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Correlation of greenhouse bioassay with field response to fertilizer by paper birch

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Summary Bulk soil samples were collected from the top 15 cm of untreated areas adjacent to field fertilizer trials at 2 locations. Amounts of N, P, K, and lime equivalent to the field treatments were mixed with the soil in 15-cm diameter pots. Paper birch (*Betula papyrifera* March.) trees were grown from seed for a greenhouse bioassay. Height and dry weight of the bioassay seedlings were significantly correlated with 3-year volume growth of 10-year-old paper birch seedlings in the field. Correlation coefficients were 0.88 for height growth and 0.91 for dry weight growth on one site, and 0.72 and 0.63 on the other. With further refinements and observations on a larger number of sites, this bioassay technique should be a valuable tool for estimating potential response to fertilizer by young paper birch in the field, and for ranking the relative productivity of different soils.

Introduction

Forest scientists have long sought to improve the methods of evaluating fertility of forest sites. The history of soil and vegetation sampling, and of bioassays utilizing 'pot culture' procedures to study the relationship between soil fertility and plant growth, goes back to the 15th century[2]. Some of the classic experiments that formed the basis of modern day plant physiology were essentially pot culture studies.

On the other hand, pot culture in greenhouse or other artificial environments can never deplicate all of the plant growth conditions in the field. Moisture availability, soil temperatures, soil disturbance, level and quality of light, microbiology of the soil, and competition from other vegetation can affect the productivity of a particular soil in pot culture compared with productivity in the field. One prominent forest scientist has said, 'The best thing that could happen in the soil fertility field would be to break all the glass in research greenhouses!' This represents one end of the spectrum of thought on the use of bioassay. The other end is the exclusive use of greenhouse or growth chamber techniques. The most realistic approach to investigating soil fertility lies somewhere between these extremes.

Mitchell[5] was one of the first to employ pot culture techniques with forest trees. He grew conifer seedlings in sand cultures provided with varying levels of N and estimated the 'optimum' supply of N under these conditions. He then grew seedlings of the same species in the soil under investigation. Nutrient solutions, complete except for the element being tested, were added to the soil. After the seedlings were harvested, nutrient content was expressed in both units of concentration (%) and total quantity in the entire plant (mg). Using this technique, he ranked 4 soils with respect to each other and with respect to the 'optimum' levels based on the solution culture.

Mead and Pritchett[4] grew slash pine (*Pinus elliottii* Engelm.) seedlings in soil samples collected from fertilizer trials at 8 locations. They observed significant correlation between 7-year height growth in the field and top weight ($r = 0.66$), total weight ($r = 0.57$), and diameter ($r = 0.56$) of seedlings grown in the greenhouse for 8 months in pots treated with fertilizer equivalent to the field treatments. Mead and Pritchett[4] concluded that pot culture could not be used to predict potential response of slash pine to fertilizer in field trials. However, they believed that pot culture might be successful in predicting the element or elements that might be limiting growth. This would reduce the number of treatments that require field testing.

I used a greenhouse bioassay technique with paper birch (*Betula papyrifera* Marsh.) seedlings to evaluate soil fertility from 2 field fertilizer trials with paper birch. There was good correlation between greenhouse and field as to which treatments produced the greatest response.

Methods

Field

Two seven-year-old seedling stands that regenerated following clearcutting and site scarification were chosen for study. One is on the Massabesic Experimental Forest in southwestern Maine. The soil is a well-drained to somewhat excessively drained sandy loam of the Hermon series (loamy-skeletal, mixed, frigid typic Haplorthod). In addition to paper and gray birch (*B. populifolia* Marsh.) seedlings, the stand contained aspen (*Populus tremuloides* Michx. and *P. grandidentata* Michx.) seedlings, and red maple (*Acer rubrum* L.) and northern red oak and white oak (*Quercus rubra* L. and *Q. alba* L.) stump sprouts. The second stand is on the Bartlett Experimental Forest in central New Hampshire. The soil is a well-drained to moderately well-drained fine sandy loam complex of the Berkshire (coarse-loamy, mixed frigid typic Haplorthod) and Peru (coarse-loamy, mixed, frigid aquic Fragiorthod) series. In addition to paper and yellow birch (*Betula alleghaniensis* Britton), pin cherry (*Prunus pensylvanica* L.) and *Rubus* sp. seedlings were abundant; there were also some red maple stump sprouts and American beech (*Fagus grandifolia* Ehrh.) root suckers.

At both locations, paper birch crop trees were selected on about a 2 × 2 m spacing and all other trees and woody shrubs were mowed down with brush saws. Mowing was repeated during the second year. After 1 year of release, the following fertilizer and lime treatments were applied to 10 × 10 m plots in a randomized complete block design, with 5 replications at Massabesic and 3 at Bartlett:

Control: All trees except selected crop trees cut, no fertilizer, no lime.
N: Thin plus 400 kg/ha nitrogen as ammonium nitrate. Half applied in late May, the remainder in late July.
P: Thin plus 200 kg/ha phosphorus as triple superphosphate. Applied in late May.
NP: Thin plus combination of N and P above.
NPK: NP as above plus 100 kg/ha potassium from muriate of potash (KCl). Applied in late May.
Lime: Each of the above plus 3.6 T/ha dolomitic limestone. Applied in October prior to fertilizer application.

Height and dbh (diameter at 1.37 m) were measured on 10 centrally located sample trees on each plot. Basal area, height, and volume growth were calculated for each tree. Mean values for each plot were analyzed by analysis of variance. Each fertilizer treatment was compared to the control by Dunnett's least significant difference[6].

Greenhouse

In late September, after heavy rainfall increased soil moisture to field capacity, bulk samples of the top 15 cm of soil (including organic horizons) were collected from unfertilized portions of the stands adjacent to the 2 fertilizer trials. Soil was collected from several locations adjacent to each block in the field. These samples became 'blocks' in the greenhouse, one for each field block. After mixing and sieving through a 1.2-cm mesh screen to remove large stones and roots, an equal quantity (1.5 kg) of moist soil was weighed into 14.5-cm diameter plastic 'azalea type' pots. Surface organic matter and fine roots that passed the screen were left in the soil. Fertilizer equivalent to the field rates on the basis of the midpoint area (12 cm diameter) of the pots ($.1184 \times 10^{-5}$ ha) was mixed into the soil. Several paper birch seed were placed on the soil in the central portion of each pot. The soil was mulched with a 0.5 cm layer of granulated clay (oilsorb) to reduce washing of seedlings during watering. Each pot was placed in a pot saucer to collect any water and nutrients that ran through during watering. The final weight of the pots was recorded so that they could be watered as needed to keep moisture at about field capacity.

Seeds germinated within 8 to 10 days and seedlings were thinned first to the largest 4 to 5 per plot after 3 weeks; then to the 2 largest seedlings after 6 weeks; and finally to the single most vigorous seedling for the remainder of the study. Pots were watered weekly for the first few weeks, and more frequently near the end of the experiment when the seedlings were large and transpired larger amounts of water. Natural day length was extended to 18 h with cool white fluorescent lamps. The temperature was 20–24°C. Tree height was measured weekly, and the experiment was terminated when the height growth of the fastest growing treatments slowed, indicating that the capacity of the soil had been exhausted. Seedlings were harvested and the weight of stems, branches, foliage, and roots were measured and recorded after drying at 70°C.

The same procedures were followed the next year with soil samples collected from the untreated stand adjacent to the fertilizer plots at the Bartlett study location. The greenhouse temperature was slightly cooler, 15–18°C, because of fuel economy measures, so it took longer for seedlings to reach maximum height. However, the decision to terminate the study was based on the same height growth reduction that occurred in the first study.

Bioassay calculations

Analysis of variance and Dunnett's least significant difference[6] were used to compare mean height and seedling dry weight from each fertilizer treatment with control. Treatment means for both field and greenhouse experiments were expressed as a ratio with control, *i.e.*, control = 1.0 in all cases. Least squares regression equations were calculated in the form $Y = bo + bl\,x$ where Y = relative volume growth in the field, and x = either relative height or relative total dry weight in the greenhouse for each location.

Results

Field

At both field locations, paper birch responded significantly to N, NP, and NPK combinations (Table 1). The trees in the best treatment at Massabesic, NL, grew about 3 times as much as the control trees. In the best treatment at Bartlett, NP, trees grew more than 2 times as much as control. Lime gave nonsignificant and inconsistent responses at both locations.

Table 1. Average 3-year relative volume growth of fertilized and limed paper birch trees at 2 field locations

Treatment	Volume growth (cm^3/100 cm^3/tree)	
	Massabesic	Bartlett
Control	668	450
Lime	845	412
P	626	501
P Lime	772	489
N	1436*	582*
N Lime	1943*	544*
NP	1772*	919*
NP Lime	1137*	658*
NPK	1886*	706*
NPK Lime	1714*	779*
Dunnett's LSD	239	92

* Significantly greater than control ($P = .05$) by Dunnett's least significant difference[6].

Table 2. Average height and weight of paper birch seedlings grown in soil from 2 locations treated with N, P, and lime

Treatment	Massabesic		Bartlett	
	Height (cm)	Weight (g)	Height (cm)	Weight (g)
Control	16	3.0	27	4.7
Lime	18	3.2	28	5.5
P	23	4.0	24	4.9
P Lime	26	4.7	29	8.4
N	39*	9.6	28	4.6
N Lime	33*	5.2	21	3.0
NP	88*	38.2*	73*	30.6*
NP Lime	70*	30.4*	66*	18.8*
NPK	81*	34.9*	67*	27.6*
NPK Lime	81*	28.3*	76*	27.4*
Dunnett's LSD	11	7.9	14	7.7

* Significantly greater than control ($P = .05$) by Dunnett's least significant difference[6].

Greenhouse

Paper birch seedlings in pot culture treatments that included both N and P were significantly taller and heavier than the controls in soils from both locations (Table 2). Seedlings reached maximum heights in about 20 weeks in the Massabesic soil, whereas it took 26 weeks to reach maximum height growth in Bartlett soil. This slower growth was primarily at the start of the experiment and probably was due to the cooler greenhouse temperatures.

In the soil from Massabesic, trees treated with N plus P were, on average, 5 times taller and 11 times heavier than control trees. Height also was significantly increased by the N and N Lime treatments on this soil. In the soil from Bartlett, trees treated with N plus P were, on average, about 3 times taller and about 6 times heavier than control trees (Table 2).

Bioassay

The regressions of normalized field growth on normalized pot culture height and weight data were highly significant (Figs. 1–4). Each equation failed to reject the null hypothesis that there was no relationship between response to fertilizer treatments in the field and in greenhouse pot culture.

Of 36 possible comparisons between field and pot culture response to fertilizer, only 5 were not in agreement (Table 3). Each of these responded to N in the field but not in the pots. Height growth in pots of the soil from Bartlett gave the only significant response to N while field height growth responded at both locations.

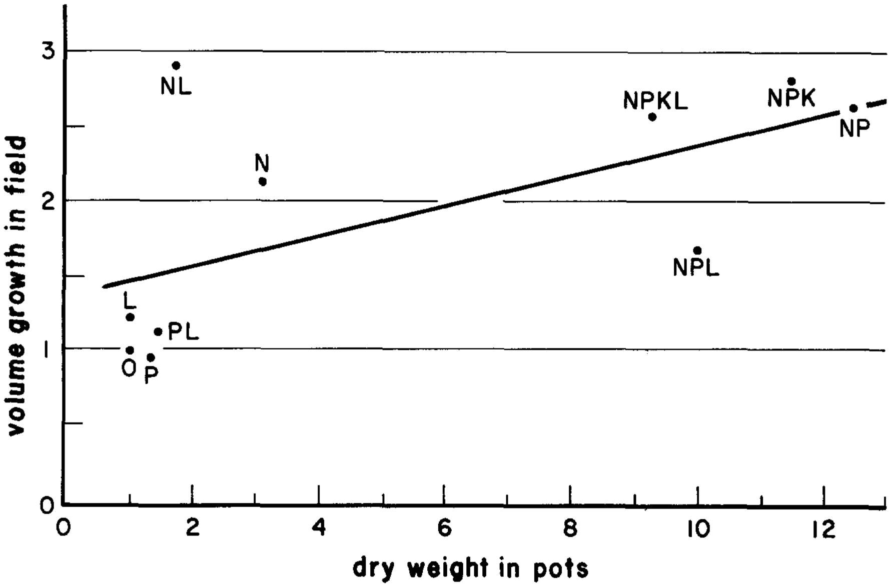

Fig. 1. Normalized 3-year volume growth of paper birch in the field as a function of total dry weight in pots. Y = 137.02 + .1034X. r = 0.63* (Massabesic soil. Units × 100)

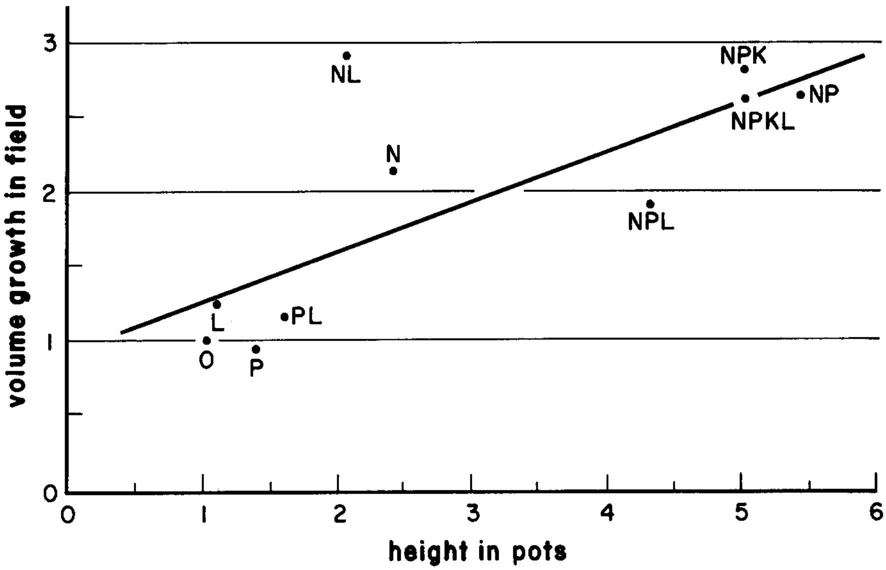

Fig. 2. Normalized 3-year volume growth of paper birch in the field as a function of total height in pots. Y = 97.585 + .3219X. r = 0.72* (Massabesic soil. Units × 100)

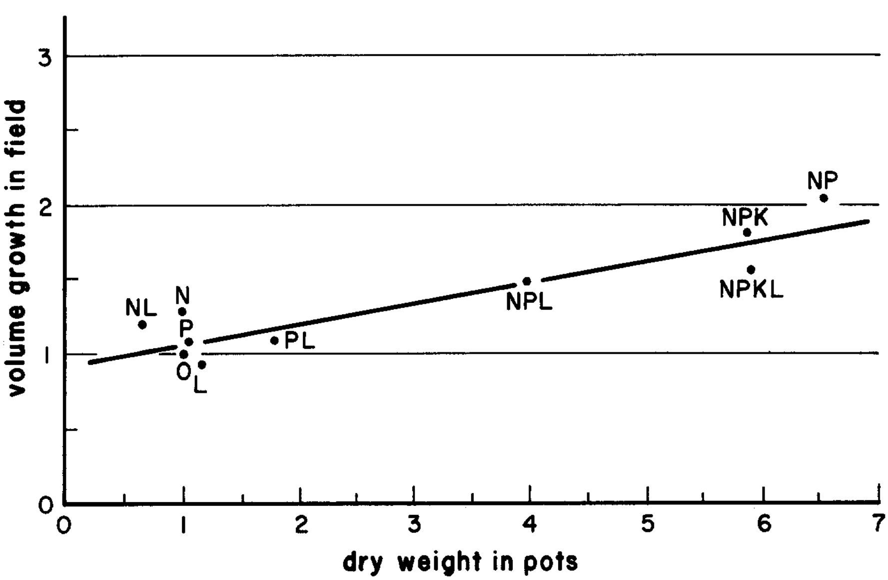

Fig. 3. Normalized 3-year volume growth of paper birch in the field as a function of total dry weight in pots. Y = 95.48 + .1351X. r = 0.91** (Bartlett soil. Units × 100)

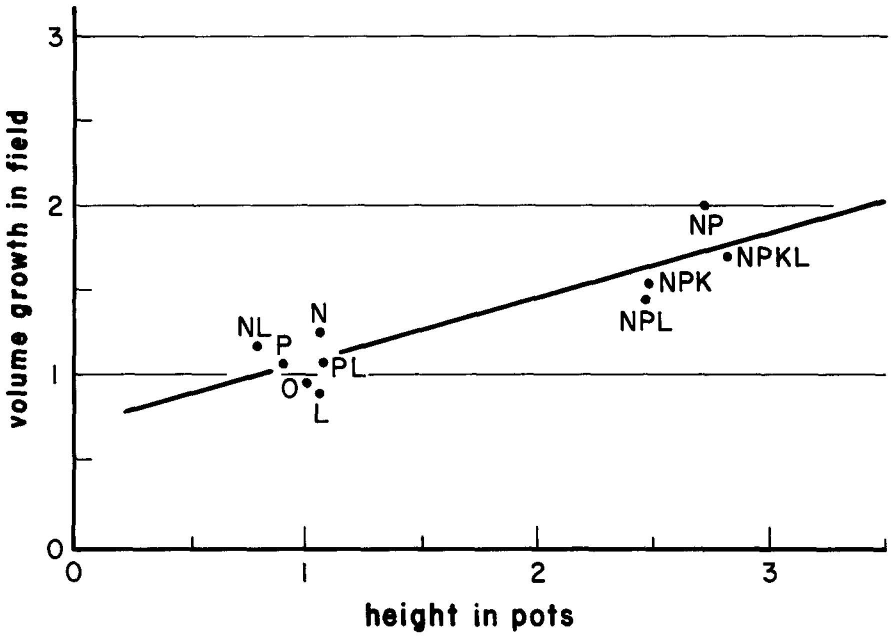

Fig. 4. Normalized 3-year volume growth of paper birch in the field as a function of total height in pots. Y = 74.69 + .3662X. r = 0.88** (Bartlett soil. Units × 100)

Table 3. Comparison of significant effects of fertilizer treatment between height and dry weight of paper birch in pot culture and volume growth in the field

Treatment	Massabesic		Bartlett	
	Height	Weight	Height	Weight
Control	–	–	–	–
Lime	o	o	o	o
P	o	o	o	o
P Lime	o	o	o	o
N	o	X	+	+
N Lime	o	+	+	+
NP	o	o	o	o
NP Lime	o	o	o	o
NPK	o	o	o	o
NPK Lime	o	o	o	o

o both significant or not significant; X pot significant, field not significant; + pot not significant, field significant.

Discussion

These results suggest that this bioassay technique may be highly useful in evaluating the potential for paper birch to respond to fertilizer treatments under field conditions on some soils. In these two tests, the method appears to be stronger qualitatively than quantitatively; that is, the technique indicates if a response will be attained, and the nutrient or combination of nutrients that have the greatest potential for response. However, the low coefficients of determination (40 to 83%) and large standard errors weaken quantitative prediction of response in the field. Also, the magnitude of response was much greater in the greenhouse than in the field, creating the possibility of overestimating the magnitude of field response.

When reanalyzed by the methods in this study, the correlation reported for pot *versus* field growth of slash pine[4] is more favorable. The correlations are significant for all but the two instances where there was a significant response in the pots but not in the field (Table 4). Considering all of the uncontrolled variables and generally small field responses obtained with slash pine, I feel that this bioassay was moderately successful. Also, since conifers are inherently well adapted to low fertility levels, and may not respond strongly to higher levels[7], correlation of greenhouse data with field data indicates that this bioassay procedure is quite sensitive.

Some hardwood species, on the other hand, tend to be very sensitive to fertility. Paper birch is one of these species. I have grown paper birch trees to a height of 5 m and 2.5 cm dbh in 9 months in the greenhouse by supplying high levels of moisture and nutrients. Thus, it appears that this bioassay procedure can be applied successfully to species with a wide range of fertility requirements.

Table 4. Correlation of normalized height of slash pine after 3 years in the field with normalized top dry weight in pots (Data from Mead and Pritchett 1971)

Soil	r	Significant response to treatment in:	
		Pots	Field
Myakka 1	.984**	Yes	Yes
Myakka 2	−.373	Yes	No
Leon	.958**	Yes	Yes
Pomello	.978**	Yes	Yes
Kershaw 1	.936*	Yes	Yes
Kershaw 2	.731	Yes	No
Rutledge	.939*	Yes	Yes
Bladon	.926*	Yes	Yes
All (n = 32)	.710***	–	–

Significant at *P*.01***, *P*.05**, *P*.10*.

Yellow-poplar (*Liriodendron tulipifera* L.) is another nutrient-sensitive hardwood species. In a field trial with yellow-poplar, Carmean *et al.*[1] showed that prior tree cover – and presumably the fertility level associated with it – greatly influenced height of trees 16 years after planting. When I treated the Carmean *et al.*[1] data with this bioassay technique, there was a very strong correlation (r = 0.93) between the 16-year height and the fresh weight of seedlings grown for 12 weeks in pot culture (Fig. 5). Thus, 16 years of field response could have been predicted after a bioassay of only 12 weeks.

Before this bioassay can be strongly advocated, additional trials will be needed to standardize methods for determining soil quantity, for controlling water, temperature, and light, and for achieving genetic uniformity of seedlings. The 'normalization' procedure I have used here to express response to treatment versus control assumes there is no interaction between uncontrolled site variables and treatment response when comparing responses between sites. A primary standard is required as a basis for comparing the variation in performance among soils as well as among fertilizer treatments within each soil. Mitchell[5] used sand culture to which he added a defined nutrient solution as a 'standard soil'. Ingestad[3] expanded this concept by adding nutrients in logarithmically increasing quantities to match logarithmic plant growth in an automated solution culture system. In either case, the 'optimum' or maximum

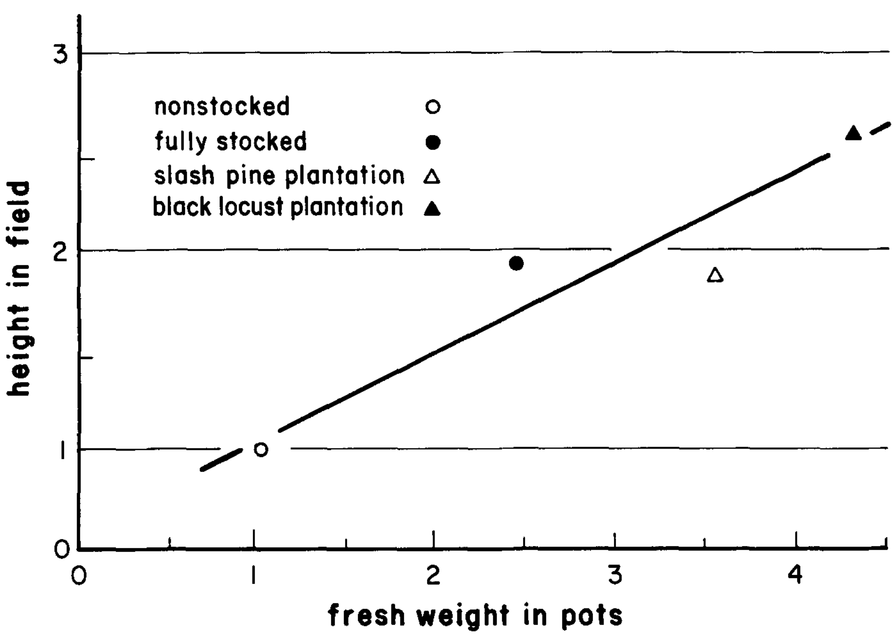

Fig. 5. Normalized height of yellow poplar at age 16 in the field as a function of fresh weight in pots. Y = 31.045 + .845X. r = 0.93* (From Carmean *et al.*[1] Units × 100)

growth potential of paper birch must be defined to allow comparison against all other levels of productivity.

It is interesting to note that height of seedlings in pots had a stronger correlation with field volume growth at Massabesic than did dry weight (Figs. 1, 2, Table 3). In the field, height is assumed to be relatively free from influence by nonsite properties such as stocking level and species composition. This also may be true for seedlings in pots. Just as height at a specified age determines site index in the field, height of seedlings in pots may provide an easily observed index of potential productivity and response to increased soil fertility.

I believe that these *preliminary* studies justify continued developed of the use of bioassay for evaluating potential response to fertilizer treatments. I also feel that there is a possibility that paper birch can be used as an indicator species to rate soils for inherent productivity.

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