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Greenhouse Screening of Citrus Rootstock for Tolerance to Bicarbonate-induced Iron Chlorosis

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Abstract. **Eighteen citrus rootstock seedling lines were tested for their tolerance to Fe chlorosis using sand culture. Potassium carbonate was used to induce Fe-deficiency chlorosis. Chlorosis was quantified by 1) visual ratings, 2) SPAD-502 chlorophyll meter readings, 3) leaf chlorophyll concentration, 4) leaf active Fe, and 5) leaf total Fe. The first four criteria were well correlated among each other but not with leaf total Fe. Although any of the first four measurements could be used to quantify chlorosis, visual ratings and SPAD-502 readings were more convenient. The rootstock that have been reported to be tolerant or very susceptible to Fe chlorosis in calcareous soils were rated similarly for tolerance to bicarbonate-induced Fe chlorosis. Nontrifoliate types such as Texas sour orange *(C. aurantium* L.), Cleopatra mandarin *(C. reticulata* Blanco), Vangasay lemon (*C. limon* Burro.), and Ridge pineapple x Milam 1578-201 *(C. sinensis* L. Osbeck x *C. jambhiri)* were tolerant to moderately tolerant. Although most of the trifoliate hybrids tested were moderately susceptible to very susceptible, Smooth Seville x Argentine trifoliate {[*C. grands* (L.) Osbeck x C. *aurantium]* x *Poncirus trifoliata* (L.) Raf.} and F-81-12 citrange *(C. sinensis* x *P. trifoliata)* exhibited relatively high tolerance to lime-induced Fe chlorosis.**

About 25% to 30% of the earth's land surface is calcareous, and many plants, including citrus, growing in these soils develop Fe deficiency (Wallace and Lunt, 1960; Vose, 1982). Although Fe chelate applications can alleviate this problem, they are expensive. Rootstock tolerant to Fe deficiency in calcareous soils are used frequently, and they are a less expensive alternative, Citrus rootstock vary in their tolerance to lime-induced Fe chlorosis (Hamze et al., 1986), from trifoliate orange *[Poncirus trifoliata* (L.) Raf.] (susceptible) to the more tolerant citrus types such as sour orange, various mandarins *(C. reticulate* Blanco, *C. nobilis* Lour., and *C. depressa* Hay), limes *(C. limonia* Osbeck), and lemons *(C. jambhiri* Tan). Sweet orange *(C. sinensis* L. Osbeck) and trifoliate hybrids such as citranges and citrumelos have intermediate to low tolerance (Cooper and Peynado, 1954; Hamze and Nimah, 1982; Hamze et al., 1986).

Sour orange is a widely used commercial rootstock because it is tolerant to salinity, soil-borne diseases, cold weather, waterlog, and soils that are calcareous and high in clay content. Trees on sour orange produce high-quality fruit but yield less than trees on various trifoliate hybrids, such as 'Swingle' citrumelo, Troyer citrange, Morton citrange, and Rangpur × Troyer (Rouse et al., 1986; Wutscher, 1979). The use of sour orange is limited to areas not invaded yet by citrus tristeza virus (CTV) and its insect vector. Some regions, such as the Lower Rio Grande Valley of Texas, are threatened as this viral problem spreads northward from Mexico (Davis, 1986; Rouse et al., 1986; Wutscher, 1979). Alternate rootstock tolerant to calcareous soils and CTV are needed.

Bicarbonate has been cited as a major causal factor of Fe chlorosis in calcareous soils and in solution culture systems (Chancy et al., 1989). Thus, bicarbonate has been used successfully in nutrient solutions to mimic the effects of calcareous soils. Good correlations between the tolerance rankings of nutrient solution and field tests (Coulombe et al., 1984; Dragonuk et al., 1989) have been reported.

The intensity of leaf Fe chlorosis is usually rated subjectively by visual scoring (Byrne, 1988; Coulombe et al., 19-84; Hamze et al.. 1986; Maxwell and Wutscher, 1976) and, less commonly, by the leaf concentrations of Fe and chlorophyll. Although total Fe concentrations of chlorotic leaves are not consistently correlated with leaf chlorosis (Abadia et al., 1985; Pierson and Clark, 1984; Rao et al., 1987; Rashid et al., 1990), acid-soluble (active) Fe is consistently correlated with leaf chlorophyll concentration in a variety of plants (Takkar and Kaur, 1984). Leaf chlorophyll concentration is measured by extraction and quantification via spectrophotometry (Hiscox and Israelstam, 1979), with reflectometers that measure leaf greenness (Singha and Townsend, 1989), or with portable chlorophyll meters (MinoltaCameraCo., Japan) that calculate the relative leaf chlorophyll content (Marquard and Tipton, 1987; Yadava, 1986).

The objectives of this study were to evaluate the usefulness of several chlorosis measurements and to determine the tolerance of

Table 1. Citrus rootstocks[z] screened for tolerance to bicarbonate-induced chlorosis.

Common name	Binomial
Nontrifoliate	
Texas sour orange	*Citrus aurantium* L.
Cleopatra mandarin	*C. reticulata* Blanco
Vangasay lemon	*C. limon* Burm.
Ridge pineapple x Milam (1578-201)	*C. sinensis* L. Osbeck x *C. jambhiri*
Trifoliate orange	
Flying Dragon trifoliate	*Poncirus trifoliata* (L.) Raf.
Trifoliate hybrid	
Citrumelo	
F-80-3	*P. trifoliata* x *C. paradisi* Macf.
F-80-5	*P. trifoliata* x *C. paradisi* Macf.
F-80-8	*P. trifoliata* x *C. paradisi* Macf.
F-80-18	*P. trifoliata* x *C. paradisi* Macf.
Citrange	
Troyer	*C. sinensis* x *P. trifoliata*
Benton	*C. sinensis* x *P. trifoliata*
F-81-12	*C. sinensis* x *P. trifoliata*
Trifoliate x Ridge pineapple (1573-26)	*P. trifoliata* x *C. sinensis*
Other	
Cleopatra mandarin x Flying Dragon trifoliate	*C. reticulata* x *P. trifoliata*
Sunki mandarin x Benecke trifoliate	*C. reticulata* x *P. trifoliata*
Rangpur lime x Swingle trifoliate	*C. limonia* Osbeck x *P. trifoliata*
Smooth Seville x Swingle trifoliate	[*C. grandis* (L.) Osbeck x *C. aurantium*] x *P. trifoliata*
Smooth Seville x Argentine trifoliate	[*C. grandis* (L.) Osbeck x *C. aurantium*] x *P. trifoliata*

[z]All seed was obtained from the Division of Plant Industry, Bureau of Citrus Budwood Registration, Winter Haven, Fla., except for the Texas sour orange, which was obtained from the Texas A&I Citrus Center, Weslaco.

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various CTV-tolerant rootstock to bicarbonate-induced Fe chlorosis under greenhouse conditions.

Materials and Methods

Seven-week-old seedlings of 18 citrus rootstock (Table 1) were planted and grown in individual plastic pots (with ≈ 0.5 liter of washed sand) in a greenhouse. During the 3-week establishment period, the plants were watered with a half-strength macronutrient and full-strength micronutrient Hoagland's solution (Hoagland and Arnon, 1938) adjusted to pH 6.0 with 1.0 N NaOH. Iron (18 ppm) was added in the form of FeEDTA.

After the establishment period, the plants were arranged in a randomized complete-block design with 10 replications and three pH/bicarbonate treatments. The basic nutrient solution was modified by adding either 0,0.068, or 0.148 g of K_2CO_3/liter of solution. The respective pH and bicarbonate levels (in millimolar) were 6.00, 0.16; 7.5, 4.00; and 8.5, 6.00. During the first 5 weeks of treatment, a minimum of 50 ml of nutrient solution was added twice a week; from week 6 until week 15, 100 ml was added twice a week. During this period, the mean air maximum and minimum temperature were 30 and 20C, respectively.

Chlorosis was measured on the youngest fully developed leaves with the SPAD-502 chlorophyll meter (Minolta) and by visual chlorosis ratings after 15 weeks of treatment. The chlorosis scale was as follows: 1 = healthy green leaves; 2 = yellowish-green interveinal areas, green veins; 3 = greenish-yellow interveinal areas, green veins; 4 = yellow interveinal areas, green veins; 5 = yellow-white interveinal areas, pale-green veins. In addition, leaves (the three youngest topmost fully expanded leaves) from three one-plant samples pooled over replications (1–3, 4-6, and 7–10) were collected for an analysis of chlorophyll, active-Fe, and total Fe concentration. For chlorophyll measurement, 100-mg leaf samples were extracted with dimethyl sulfoxide (Hiscox and Israelstam, 1979) and quantified spectrophotometrically at 645 and 663 nm (Bruinsma, 1963). Active Fe (acid-soluble Fe) was determined using the method described by Takkar and Kaur (1984) and Katyal and Sharma (1980), and total Fe, P, K, Ca, Mg, Mn, and Zn were determined on leaf samples that were washed (Smith and Storey, 1976), dried, ground, and digested (Gallaher et al., 1975). The digested samples were quantified with an Applied Research Labs inductively coupled plasma atomic spectrophotometer (model 3510; Sunland, Calif.). The nutrient solutions were also quantified for the previously listed elements.

The chlorosis measurements were subjected to correlation analysis. The bicarbonate × rootstock factorial was tested with analysis of variance. The pH 6.0 treatment mean within a rootstock was separated from other treatment means by orthogonal contrasts. The citrus rootstock were placed into tolerance categories with respect to their performance relative to three standard rootstock: Texas sour orange (tolerant), Troyer citrange (moderate), and Flying Dragon trifoliate (very susceptible). Each rootstock was compared to all other rootstock in the trial with paired contrasts ($\alpha \leq 0.05$). If the rootstock was not distinguishable from Texas Sour orange, Troyer citrange, or Flying Dragon trifoliate, it was rated 1, 3, or 5, respectively. Two and 4 were used for the intermediate categories. If a given rootstock fell into two categories, the average score was used.

Results and Discussion

Nutrient concentrations in solutions and tissue. Adding K_2CO_3 to the basic nutrient solutions raised the solution concentration of K but lowered P, S, Ca, Mg, Mn, and Zn concentrations. The Fe concentration remained stable because of the chelated form used (Table 2). The most marked decreases in solution nutrient concentration from pH 6.0 to pH 8.5 on a percent basis was for P (–71 %), Mn (–85%), and Zn (-88%). Although this same trend was apparent in the tissue nutrient concentrations, changes were generally not as extreme (Table 2). Despite these trends, only active-Fe tissue concentration was well correlated with chlorosis. Although Mg was significantly correlated with chlorosis, the correlation was weak (Table 2), and no Mg deficiency symptoms were apparent during the experiment. The only recognizable nutrient deficiency symptom was Fe deficiency, which was alleviated when Fe chelate was applied.

Chlorosis indicators. All chlorosis indicators, except total Fe concentration of the leaves, were correlated with each other (Table 3). Total Fe concentration is not correlated with leaf chlorophyll concentration or chlorosis in several crops (Abadia et al., 1985; Rao et al., 1987; this study); thus, it is not useful for measuring leaf chlorosis. This noncorrelation led Chancy (1984) to define chlorosis due to Fe deficiency as any leaf chlorosis that regreens when FeEDDHA or $FeSO_4$(not when other nutrients) are" applied. Active Fe and chlorophyll were highly correlated with each other and with SPAD readings. The correlations between chlorophyll and active-Fe concentrations with the chlorosis ratings were weaker (Table 3). This relationship indicates that the SPAD chlorophyll meter readings are better indicators of leaf chlorophyll and active-Fe concentrations than are chlorosis ratings.

SPAD readings are influenced by leaf condition (Campbell et al., 1990). Thus, although leaf SPAD meter offers an. easy means of measuring leaf chlorophyll, the values collected are relative, and a standard curve must be constructed for each experiment if absolute values are required. The accuracy of leaf chlorosis ratings is limited by the precision of the chlorosis scale and the experience of the researcher. Nevertheless, when dealing with large plants, chlorosis ratings are more efficient than SPAD readings and generally summarize the condition of new growth. In contrast, SPAD readings attempt to characterize plant condition by subsampling smaller regions.

All the evaluation attributes that were correlated with leaf chlorosis showed a significant rootstock × pH/bicarbonate interaction (Table 4). This result indicates that the rootstock showed unequal development of bicarbonate-induced chlorosis in response to increasing bicarbonate concentration. Thus, rootstock possess different tolerance levels to this type of stress. Most rootstock had more leaf

Table 2. Nutrient concentration of nutrient solutions and corresponding plant tissue, and the correlation of nutrient concentration of tissue with leaf chlorosis.

	Solution concn (μM)[z]			Tissue concn (μM)[z]			Correlation with
Element	6.0	7.5	8.5	6.0	7.5	8.5	chlorosis[y]
P	720	440*	210*	9.7	8.4	8.7	NS
K	3820	4320*	5480*	87	110*	121*	NS
S	1190	1020*	1080	---	---	---	---
Ca	3340	2740*	2300*	31.7	25.9*	21.2*	NS
Mg	1380	1110*	1130*	7	5.8*	4.5*	–0.4**
Fe	17	16.6	15.8	1850	1750	1790	NS
Active Fe	---	---	---	344	299*	254*	–0.7***
Mn	13.1	6.6*	2.0*	894	795*	692*	NS
Zn	4.3	1.1*	0.5*	421	307*	374	NS

[z]Contrast comparing modified solutions to basic half-strength Hoaglands solution.
[y]Correlation between chlorosis ratings and tissue nutrient concentration (n = 54).
[NS],*,**,***Nonsignificant or significant at F' <0.05,0.01, or 0.001, respectively.

Table 3. Correlations among chlorosis indicators and foliar active-Fe concentration,

	Chlorosis indicators			
Variable	Chlorophyll	Chlorosis rating[z]	SPAD-502 reading	Total Fe
Active Fe	0.87***	0.71***	0.86***	–0.23
Chlorophyll	---	–0.69***	0.94***	–0.15
Chlorosis rating	---	---	0.80***	0.08
SPAD-502	---	---	---	–0.13

[z]Chlorosis rating scale: 1 = healthy green leaves; 3 = greenish-yellow interveinal areas, green veins; 5 = yellow-white interveinal areas, pale-green veins.
***Significantly different from zero at $P \leq 0.001$; n = 54.

Table 4. Leaf chlorosis ratings, chlorophyll concentration, foliar active Fe, and ASPAD-502 readings of citrus rootstock seedlings grown in sand culture at three pH/bicarbonate levels.

	Leaf chlorosis ratings[z]			Chlorophyll concn ($mg{\cdot}kg^{-1}$)			Active Fe concn (μM)			SPAD-502 reading		
	pH[y]											
Rootstock	6.0	7.5	8.5	6.0	7.5	8.5	6.0	7.5	8.5	6.0	7.5	8.5
Nontrifoliates												
Texas sour orange	1.0	1.2	1.0	2667	2621	2307**	663	663	645	65	65	63
Cleopatra mandarin	1.0	1.0	1.0	1692	1267**	683**	609	609	448**	57	48**	31**
Vangasay lemon	1.1	1.3	1.4	1466	1435*	1400**	466	358**	286**	53	53	52**
Ridge pineapple x												
Milam (1578-201)	1.0	1.2	1.5	1379	1371	1301**	394	340**	269**	51	50	48**
Trifoliate hybrids												
Citrumelo												
F-80-3	1.3	1.4	1.7	1300	927*	553**	340	286	251	48	39**	25**
F-80-5	1.2	2.2*	2.2*	967	944	927*	251	215	179	41	40	39**
F-80-8	2.0	2.4	3.8*	841	793**	648**	215	179	143**	37	35**	30**
F-80-18	2.6	3.3*	3.8*	589	485**	417**	161	161	143	25	20**	16**
Citrange												
Troyer citrange	1.0	1.4	1.6*	1340	1324	1361	340	304	269**	50	49	50
Benton citrange	1.2	1.6	1.8*	1198	1141**	1109**	322	286**	215**	47	46	45*
F-81-12 citrange	1.0	1.3	1.4	1570	1514**	1488**	519	376**	304**	55	55	54**
Trifoliate x												
Ridge pineapple (1573-26)	1.1	1.5	2.7*	811	756*	515**	197	161	143**	32	31*	23**
Other												
Cleopatra x												
Flying Dragon trifoliate	2.0	2.2	2.6	728	674*	703	179	161	143	32	31*	33
Sunki mandarin x												
Benecke trifoliate	1.5	2.3	2.5*	904	883	868**	233**	197**	161**	39	38	37**
Rangpur x												
Swingle trifoliate	1.2	1.9*	1.9	1050	1011	998**	286	233*	197**	44	42*	41*
Smooth Seville x												
Swingle trifoliate	1.1	1.9*	1.9*	1095	1085	1063**	304	233**	215**	46	45	44**
Smooth Seville x												
Argentine trifoliate	1.1	1.1	1.3	1721	1660*	1681**	555	501**	430**	59	58*	57**
Trifoliate orange												
Flying Dragon	2.5	2.7	4.1*	629	418**	297**	161	145	125*	28	16**	15**
Mean	1.4	1.8**	2.1**	1218	1128**	1029**	342	299**	254**	45	42**	40**

[z]Chlorosis rating scale: 1 = healthy green leaves; 3 = greenish-yellow interveinal areas, green veins; 5 = yellow-white interveinal areas, pale-green veins.
[y]Means for higher pH levels separated from pH 6.0 with orthogonal contrasts; observations per mean with rootstock: leaf chlorosis rating = 10; chlorophyll, active Fe, and SPAD-502 = 3.
**,*Significant at *P* £ 0.01 or 0.05, respectively. The interaction effect is significant at *P* £ 0.07,0.001,0.001, or 0.001 for chlorosis ratings, chlorophyll concentration, active Fe, and SPAD-502 readings, respectively.

chlorosis, less chlorophyll, less active Fe, and lower SPAD-502 chlorophyll meter readings at pH 8.5. than at pH 6.0. The most tolerant rootstock (e.g., Texas sour orange) showed little change in chlorosis, active-Fe concentration, chlorophyll concentration, and SPAD-502 readings as the pH/bicarbonate level increased from pH 6.0 to pH 8.5 (Table 4). In contrast, for the most susceptible rootstock (e.g., Flying Dragon), the level of all the chlorosis indicators decreased with increasing pH/bicarbonate levels. Although all plants were green at the beginning of the treatment, after 15 weeks five of the rootstock (F-80-8, F-80-18, Cleopatra x Flying Dragon, Sunki mandarin x Benecke, and Flying Dragon) showed chlorosis ratings ≥ 1.5 at the lowest pH/bicarbonate treatment. These rootstock generally had low chlorophyll concentrations (< 910 $mg{\cdot}kg^{-1}$), active-Fe concentrations (< 233 μM), and SPAD-502 readings (<39). Although Trifoliate x Ridge pineapple (1573-26) rootstock did not exhibit appreciable leaf chlorosis, its chlorophyll content, active-Fe concentration, and SPAD-502 reading were low. The nutrient-solution concentration Fe level of 18 μM was insufficient to maintain healthy green growth characteristics of these genotypes, suggesting their intolerance to low Fe levels.

Texas sour orange, a rootstock tolerant to calcareous soils, possessed the highest levels of chlorophyll, active Fe, and SPAD-502 readings. In contrast, Flying Dragon trifoliate, a rootstock susceptible to calcareous soils, had the lowest chlorophyll levels (Table 4). The trifoliate hybrids (citrumelos, citranges, and other hybrids) generally exhibited intermediate values for chlorosis, chlorophyll, active Fe, and SPAD-502 readings. The trifoliate hybrids that possessed the highest levels of chlorophyll, active Fe, and SPAD-502 readings were Smooth Seville x Argentine trifoliate and F-81-12 citrange, whereas F-80-18 citrumelo had the lowest values for these variables (Table 4).

Critical active-Fe tissue concentrations. Foliar active-Fe concentrations of the rootstock ranged from 125 to 663 μM on a fresh-weight basis (Table 4). Because the chlorosis symptoms were caused by Fe deficiency, the critical tissue concentrations for the development of Fe chlorosis can be established by comparing chlorosis symptoms with active-Fe concentrations (Table 4). In the chlorotic plants (ratings ≥ 2), the active-Fe levels ranged from 125 to 215 μM with a mean of 161 μM; most of these plants were < 179 μM, which was designated as the deficiency level. The plants with marginal chlorosis (rating of 21.5 and <2.0) had active-Fe levels ranging from 161 to 269 μM with a mean of 233 μM. Only one was <179 μM. The low active-Fe levels ranged from 197 to 269 μM. Above this value, Fe is sufficient for normal growth and development. Among the plants that were not chlorotic (rating <1.5), there was a wide range (197-663 μM) of active-Fe content; however, in all but two instances (F-80-5 and 1573-26), the active-Fe content was 2286 μM.

Relative chlorosis tolerance of 18 citrus rootstock. Relative rootstock tolerance to bicarbonate-induced chlorosis was assessed using the plant performance at the high pH (8.5)/bicarbonate level because it best resembled the pH and bicarbonate levels in the calcareous soils (pH 7.8 to 8.5; bicarbonate 4.5 to 5.5 μM) found in the citrus-growing area of the Lower Rio Grande Valley. Although the ranking with chlorosis ratings clearly distinguished between extreme responses, the top 10 ranked rootstock were not different from either Texas sour orange or Troyer citrange (Table 5). Thus, all were ranked as 2. The three other chlorosis indicators (active Fe, chlorophyll, and SPAD readings) were consistent in their rankings except for Cleopatra mandarin. The reason for the inconsistent responses of Cleopatra man-

Table 5. Relative tolerance to bicarbonate-induced chlorosis for 18 citrus rootstock grown in greenhouse sand culture at pH 8.5.[z]

Rootstock	Basis for ranking tolerance: Chlorosis ratings	Active Fe	Chlorophyll content	SPAD-502
Texas sour orange	2	1	1	1
Smooth Seville x Argentine trifoliate	2	2	2	2
F-81-12 citrange	2	2	2	2
Cleopatra mandarin	2	2	4	4
Vangasay lemon	2	3	2	3
Ridge pineapple x Milam (1578-201)	2	3	2	3
Troyer citrange	2	3	2	3
Benton citrange	2	3	2	4
Rangpur lime x Swingle trifoliate	2	4	4	4
Smooth Seville x Swingle trifoliate	2	4	4	4
F-80-3 citrumelo	3	3	4	4
F-80-5 citrumelo	4	4	5	4
Cleopatra mandarin x Flying Dragon trifoliate	4	5	4	4
Sunki mandarin x Benecke trifoliate	4	4	4	4
Trifoliate x Ridge pineapple (1573-26)	4	5	4	4
F-80-8 citrumelo	5	5	4	4
F-80-18 citrumelo	5	5	5	5
Flying Dragon trifoliate	5	5	5	5

[z]Tolerance grouping, 1 = tolerant, as Texas sour orange; 2 = moderately tolerant; 3 = moderately susceptible, as Troyer citrange; 4 = susceptible; and 5 = very susceptible, as Flying Dragon trifoliate,

darin is not known. Given the ease of taking SPAD readings and its high correlation with active Fe and chlorophyll (Table 3), SPAD could be used in place of chlorophyll and active-Fe measurements.

The relative tolerance of Texas sour orange, Cleopatra mandarin, Vangasay lemon, Troyer citrange, Benton citrange, and Flying Dragon trifoliate (Table 5) agreed with previous reports (Wutscher, 1979). Susceptible and very susceptible rootstock include F-80-5, Cleopatra mandarin x Flying Dragon trifoliate, Sunki mandarin x Benecke trifoliate (1573-26), F-80-8, F-80-18, and Flying Dragon trifoliate. The other trifoliate hybrids were moderately susceptible to bicarbonate-induced chlorosis except for two (Smooth Seville x Argentine trifoliate and F-81-12), which appeared moderately tolerant. No rootstock was as tolerant to bicarbonate-induced chlorosis as Texas sour orange (Table 5).

This study indicated that total leaf Fe concentration is not a good indicator of chlorosis and that SPAD-502 readings can be used instead of leaf active Fe and leaf chlorophyll concentration as measurements of leaf chlorosis. The tolerance to bicarbonate-induced chlorosis among the trifoliate hybrids tested ranged from very susceptible to moderately tolerant. The two most tolerant lines, Smooth Seville x Argentine trifoliate and F-81-12 citrange, may have tolerance to CTV and cold-hardiness and foot rot resistance because these characteristics are found in their parents. Thus, the development of a CTV-resistant sour orange replacement with adaptation to calcareous soils may be possible.

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