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Field Tests of Graft Compatible Douglas-fir Seedling Rootstocks

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Summary

Rootstocks from control- and wind-pollinated progeny of highly compatible Douglas-fir parents averaged 90.9 percent and 76.4 percent graft compatibility, respectively, after 2-3 years; unselected, local trees averaged 64.8 percent. Rootstocks were tested at six orchards from Lake Cowichan, B. C., to Chico, California. No significant orchard by rootstock interactions were found although the orchard by standard-scion clone interaction was significant. Results demonstrate the practicality of establishing a regional program for rootstock breeding to produce compatible rootstocks for general use in seed orchards.

Key words: Incompatibility, compatibility, graft, propagation, stock, seed orchards, rootstock, Douglas-fir, seedling, pollination.

Zusammenfassung

Mit 2-3jährigen Pfropfunterlagen aus kontrolliert bestäubten Nachkommenschaften von *Pseudotsuga menziesii* (Mirb.) Franco und frei abgeblühten Nachkommenschaften von in hohem Maße pfropfverträglichen Eltern wurde ein Anwachsprozent von im Durchschnitt 90,9% erreicht, mit Pfropfunterlagen aus einer frei abgeblühten Nachkommenschaft nur 76,4%, mit unselektierten lokalen Pfropfunterlagen nur im Durchschnitt 64,8%. Die Pfropfunterlagen wurden später in sechs Samenplantagen von Lake Cowichan, in British Columbia, bis Chico, California getestet. Es wurden keine signifikanten Interaktionen zwischen den Pfropfunterlagen und Plantagen gefunden, obwohl eine solche zwischen den Standard-Pfropfklonen und den Plantagen signifikant war. Die Ergebnisse zeigen, daß ein regionales Programm zur Erstellung pfropfverträglicher Sämlingsnachkommenschaften zweckmäßig ist.

Introduction

A breeding program for production of graft-compatible Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) rootstocks was begun after strong additive inheritance was demonstrated for graft compatibility (Copes, 1974). Trees grown from seed of pollinations between highly compatible parents should thus yield rootstocks that would be highly graft compatible with most scion clones. Whether seedling rootstocks would be able to overcome the incompatibility factors of severly incompatible scion clones is not known (Copes, 1974). Extensive field tests of select rootstock progeny would be needed to resolve this uncertainty.

Another problem that has hindered general use of compatible seedling rootstocks has been the varied environments to which rootstocks must be adapted. Environmental effects on graft compatibility have not been evaluated in forest trees. Indirect evidence of graft / environment interactions could arise from comparison of incompatibility in orchards that contained the same clones, yet had very different incompatibility environments. In radiata pine seed orchards containing identical clones, incompatibility seemed to be most severe on sites subject to greatest stress (Pederick and Brown, 1976). Results of research on fruit trees provide further insight. Moisture induced environmental interactions were reported in grafts of lemons (Kirkpatrick and Bitters, 1965) and soil induced interactions were reported in grafts of apricots (Carlson, 1965).

My study was designed to determine suitability of using rootstocks resulting from controlled crosses in production seed orchards and to evaluate the effect of different environments on rootstock compatibility. Nearly 2800 control- and wind-pollinated seedlings from three highly compatible parent trees and 600 local seedlings were used as rootstocks. These were graft-tested at six seed orchard sites ranging from British Columbia to northern California, a range of 9° latitude. Rootstocks were tested at all orchards with a group of five "standard-scion" clones and with ten "local-scion" clones.

Methods

Seeds for rootstocks were obtained in 1969 from pollinations among three highly graft-compatible trees. Parent

selection for pollination was based solely upon their having superior ramet survival and few external symptoms of graft incompatibility after 8 to 11 years of growth. The actual internal compatibility status of the parents was not determined by anatomical test. Each clone was selected by an orchardist as the most highly compatible clone in his orchard. Selected clones were No. 17 from the Dennie Ahl Seed Orchard (USDA Forest Service), Shelton, Washington; No. 165 from the David T. Mason Seed Orchard (Barringer and Associates), Sweet Home, Oregon; and No. 65 from the Row River Seed Orchard (Georgia-Pacific Corporation), Cottage Grove, Oregon. Seed collections included three wind-pollinated families (17 × W, 165 × W, and 65 × W) and three full sib families (17 × 65, 17 × 165, and 165 × 65).

In 1972 and 1974, seeds from the six families were germinated, and the resulting seedlings were grown in a greenhouse at Corvallis, Oregon. After the first growing season, they planted in an outdoor transplant bed for an additional 1 to 3 years.

In 1976, the 3- to 5-year-old trees were dug and planted at six orchard sites. Location and latitude of each orchard were as follows: Lake Cowichan, British Columbia (48° 49'); Sequim, Washington (48° 05'); Shelton, Washington (47° 20'); Monmouth, Oregon (44° 50'); Cottage Grove, Oregon (43° 45'); and Chico, California (39° 30'). At each site, 465 seedlings of the full-sib and wind-pollinated families and 100 local rootstock trees were planted [at each site]. The local rootstocks--the usual type of unselected seedlings used at each specified orchard--served as controls. Dead trees were replaced in 1977.

Trees at each orchard were planted in two completely randomized blocks of 283 rootstocks. Spacing between trees and rows was 0.9 × 1.2 m. Technicians from most orchards tended the trees with their standard cultural techniques for weed control, fertilization, and irrigation.

Grafting took place in March and April of 1977 and 1978. Tip-cleft grafts were placed on the terminal leader and on lateral branch tips of each tree. As many as eight individual scion clones were grafted on individual rootstocks. Two types of scion clones were grafted: standard and local. The standard clones came from five trees growing near Corvallis, Oregon. This same group of five clones was grafted at all six orchard sites and was used specifically to evaluate effects of varied environment. At each orchard, ten scions from each of the five clones were grafted on all six rootstock families (10 × 5 × 6).

Local scion groups consisted of ten clones selected at random from each orchard. They were grafted on the same six rootstock families as were the standard scion clones, but also on the local rootstocks. Their primary purpose was to test average compatibility or general suitability of the six rootstock families for local use at each orchard site. Local-scion clones were grafted only in the orchard where they originated. Ten scions of each of the ten local clones were grafted on the seven types of rootstocks (10 × 10 × 7) at each orchard. Thus, about 6000 grafts were made (about 300 standard-scion plus 700 local-scion grafts at each of the six orchards).

In 1978 and 1979, all 2- and 3-year-old graft unions were cut from the rootstocks and preserved in 50 percent ethanol. The numbers of grafts from each orchard that were examined in the laboratory ranged from 613 and 671 from Cottage Grove and Chico, respectively, to 952 from Lake Cowichan. Smaller samples were collected in Cottage Grove and Chico because of poorer tree survival after planting in 1976. The preserved unions were microtomed into 20- to 25- μm thick transverse sections, stained with safranin 0 and fast green FCF, and examined under a light microscope at 20-100 X. Microtechnique procedures were described by Gnose and Copes (1975). Anatomical criteria for determining compatibility or incompatibility of each graft union were reported by Copes (1970).

Compatibility results were evaluated by analysis of variance after arcsin transformation of percentage data.

Results

Rootstocks grown from both wind- and control-pollinated seed of three highly compatible parents were more compatible than those grown from seed of unselected parents *(Tables 1 and 2)*. Average compatibility of 2132 grafts made on control-pollinated rootstocks was 90.1 percent, but 2279 grafts made on wind-pollinated rootstocks of the

Table 1. — Average compatibility and sample size for seven rootstock types graft-tested with a different group of ten local-scion clones at each orchard.

Orchard	Rootstocks									
	Local controls	Wind-pollinated				Control-pollinated				Wind- and control-pollinated
		165 × W	65 × W	17 × W	$\bar{x}$ / n	165 × 65	17 × 165	17 × 65	$\bar{x}$ / n	$\bar{x}$ / n
	(percent / n)									
Lake Cowichan, BC	62.6 / 99	65.0 / 146	73 5 / 102	65.9 / 88	68.3 / 287	96.0 / 99	84.8 / 92	94.6 / 99	93.0 / 290	80.2 / 577
Sequim, WA	60.9 / 87	75.5 / 98	78.3 / 92	84.4 / 90	79.3 / 280	88.5 / 78	89.1 / 92	100.0 / 98	92.9 / 268	86.0 / 548
Shelton, WA	67.1 / 76	78.6 / 84	77.4 / 84	75.0 / 84	77.0 / 252	92.1 / 88	79.2 / 76	85.9 / 78	86.1 / 242	81.4 / 494
Monmouth, OR	67.8 / 87	77.7 / 94	89.3 / 84	79.5 / 83	82.0 / 261	98.0 / 99	86.8 / 91	96.7 / 90	93.9 / 280	88.9 / 542
Cottage Grove, OR	62.1 / 66	77.4 / 62	65.6 / 61	50.0 / 74	63.5 / 197	80.0 / 50	75.0 / 48	88.2 / 51	81.2 / 149	73.1 / 346
Chico, CA	70.4 / 54	79.1 / 110	80.9 / 84	77.2 / 57	79.3 / 251	91.2 / 113	92.4 / 92	92.9 / 28	91.9 / 233	85.4 / 484
$\bar{x}$ / n	64.8 / 469	75.4 / 545	77.9 / 507	72.3 / 476	75.3 / 1528	92.1 / 527	85.6 / 491	93.9 / 444	90.6 / 1462	84.3 / 489

Table 2. — Average compatibility and sample size for seven rootstock types graft-tested at six orchards with the five standard-scion clones.

Orchard	Rootstocks								
	Wind-pollinated				Control-pollinated				Wind- and control-pollinated
	165 x W	65 x W	17 x W	X / n	165 x 65	17 x 165	17 x 65	X / n	X / n
	(percent / n)								
Lake Cowichan, BC	79.6 / 49	78.3 / 46	74.5 / 47	77.5 / 142	93.8 / 48	92.9 / 42	93.2 / 44	91.9 / 134	85.2 / 276
Sequim, WA	69.8 / 53	79.6 / 44	75.6 / 41	74.6 / 138	89.6 / 48	88.4 / 43	90.9 / 44	89.6 / 135	82.1 / 273
Shelton, WA	74.4 / 43	77.8 / 45	76.1 / 46	76.1 / 134	90.2 / 41	77.5 / 40	89.6 / 48	86.0 / 129	80.9 / 263
Monmouth, OR	76.0 / 36	91.5 / 47	76.6 / 47	81.8 / 130	96.0 / 50	93.5 / 46	98.0 / 49	95.9 / 145	89.2 / 275
Cottage Grove, OR	83.3 / 36	85.7 / 42	80.0 / 35	83.2 / 113	96.3 / 27	91.2 / 34	96.3 / 27	94.3 / 88	88.1 / 201
Chico, CA	79.3 / 53	81.3 / 32	77.8 / 9	79.8 / 94	94.1 / 17	80.0 / 20	100.0 / 2	87.2 / 39	82.0 / 133
X / n	76.8 / 270	82.5 / 256	76.9 / 225	78.6 / 751	93.1 / 231	88.0 / 225	93.5 / 214	91.5 / 670	84.7 / 1421

same clones averaged 76.4 percent. Unselected local rootstocks averaged only 64.8 percent.

Average rootstock compatibility was approximately the same for grafts tested with either the five standard- or the 60 local-scion clones (84.7 and 84.3 percent). The control-pollinated families averaged 91.5 and 90.6 percent with local- and standard-scion clones, respectively; corresponding values for wind-pollinated families were 78.6 and 75.3 percent *(Tables 1 and 2)*. Variation between orchards for each type of rootstocks ranged from 0.1 to 33.2 percent.

Analysis of variance of local-scion clone grafts showed control- and wind-pollinated rootstock families from highly graft-compatible parents to be significantly more compatible at four of of six orchards than were the unselected, local rootstocks *(Table 3)*. Results obtained at Cottage Grove were not significant because compatibility of the wind-pollinated rootstock families was atypically low *(Table 1)*. At Chico, significance was not detected even though the wind- and control-pollinated family results had higher than average values for this study. Lack of significance occurred because of the unusually high compatibility values of the local rootstocks (70.4 percent).

Control-pollinated rootstocks tested with local scion clones were significantly more compatible than wind-pollinated rootstocks at five of six orchards *(Table 1)*. Relatively uniform compatibility values were obtained at each orchard from the three wind- or the three control-pollinated rootstock families. This was indicated by the lack of significant variation between the three wind- or three control-pollinated families *(Table 3)*. Evidence that a random collection of compatible and incompatible local-scion clones had been grafted at each orchard was shown by significance for clones at each orchard.

Rootstock families tested with the same five standard-scion clones at all six orchards also showed the control-pollinated trees to be significantly more compatible than wind-pollinated trees of the same parents *(Table 4, Fig. 1)*. Significant variation was found for orchards and for scion

Table 3. — Analysis of variance of six local scion groups (one group of ten from each orchard) graft tested on three control- and three wind-pollinated rootstock families and unselected local rootstocks.

Source of variation	DF	Lake Cowichan BC		Sequim, WA		Shelton, WA		Monmouth, OR		Cottage Grove, OR		Chico, CA	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Residual	54	.0537		.0331		.0741		.0297		.0685		.0715	
Local-scion clones	9	.4644	8.65**	.0545	1.65	.1920	2.59**	.2174	7.33**	.4366	6.38**	.1488	2.08*
Rootstocks	(6)												
Wind- and control-pollinated versus local rootstocks	1	.5002	9.31**	1.4595	44.11**	.5021	6.77**	.8964	30.20**	.1649	2.41	.1734	2.43
Control-pollinated versus wind-pollinated	1	2.1545	40.11**	.8072	24.40**	.2082	2.81	1.3478	45.20**	.4038	5.90*	.5431	7.60**
Among wind-pollinated	2	.0631	1.18	.0777	2.35	.0035	.05	.0393	1.32	.1276	1.86	.0212	.30
Among control-pollinated	2	.1104	2.06	.2007	6.06**	.0989	1.33	.0061	.21	.0525	.77	.1382	1.93
	69												

* Significant at .05 level.
** Significant at .01 level.

Table 4. — Analysis of variance of five standard scion clones graft tested at all six orchard sites on three control- and three wind-pollinated rootstock families.

Source of variation	D.f.	Mean square	F values
Residual	100	.0377	--
Orchards (O)	5	.1575	4.17**
Standard-scion clones (SC)	4	.6926	18.36**
Rootstocks	(5)		
Control-pollinated (CP) vs. wind-pollinated (WP)	1	1.6756	44.41**
(17x65 and 17x165) vs. 165x65	1	.0565	1.51
17x65 vs. 17x165	1	.0833	2.21
Between WP	2	.0834	2.21
Orchard x Standard-scion clones	20	.0705	1.87*
Orchard x rootstocks	(25)		
O x (CP vs. WP)	5	.0398	1.05
O x (17x65 and 17x165 vs. 165x65)	5	.0127	.34
O x (17x65 vs. 17x165)	5	.0274	.73
O x WP	10	.0298	.79
Standard scion clones x rootstocks	(20)		
SC x (CP vs. WP)	4	.1589	4.21**
SC x (17x65) and 17x165 vs. 165x65)	4	.0207	.55
SC x (17x65 vs. 17x165)	4	.0094	.25
SC x WP	8	.1041	2.76**
	179		

* Significant at .05 level.
** Significant at .01 level.

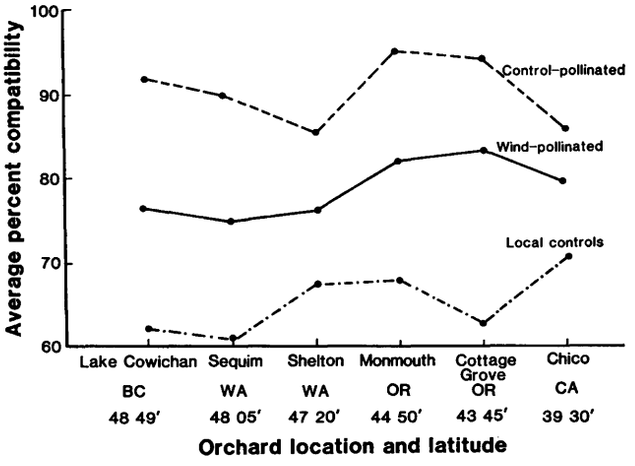

Figure 1. — Average percent compatibility of control- and wind-pollinated rootstock families tested at six orchards with five standard-scion clones. Local-control rootstocks were not tested with standard-scion clones.

clones *(Table 4)*. Average orchard compatibility for standard-scion clones grafted on wind- and control-pollinated rootstocks varied from 80.9 percent at Shelton, Washington to 89.2 percent at Monmouth, Oregon *(Table 2)*. Compatibilities of the five standard-scion clones tested at all six orchards on the control-pollinated and wind-pollinated rootstock families varied from 68.1 to 91.4 percent.

Some influence of environment on graft compatibility was shown by the significant orchard by standard-scion clone interaction, but no significant orchard by rootstock effect was found. Differences in compatibility among standard-scion clones depended somewhat upon the orchard *(Fig. 2)*. Evidence of within orchard interaction can be found in the response of standard-scion clones with control versus wind-pollinated rootstocks where differences depended upon which standard-scion clone was grafted *(Table 4)*.

Performance of rootstock families at each orchard revealed some families to be more susceptible to environmental modification, even though the orchard-rootstock comparison was not statistically significant *(Table 4)*. Rootstocks of 17 × 165 parentage were subject to greater fluctuations in average compatibility between orchards than were 17 ×65 or 165 × 65 rootstocks. Likewise, average compatibilities of 65 × W rootstocks varied more between orchards than did 165 × W or 17 × W rootstocks *(Fig. 3)*. Similar variability was exhibited by the most incompatible standard scion clone, S - 1 *(Fig. 2)*.

The six local rootstock populations demonstrated little variation in overall average compatibility even though a different rootstock population was grafted with ten different local-scion clones at each orchard. The shortcoming of using unselected, local rootstocks in orchards was indicated by their low average compatibility, ranging between 60.9 percent and 70.4 percent *(Table 1 and Figs. 1, 3)*.

Discussion

The results demonstrate the practicality of breeding rootstocks for general seed orchard use. This was first suggested in 1974 when graft incompatibility was found to be a highly inherited trait (Copes, 1974). In the present study, grafts made on control-pollinated rootstocks averaged 25.3 percent more compatible than grafts made on local, unselected rootstocks. In similar manner, wind-

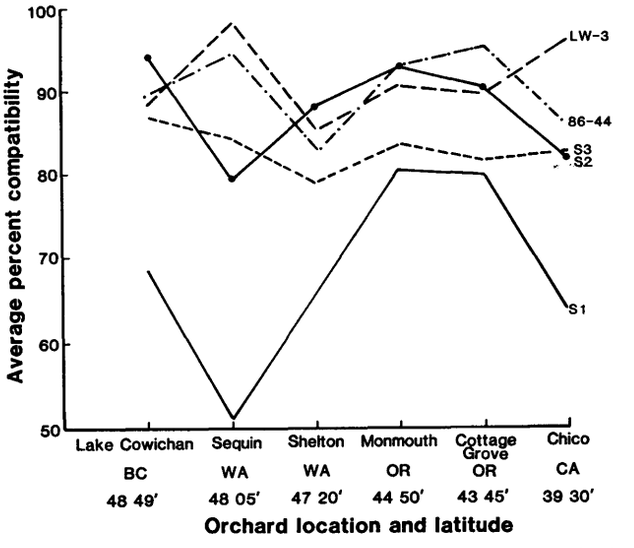

Figure 2. — Average percent compatibility of the five standard-scion clones tested at six orchards on three control- and three wind-pollinated rootstock families. No local rootstocks were evaluated.

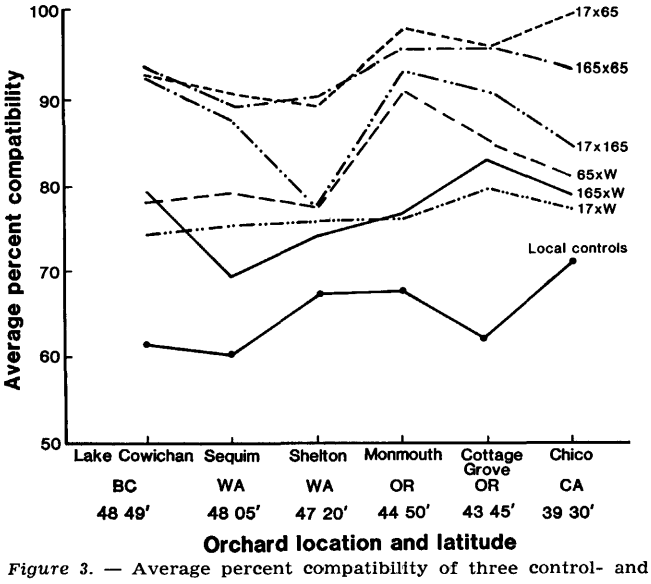

Figure 3. — Average percent compatibility of three control- and three wind-pollinated rootstock families tested at six orchards with five standard-scion clones. Local control rootstocks were tested with local scion clones, not with standard-scion clones.

pollinated rootstocks were 11.6 percent more compatible than the local, unselected rootstocks. Extensive use of wind-pollinated rootstocks is not suggested as a practical alternative to using control-pollinated families, because benefits from higher compatibility by control-pollinated rootstocks far exceed additional costs of producing such seed.

The three control-pollinated families demonstrated high average compatibility when tested over an area covering more than 9° latitude. No significant environmental interactions were found with rootstocks. Rootstocks that will retain high compatibility over a wide geographic area can be bred. Thus, breeding rootstocks for small local areas in the coastal Douglas-fir zone should not be necessary. Testing of new families can be done in one convenient area and results extrapolated to other areas. This should make development of additional rootstock families less expensive and time consuming.

Movement of rootstocks great distances from the indigenous locations of the parents must be looked upon with caution, but survival and growth of study trees for 2-3 years after grafting, indicate no major adaptive problems. The trees grew satisfactorily, and internal examinations of tracheids in rootstock and scion areas of graft unions revealed no increase in the incidence of frost damage. Seedlings from the cross between the two Oregon parent trees (165 × 65) burst vegetative buds at the Shelton, Washington, orchard 2-3 weeks before the 17 × 65 and 17 × 165 seedlings. Atypical early growth might be subject to spring frost damage, but only more extensive field tests will reveal if this is indeed a major problem.

Detection of significant environmental interaction for standard-scion clones was not unexpected because great differences existed in climate, soil and cultural treatment. If environment had no effect, clonal averages at each orchard should have been about the same, but they were not. Environmental and cultural treatments modified the expression of incompatibility symptoms. The question of why significance resulted for standard-scion clones with orchards and not for rootstocks with orchards can probably be explained through test sensitivity. Tests made with standard-scion clones were less variable, and they more accurately assessed the influence of different environments. Tests made with rootstocks were confounded by the effects of within family seedling variation, which would result in a less sensitive test.

Copes (1974) has suggested that extremely incompatible scion clones might have genetically controlled incompatibility factors which might not be overcome by seedling rootstocks of highly compatible parents. Data from eight of the most incompatible scion clones averaged only 52.7 percent compatibility when grafted on the unselected local rootstocks, but they were 70·5 percent compatible on the three control- pollinated families. (Data not presented for individual local scion clones). One or two of the three rootstock families was at least 90 percent compatible with each problem clone, but sampling at this level was not extensive enough to permit firm conclusions. Apparently, however, control-pollinated families were somewhat successful in overcoming the compatibility problems of the most incompatible clones.

Summary and Conclusions

Grafting tests at six orchard sites were made on three control- and three wind-pollinated rootstock families from highly compatible parents. Tests were made at each orchard with ten local scion clones and with five standard-scion clones. Orchard locations ranged from 39° latitude at Chico, California, to 48° latitude at Lake Cowichan, B. C. Rootstocks grown from control-pollinated seed of graft-compatible parents averaged 90.9 percent compatible after 2-3 years; wind-pollinated families of the same parents averaged 76.4 percent compatible. Both values were considerably better than that of the local rootstock populations, which averaged only 64.8 percent compatible. Usually at least one of the three control-pollinated families was more than 90 percent compatible at each orchard. Significant environmental effects between orchards were demonstrated with standard-scion clones. No significant interaction between orchards and rootstocks were detected. Breeding rootstocks for production orchards can be an effective management tool for reducing graft-incompatibility losses. Apparent wide adaptability of the rootstock families to a number of environments should allow the breeding and testing of new families to be done from one convenient location and should permit extrapolation of results from there to other sites in California, Oregon, Washington, and British Columbia.

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