International Scholars Journals

International Journal of Enology and Viticulture Vol. 7 (4), pp. 001-007, April, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Vigour and behaviour of fifteen citrus varieties against tristeza in the forest zone of Cameroon

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Accepted 19 April, 2019

Fifteen varieties or combinations of citrus (lime, grapefruit, lemons, oranges, mandarin and hybrids) were characterized in the forest zone of Cameroon. Characterization was carried out based on the behaviour of these citrus varieties against citrus tristeza virus (CTV). Foliar and cortical symptoms were evaluated. Six years old plants did not show any immune reaction against the disease. Unusual mild symptoms were observed in varieties normally tolerant to the disease, which constitute an indicator of the presence of a virulent viral complex. Satsuma mandarin, Lisbon lemon, Eureka lemon and in some aspects Tahiti lime showed a satisfactory behaviour for their vigour and reaction against CTV.

Key words: Tristeza, citrus, symptom, vigour.

INTRODUCTION

The ecological diversity of Cameroon allows a comp-etitive citrus production as well as the preliminary charac-terizing the phytosanitary constraints specific to each area. This characterization allows for the identification of the varieties adapted to each agro ecologic zone.

Tristeza, caused by the citrus tristeza virus (CTV), is classified among the major phytosanitary constraints of citrus production (Bové, 1995; Roberts et al., 2001; Brlansky et al., 2005). The symptomatologic observations carried out show that the stem-pitting effect of the dise-ase occurs in all the south zone of the northern limit of the buttresses of Adamawa massive (Aubert, 1986; Rey, 1988). The disease vector, brown citrus aphid *Toxoptera citricidus* Kirk, is endemic in the part of Cameroon men-tioned above.

The evaluation of the reaction of citrus seedlings against tristeza could be used for the screening of toler-ant varieties or combinations (Carpenter et al., 1983; Grisoni and Sporrer, 1988). This temporal assessment of the behaviour of varieties against the disease could be applied as criterion for the partial replacement of the citrus orchards. The trees from which the scions were taken can also be selected on this basis. Such strategies could bring about profitable citrus production area where the disease is endemic.

The aim of this study was to classify the varieties or combinations of citrus based on their vigour and various reactions characteristic of tristeza in the ecosystem orchard of Yaoundé.

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MATERIAL AND METHODS

Study area and plant material

The study was carried out, in forest area under subequatorial climate of Yaoundé. This site on latitude 3° 55’ north is charac-terized by an altitude of 733 m, a rainfall of 1600 mm, an average temperature of 23.5°C, and a relative humidity of 80%.

Six-year old plants were retained for this study. The combinations or varieties were planted in complete blocks experimental design with five replications, and two trees for each experimental unit. Fifteen citrus varieties or combinations were observed (limettiers, pomelos, lemon trees, orange trees, mandarin trees and some hybrids). The scions of the combinations were taken in Corsica (France) on trees having undergone an indexing against tristeza by using Mexican lime (Rey, 1989). The stocks used were *Citrus volkameriana* in association with the lemon trees and the limettiers; Citrange “Carrizo” in association with the other varieties.

Foliar and cortical symptoms notation technique

The intensity of the tristeza symptoms is evaluated based on the size and density of cortical or foliar anomalies (stem-pitting or vein clearing) (Table 1). Stem-pitting indicates bark projections in wood, with formation of invaginations on the surface of sapwood (Salibe, 1977). Vein clearing on the other hand, indicates observable clear transparent dashes on the segments of veins of the leaves have just reached their physiological maturity. These segments of vein are abnormally gorged with water (Aubert, 1982).

The notation scale includes five levels of reaction varying from 0 (absence of reaction) to 4 (very severe reaction). The cortical symptoms on the trunk were evaluated on 10 trees for each variety. A bark gap of approximately 12 cm^2 exposes wood-and-bast sat at the line graft level. The level of reaction is evaluated on the scion and stock.

The evaluation of symptoms intensity at the canopy level takes into account its growth stage. The canopy is subdivided in eight sub-sectors according to the tree orientation compared to sun direction (Aubert, 1985). An 8 cm long branch (8 to 12 months-old), is taken at the level of each sub sector for the evaluation of stem-pitting reaction level after removing the bark. Eight branches were observed per tree, and four new mature leaves were observed by sub-sector of the canopy. Twenty four leaves were thus observed per tree (240 leaves by variety). The leaves were examined under the sun for evaluation of the vein clearing reaction.

The percentages (P_i) of organs (leaves, branches and stems) by type and level of reaction were calculated for each variety (Table 1). These percentages allow calculating a compound tolerance index (CTI), which is a weighted average note by the above mentioned percentages. This index allows the establishment of a scale of tolerance defined by «+» and «±». The sign «+» correspond to one point of tolerance index, and sign «±» correspond to the fractions of tolerance index varying from 0.25 to 0.75. The other fractions were round by defect or excess (zero for fractions of tolerance index comprised between 0 and 0.25, and one for those comprised between 0.75 and 1):

$$P_i = \frac{n_i}{N}$$

With n_i = number of samples of an organ (leave, branch or stem) having tolerance index i (i varying from 0 to 4) and N = total number of samples of that organ examined.

$$CTI = \sum_{i=0}^{4} P_i \, TI_i$$

With P_i = percentage of an organ (leave, branch or stem) having tolerance index i (i varying from 0 to 4) and TI_i = tolerance index i (i varying from 0 to 4).

Vigour notation technique

The measurement carried out on the crown or bark does not always give an exact idea of the plant port. The reduction of the foliar density as an indicator of premature ageing of the tree, for example, cannot be measured appreciably. A vigour scale with 5 levels which takes into account the canopy volume, the density of leaves, and their colouring was defined:

Level 0: Tree dying and requiring a replacement;
Level 1: (low vigour): Canopy of low volume, very much dismantled, with branches desiccated on the totality of crown;
Level 2: (average vigour): Moderately dismantled canopy, of average volume, with poor coloured leaves;
Level 3: (high vigour): Very slightly dismantled canopy, bulky, with well-coloured leaves;
Level 4: (very high vigour): Very bulky canopy, strong leaves density presenting very beautiful greenery.

The percentages of trees by level of vigour were calculated for each variety. An average vigour index and a vigour scale were defined according to the principle established for symptoms notation.

RESULTS

Vein clearing symptoms on leaves

The intensity of vein clearing for sensitive species was variable between species, within the clones of the same species, and between the trees of the same clone. According to the intensity of this reaction, the following species or varieties classification was observed (Tables 2 and 3).

Moderate reaction to severe reaction: Mexican lime and Tahiti lime.
Weak reaction to moderate reaction: Redblush pomelo, Pineapple orange, Valencia late orange, Hamlin orange and Tangor ortanique.
No reation to weak reaction: Orlando Tangelo, Shambar pomelo and Marsh pomelo.
No reaction: Lisbon lime, Eureka lime, Osceola mandarin and Satsuma mandarin.

Stem-pitting symptoms on branches of the grafted varieties

The intensity of this reaction was variable between species or varieties, clones of the same species and trees of the same variety. The following classification was therefore made (Tables 2 and 3).
Moderate reaction to severe reaction: Mexican lime.
Weak reaction to moderate reaction: Redblush pomelo and Hamlin orange.
No reaction to weak reaction: Eureka lemon, Shambar pomelo, Marsh pomelo, Pineapple orange, Valencia late Orange, Tangor ortanique and Orlando Tangelo.
No reaction: Lisbon lemon, Osceola mandarin, Fortune mandarin and Satsuma mandarin.

Stem-pitting symptoms on trunk of grafted varieties

The presence of the stem-pitting on trunk was revealed on the varieties supposedly sensitive and a number of the varieties supposedly tolerant. However, expression of this symptom was very discrete on varieties supposedly tolerant (Tables 2 and 3). According to Stem-pitting on trunk, the combinations or varieties were classified as follows:

Severe reaction: Mexican lime and Redblush pomelo
Moderate reaction: Tahiti lime, Marsh pomelo, Shambar

Table 1. Foliar and cortical notation scale of tristeza symptoms.

Reaction level	Tolerance index	Vein clearing on foliar veins	Stem pitting on wood-and-bast sat
0 Absent	4 (++++)	No symptom	No symptom
1 Weak	3 (+++)	Scare discoloured dashes on foliar veins	Few dense little cortical growths
2 moderate	2 (++)	Discoloured dashes of average size but not very dense	Small and very dense cortical growths or growths of average size but few dense
3 Severe	1 (+)	Discoloured and dense dashes of average size but without coalescence	Big and dense cortical growths but without invaginations of peel back
4 Very severe	0 (-)	Enlightenment of all vein length	Dense cortical growths of big size and presence of hollow on peel back

Table 2. Index and scales of symptomatologic tolerance and vigour of varieties supposedly sensitive to tristeza.

Varieties[a]	Vein clearing on leaves		Stem-pitting on scions		Stem-pitting on branches		varieties or combinations vigour	
	T.I.[b]	T.S.[c]	T.I.	T.S.	T.I.	T.S.	V.I.[d]	V.S.[e]
Mexican lime	0.72	±	1.70	+±	1.75	+±	2.38	++±
Tahiti lime	1.05	+	3.00	+++	3.80	++++	3.75	+++±
Shambar pomelo	3.87	++++	3.40	+++±	3.47	+++±	2.8	+++
Marsh pomelo	3.92	++++	2.60	++±	3.72	+++±	3.25	+++±
Redbush pomelo	3.12	+++	2.00	++	2.30	++±	1.25	+±

[a]Tolerance scale varying from +++± to ++++ on stocks (The stocks have tolerance indexes varying from very high to immune).
[b]Tolerance Index: 0 (very severe reaction) to 4 (no reaction).
[c]Tolerance Scale: - (very severe reaction) to + (severe reaction) to ++++ (no reaction).
[d]Vigour Index: 0 (tree dying) to 4 (very high vigour).
[e]Vigour Scale: - (tree dying) to + (weak vigour) to ++++ (very high vigour).

pomelo, Eureka lime, Hamlin orange and Osceola mandarin. symptom, the following classification was done:

No reaction to weak reaction: Pineapple orange, Valencia late orange, Tangor ortanique, Lisbon lemon, Orlando Tangelo and Fortune mandarin.

The general behaviour of the varieties or species with respect to trunk stem-pitting was satisfactory (Tables 2 and 3). The intensity of these reactions was variable between species or varieties, clones of the same species, and trees of the same variety. The presence and intensity of this symptom on trunk were not always correlated with the observations made on the branches of the varieties or species examined.

Stem-pitting symptoms on stocks

The two stocks used were *C. volkameriana* (associated with the lime and lemon trees) and Troyer Citrange (associated with other species or varieties). These stocks occasionally showed some peaks of barks being inserted in wood. The general behaviour of these stocks against tristeza was very satisfactory at the time of observations.

At the level of the general behaviour, varieties supposedly tolerant were different from the varieties supposeedly sensitive with regard to the vein clearing effect (Figure 1), stem- pitting on branches (Figure 2), and on trunk (Figure 3). More than 50% of examined organs show none of the three types of reaction for the varieties supposedly tolerant. The number is even less than 50% for the varieties supposedly sensitive. The average reaction of the poly-varietals orchard against the disease remains satisfactory, considering the three types of reaction six years after planting (Figures 1, 2 and 3).

Correlations between symptomatologic tolerance indexes

Spearman correlation coefficients based on the index of symptomatologic tolerance were calculated. A significant

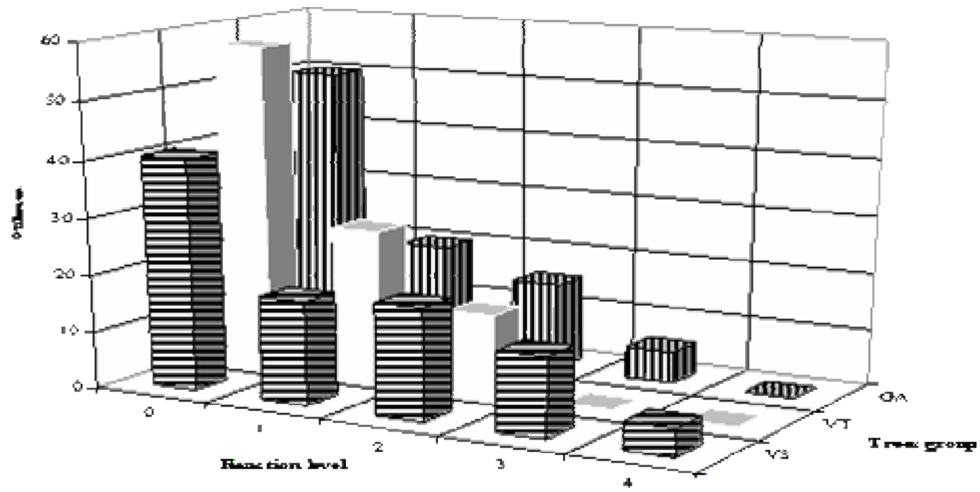

Figure 1. Average distribution of leaves as a function of vein clearing reaction level and trees group. VS = Varieties presumed sensitive, VT = varieties presumed tolerant, GA = general average, 0 = no reaction, and 4 = very severe reaction.

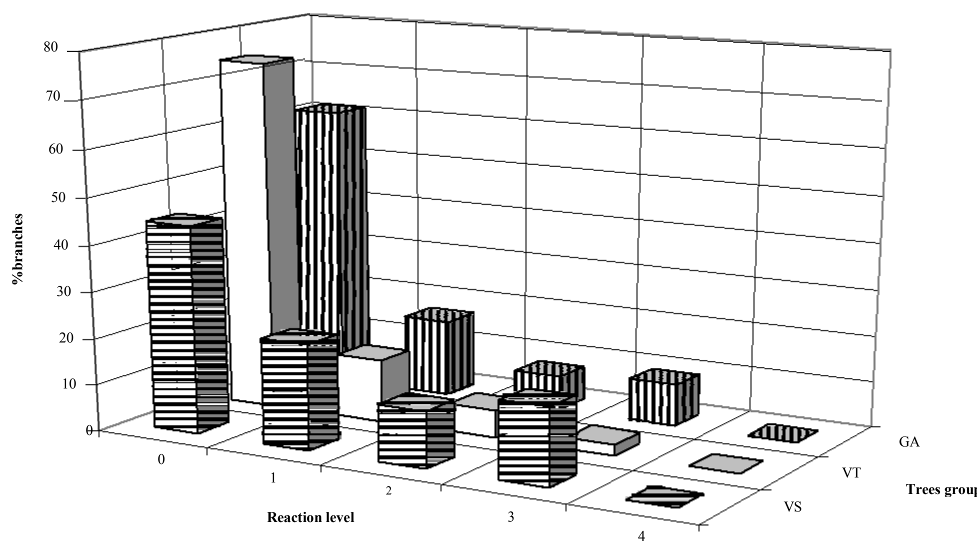

Figure 2. Average distribution of branches as a function of stem pitting reaction level and trees group. VS = Varieties presumed sensitive, VT = varieties presumed tolerant, GA = general average, 0 = no reaction, and 4 = very severe reaction.

correlation ($P = 0.05$, $r^2 = 0.53$) between the stem-pitting on branches, and the vein clearing on leaves was recorded.

Combinations port

The differences observed on port of the combinations were as important as on the foliar and cortical symptoms. An intraclone and intravarietal variation was observed. However, as shown by the poor port of certain tolerant combinations, there is not always a correlation between the presence and severity of the symptoms and vigour (Table 3). According to their vigour, the combinations or varieties were classified as follows:

Low vigour: Redblush pomelo.
Low vigour to average vigour: Fortune mandarin and Pineapple orange.
Average vigour to high vigour: Mexican lime, Osceola mandarin, Orlando Tangelo, Tangor ortanique, Hamlin orange, Valencia late orange and Shambar pomelo.

Table 3. Index and scales of symptomatologic tolerance and vigour of varieties supposedly tolerant to tristeza

Varieties[a]	Vein clearing on leaves		Stem-pitting on scions		Stem-pitting on branches		varieties or combinations vigour	
	T.I.[b]	T.S.[c]	T.I.	T.S.	T.I.	T.S.	V.I.[d]	V.S.[e]
Lisbon lemon	4.00	++++	3.90	++++	4.00	++++	2.80	+++
Eureka lemon	4.00	++++	3.40	+++±	3.87	++++	3.35	++++
Pineapple orange	3.07	+++	3.50	+++±	3.37	+++±	2.15	+±
Valencia late orange	3.58	++±	3.50	+++±	3.45	+++±	2.35	++±
Hamlin orange	2.50	++±	3.30	+++±	2.12	++±	2.30	++±
Tangor Ortanique	2.50	++±	3.50	+++±	3.45	+++±	2.40	++±
Orlando tangelo	3.75	+++±	3.70	+++±	3.55	+++±	2.50	++±
Osceola mandarin	4.00	++++	3.00	+++	4.00	++++	2.10	++
Fortune mandarin	4.00	++++	3.90	++++	4.00	++++	1.62	++
Satsuma mandarin	4.00	++++	3.60	+++±	4.00	++++	3.90	++++

[a]Tolerance scale varying from +++± to ++++ on stocks (The stocks have tolerance indexes varying from very high to immune).
[b]Tolerance Index: 0 (very severe reaction) to 4 (no reaction).
[c]Tolerance Scale: - (very severe reaction) to + (severe reaction) to ++++ (no reaction).
[d]Vigour Index: 0 (tree dying) to 4 (very high vigour).
[e]Vigour Scale: - (tree dying) to + (weak vigour) to ++++ (very high vigour).

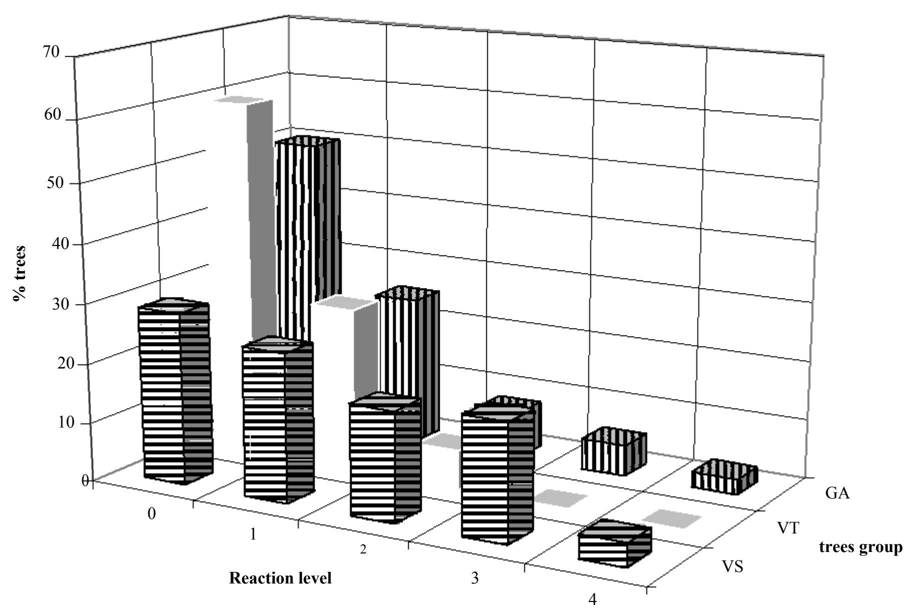

Figure 3. Average distribution of trees as a function of stem pitting reaction level on scion and trees group. VS = Varieties presumed sensitive, VT = varieties presumed tolerant, GA = general average, 0 = no reaction, and 4 = very severe reaction.

High vigour to very high vigour: Marsh pomelo, Lisbon lemon, Eureka lemon, Satuma mandarin and Tahiti lime.

DISCUSSION

The vein clearing seems to be the only specific tristeza symptom. The cortical symptoms although indicating of virulence, were not specific to disease. For example, all isolates of the cristacortis agent induce severe stem-pitting on Orlando and Wekiwa tangelos, and sour orange, while only some isolates cause symptoms on Clementine and a single isolate produces symptoms on lemon (Bové, 1995). However, knowledge on horticultural techniques and indicator plants of diseases that cause similar cortical symptoms allows for assigning these sym-

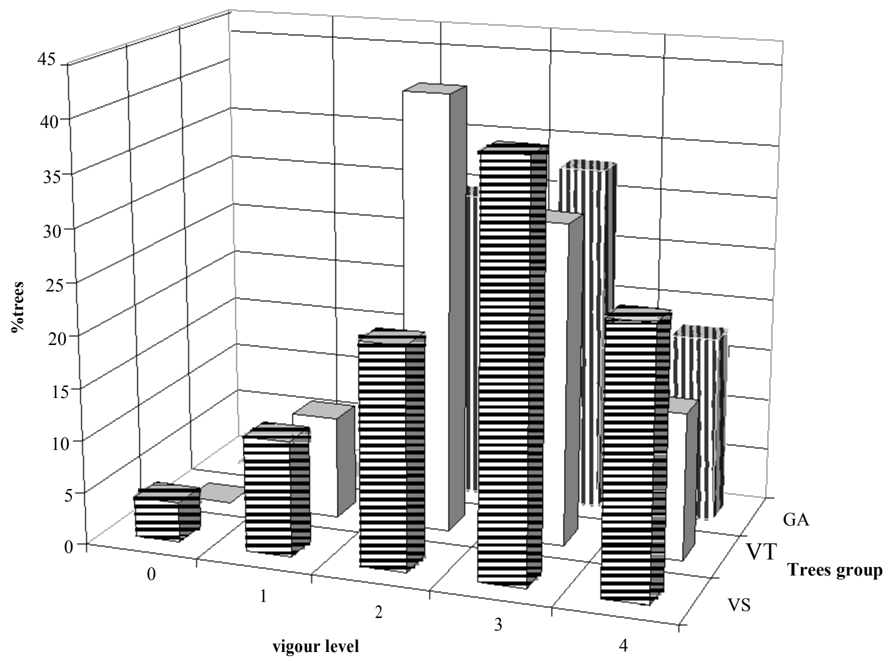

Figure 4. Average distribution of trees as a function of vigour reaction level and trees group. VS = Varieties presumed sensitive, VT = varieties presumed tolerant, GA = general average, 0 = no reaction, and 4 = very severe reaction.

ptoms a distinct pathological significance.

Trees observed in this study were obtained either by sowing or grafting. In grafting case, the stocks result from sowing and the scions were indexed in Corsica against the principally known viral diseases. Among viral diseases, those causing the same cortical reactions than the tristeza were transmissible by graft and not by vectors (Toumey, 1980). The current mode of propagation of citrus in rural area of Cameroon is sowing. All these considerations make uncertain the presence on wood-and-bast sat of a stem-pitting aetiology other than tristeza. The behaviour of the varieties assessed, except for Mexican lime and for Redblush pomelo, is satisfactory concerning the tristeza.

Significant correlation between stem-pitting on branches, and vein clearing on leaves could be due to the fact that the viral complex, which infects a tree, has a differential acropetal migration depending on the growth seasons (Raccah et al., 1980; Roistacher et al., 1982). The leaves lodge the same viral strains complex as the branches (8 to 12 months old) of the previous growth season, which carry them.

Observation of the average vigour of trees by reaction group shows that tristeza is not the principal constraint which determines the strength of the varieties (Figure 4). The average of the five varieties supposedly sensitive seems to exceed that of the 10 varieties supposedly tolerant. On individual basis, the poor vigour of certain varieties (Mexican lime and redblush pomelo) obviously seems to result from the damage due to the tristeza. Concerning tolerant varieties, the poor vigour of the Fortune and Osceola mandarin, oranges and hybrids (Tangor ortanique; Orlando tangelo) could be explained by physiological, genetic, pedoclimatic (soil type, temperature) causes and other infections different from tristeza (*Phytophthora* foot rot, blight, burrowing nematodes) (Ferguson, 2002).

Conclusion

Observations and follow-up of the varieties behaviour against tristeza in natural conditions can contribute to the elaboration of strategies for the partial replacement of citrus orchards. The cross protection technique can be also based on these observations. A combination of these strategies associated with the taking away of the scions on infected but vigorous trees can result in increased and profitable citrus production in zone where tristeza is endemic. The presence of unusual stem-pitting and vein clearing symptoms on varieties supposedly tolerant could reveal the presence of some relatively virulent viral strains. Cameroon has a great ecological and climatic diversity with a northern zone supposedly disease- free. This natural advantage enables the development of a competitive citrus production, which consists in using varieties or combinations adapted to each agro-ecological zone. The identification of the various phytosanitary and agronomic constraints is an essential necessity for economic development of citrus production.

ACKNOWLEDGMENT

The authors are grateful to IRA-IRFA programme for the funding of this work.

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