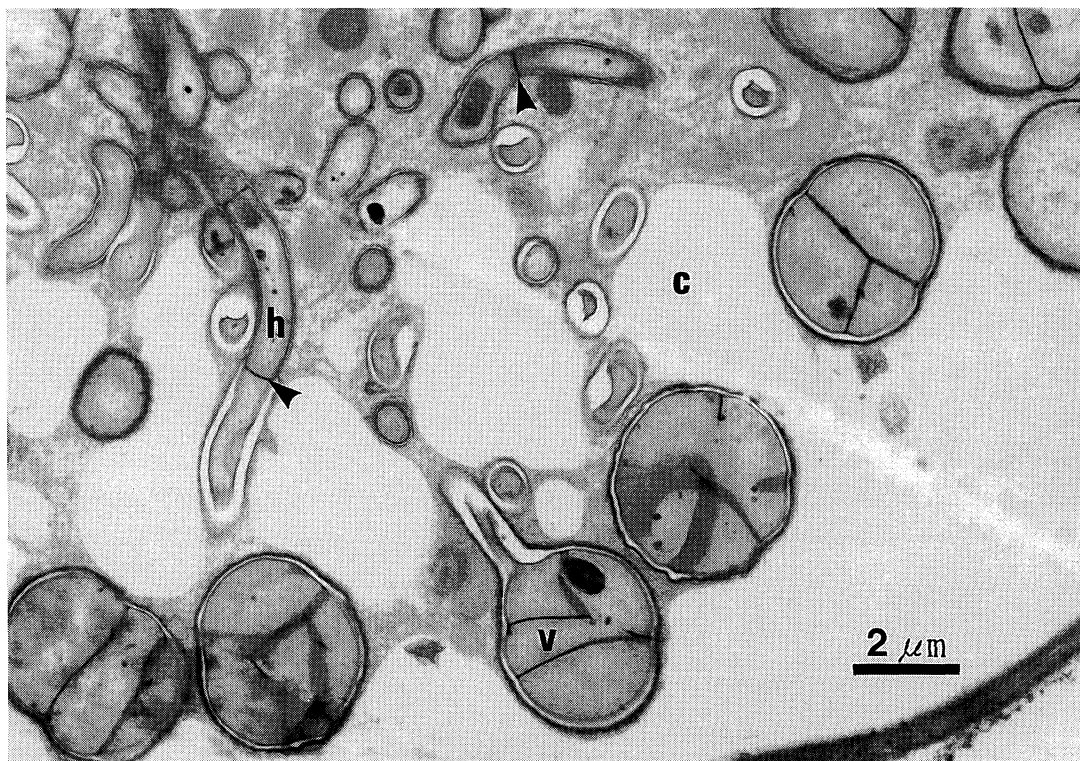


Fig. 5 Transmission electron micrograph of a *Frankia* in root nodule cell of *Alnus hirsuta*. h, hyphae of *Frankia*; v, vesicle; c, cytoplasm of root nodule cell; arrowheads indicate septa in *Frankia* hyphae.

(1987) reported that the actinomycete, *Frankia*, were found in three morphological forms in root nodules of *Alnus*: filamentous hyphae, spherical vesicles and sporangia. Only the vesicles contain nitrogenase and are the sites where fixed nitrogen is transformed to ammonium by *Frankia* cells. Subsequently, the ammonium is diffused to the host plant (Vikman *et al.* 1990). A transmission electron micrograph of an *A. hirsuta* root nodule cells containing *Frankia* hyphae and vesicles is shown in Fig. 5. These vesicles may exhibit high levels of nitrogen fixing activity, since the vesicles do not show irregular septa and flocculent granular cytoplasm in the cells, as were observed under unusual growth conditions as described by Vikman *et al.* (1990).

Analysis of soils

On the two islands, the planted *Alnus* trees were grown for 2 years at p3Y, 3 years at p2Y, 5 years at p1Y, 11 years at p1T and 26 years at p2T. Two effects on soil nitrogen of planting *Alnus* were investigated: first, how much nitrogen was contained in the soil under taproot system as compared to a distant control position in the same plot, and second, whether levels of soil nitrogen was increased in proportion to the age of the *Alnus* planting. For control measurements of the soil condition, near native *Alnus* plants plots, p1S and p1A were selected on the banks of the Furano river near Mt. Tokachidake, an active volcano. The pH values of these soils were strongly acidic, especially pH 3.0, at p1S. *Alnus* plants

were found to form large numbers of root nodules in spite of this acidic soil condition. The concentrations of inorganic salts and metal ions in all the soil samples tested are listed in Table 1. At p1T and p2T on Teuri island, the soil samples contained exchangeable K^+ , Mg^{++} , Ca^{++} in remarkably high concentrations. Mn^{++} content was also high at these two plots (p1T and p2T) and at p2Y on Yagishiri island. The concentrations of inorganic and metal ions were comparable to the satisfactory values for arable lands. Generally, the concentrations of several forms of nitrogen were high under taproot systems when compared to the controls, except for p1T. The concentration of hot water extractable nitrogen in the soil samples from under the taproot system was 4.5 folds higher than levels in control soil from p2T (25 year-old *A. hirsuta* plantation). The average value of hot water extractable nitrogen in arable land in Hokkaido is 6-7 mg per 100 g soil (personal communication, Y. Ichikawa, Tokachi Federation of Agricultural Cooperatives). Therefore, the value of 19.5 mg per 100 g soil at p2T is excellent. In this plot, the contents of NO_3-N and NH_4-N in the soil were higher under taproot systems than in the control soil. The soil specimen from p2T was soft instinctively and many slaters were found living in it. These results suggest that the nutrient poor soil has changed to a richer humic soil during 28 years of *Alnus* plantation.

Natural communities of growing *Alnus* were identified on the infertile soil of plot p1S and p1A, confirming that *Alnus* has the ability to adapt to a wide range of habitats.

Natural abundance of ^{15}N ($\delta^{15}N$) in leaves

Amarger *et al.* (1979) and Shearer and Kohl (1986) measured natural ^{15}N abundance ($\delta^{15}N$) of N_2 -fixing and non- N_2 -fixing plants. Yoneyama *et al.* (1990) also measured $\delta^{15}N$ values from leaves and nodules of many tropical legumes. From the $\delta^{15}N$ values of N_2 -fixing and neighboring non- N_2 -fixing plants, the dependence of symbiotic plants on fixed- N_2 (%Ndfa) was estimated. Table 2 shows the $\delta^{15}N$ values from leaves of *A. hirsuta* and reference plants at plots p1Y and p2T. The %Ndfa of *A. hirsuta* at p1Y and p2T were 94 and 85%, respectively. The difference in %Ndfa from *Alnus* at these two plots may be related to the availability of nitrogen in rhizosphere in these soils.

A relationship between increased soil fertility and presence of *Alnus* plantations is demonstrated by the results. It was observed that *Q. dentata* Thumb. and *P. glehnii* (Fr. Schm.) Mast. have grown satisfactorily among the planted *Alnus*. These restored forests also improved the condition of the dried up headwaters. These results were most likely due to application of the earthwork or hurdle-fence method, that protected the planting stock from cold gales in winter.

Table 2 Natural abundance of ^{15}N ($\delta^{15}\text{N}$) in leaves

Site of sampling	Plant species	Leaf N%	Leaf $\delta^{15}\text{N}$ (‰)	%Ndfa**
Yagishiri Is. (p1Y)	<i>Alnus hirsuta</i> (Spach) Rupr.	2.86	-1.4	94%
	<i>Quercus dentata</i> Thunb. (Ref.*)	1.34	+4.0	
	<i>Salix sachalinensis</i> Fr. Schm. (Ref.*)	1.57	+6.4	
Teuri Is. (p2T)	<i>Alnus hirsuta</i> (Spach) Rupr.	2.29	-0.7	85%
	<i>Salix miyabeana</i> Seem. (Ref.*)	2.74	+5.4	

* Ref. = reference plant.

** The %N derived from atmospheric N_2 (%Ndfa) was calculated with the following equation (Amarger *et al.* 1979), using the $\delta^{15}\text{N}$ values of N_2 -fixing plants ($\delta^{15}\text{Nf}$) and neighboring reference plants ($\delta^{15}\text{Nr}$). When two $\delta^{15}\text{Nr}$ values were available, their averages were used.

$$\% \text{Ndfa} = \frac{\delta^{15}\text{Nr} - \delta^{15}\text{Nf}}{\delta^{15}\text{Nr} - \delta^{15}\text{Na}} \times 100$$

where $\delta^{15}\text{Na}$ is the $\delta^{15}\text{N}$ value of plant N derived from atmospheric N_2 , which is determined by culturing N_2 -fixing plants solely depending on atmospheric N_2 .

The $\delta^{15}\text{Na}$ values for *Alnus hirsuta* and *A. serrulatooides* were -1.8‰ (Domenach *et al.* 1989).

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