BLIGHT

Screening of 26 Rootstocks for Declinamiento Tolerance in Misiones, Argentina

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ABSTRACT. A rootstock trial was planted in 1979 to test Valencia sweet orange on 26 different rootstocks for tolerance to declinamiento, a disease of unknown etiology, in Misiones, Argentina. This disease affects sweet orange on trifoliate orange rootstock and its most conspicuous symptom is the production of small fruits. The criteria used to evaluate the rootstocks included annual yield and fruit size, the tree vigor as measured by canopy volume, annual decline incidence, and a western blot test for the presence of the citrus blight protein. The most tolerant rootstocks were Red rough lemon which is a local selection of rough lemon, Volkamer lemon, Orlando tangelo, Rangpur lime, and Cleopatra mandarin among the more vigorous rootstocks, and the Rubidoux trifoliate orange and the citranges C-35, C-84, and Troyer among intermediate vigor trees. All trees on local trifoliate orange rootstocks which served as positive controls showed characteristic decline symptoms and small fruit size after 4 to 5 yr.

Citrus production in the Misiones has been mainly based on growing Calderón sweet orange budded on trifoliate orange rootstock (6). This scion-rootstock combination is outstanding in relation to the fruit quality, yield, cold hardiness, and resistance to tristeza and foot rot. Of a total planting of 12,000 ha recorded at the end of the 1960's, approximately 8,000 ha were planted with this scion/stock combination. In the mid 1960's, a new disease appeared which produced a decline in the tree canopy in citrus plantations on this stock/scion combination. Within 15 yr, the disease had spread to practically all sweet orange plantations on trifoliate orange rootstock in Misiones and had became a limiting factor for citrus production. This disease is known as declinamiento or, locally, as fruta bolita and its symptoms have been described by Schwarz et al. (7).

Frequently, physiological or pathological problems of citrus can be solved by changing to a resistant or more tolerant rootstock (2). It was assumed that this could be a possible solution for declinamiento.

Although the cause of declinamiento is not known, its symptoms and the diagnostic test profiles are the same as for citrus blight of Florida and declinio in Brazil (e.g. zinc analysis, water uptake) and suggests that declinamiento is closely related to these other declines (8, 9, 13). However, rootstock susceptibility for declinamiento is somewhat different than for declinio in Brazil or blight in Florida, USA (9, 12, 14).

The objective of this study was to introduce and evaluate new rootstocks for the region in relation to declinamiento resistance and their adaptation to local climatic conditions.

MATERIALS AND METHODS

A nucellar Valencia sweet orange scion obtained from Limeira, São Paulo, Brazil was used throughout. At least 20 seedlings of each rootstock variety were budded with Valencia scion when the seedlings were 12-mo-old. The budded plants were grown in 10 L plastic bags and grown in a screenhouse until field planting after approximately 12 mo. Twenty-six rootstock varieties were used in the experiment and trees budded on a local trifoliate orange were used as a susceptible control. The varieties and seed sources are listed in Table 1.

Trees were planted on virgin soil in October 1979 in the Montecarlo area. The plot design was a randomized complete block with four trees per rootstock per block and four replications on a spacing of 7 × 6 m. Tree development was measured periodically on each tree and the data expressed as canopy volume in m^3 (V), based on the formula V = 2/3 pr^2h where r = the radius of the canopy; and h = tree height.

The trees first bloomed in 1983 and fruit production and fruit size were recorded yearly. The fruit production was expressed as average kg/tree/year and each fruit was sized by use of a caliper into categories from 4.5 cm to 8.5 cm in diameter.

The declinamiento incidence was rated annually according to symptom development on a scale as follows: 0 = healthy to 4 = a severely declined tree.

Western blot assays were carried out when the trees were 16-yr-old, using the monoclonal antibody to the 12 Kd protein for blight with the methodology described by Derrick et al. (4) in crude extract from leaves of selected trees in the experiment. Each block of every rootstock was assayed using at least five leaves of the scion from selected trees.

TABLE 1
LIST OF ROOTSTOCKS, CULTIVAR NAMES, AND SEED SOURCES USED IN THE EVALUATION OF DECLINAMIENTO TOLERANCE IN VALENCIA SWEET ORANGE IN MONTECARLO, MISIONES

Horticultural groups	Cultivars	Seed source
Trifoliate orange	Local	Local
	Pomeroy	California, USA
	Beneke	California, USA
	Barnes	California, USA
	Rubidoux 35	California, USA
	Rubidoux 15	California, USA
Trifoliate orange hybrids		
Citrange	Troyer	California, USA
Trifoliate orange × Washington Navel	Carrizo	California, USA
Trifoliate orange × Washington Navel	Yuma	California, USA
Citrange	C 32	California, USA
Trifoliate orange × Ruby orange	C 35	California, USA
Trifoliate orange × Ruby orange	C 84	California, USA
Citrumelo	Swingle	California, USA
Trifoliate orange × Grapefruit	Sacaton	California, USA
Lemon like fruits		
Rough lemon	Common	Local
	Red rough lemon	California, USA
Volkamer lemon		California, USA
Sweet orange	Common	Local
	Koethen sweet	California, USA
	Koethen sweet 13	California, USA
	Koethen sweet 34	California, USA
Tangerine like fruits		
C. amblicarpa Ochse		California, USA
Cleopatra		Local
Rangpur lime		Local
C. taiwanica Tan & Shim.		California, USA
Tangerine hybrids		
Tangelo tangerine × Grapefruit	Orlando	California, USA

RESULTS

After 16 yr in the field, we classified three groups of trees based on the final tree size: vigorous; intermediate; and declining. Those whose canopy volume had reached more than 30 m^3 were classified as vigorous trees (Fig. 1, Table 2); whereas those whose canopies were 16 to 25 m^3 were considered as intermediate; and those with a volume less than 10 m^3 were considered declining trees. The following rootstocks were classified in the vigorous trees group: Orlando tangelo, local and Red rough lemon, Volkamer lemon, Rangpur lime, and Cleopatra mandarin.

The intermediate group included trifoliate orange hybrids such as Troyer citrange, C-35, and C-84, *Citrus amblicarpa* and *C. taiwanica*, and the different sweet orange rootstocks such as the "comun" and various Koethen selections. The only trifoliate rootstock included in this group was Rubidoux trifoliate orange 35.

The remaining trifoliate oranges such as Local, Pomeroy, Beneke, Barnes, and Rubidoux 15 were included in the decline group because they were very susceptible and had the smallest trees. The other rootstocks such as the Carrizo citrange, Yuma, C-32, Sacaton, and Swingle citrumelo were also susceptible, but they were less so than the trifoliate oranges above. Trees on Yuma citrange were dwarfed. The behavior of Valencia on Carrizo citrange rootstock was not uniform for every tree in the trial, however, nearly 75% of the trees showed severe symptoms of declinamiento before the trees were 10-yr-old.

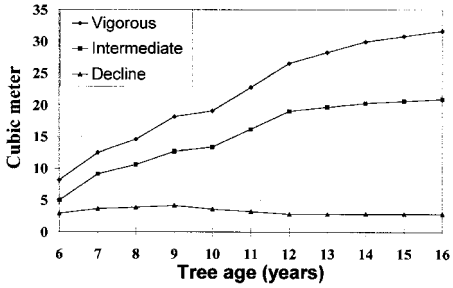

Fig. 1. Average composite canopy volume through the time of Valencia trees on different rootstocks classified according to their growth pattern in relation to declinamiento as vigorous, intermediate, or declining in Misiones, Argentina.

The first symptoms on the susceptible rootstocks appeared when the trees were about 5-yr-old which is the age when the tree develops its first important crop (Fig. 2). The development declinamiento severity increased rapidly on the susceptible rootstocks. Although all severely declining trees were still alive at the end of the experiment, they were of no commercial value. Some combinations of sweet orange on vigorous or intermediate rootstocks also developed zinc deficiency symptoms and small leaves when the trees were young. However, the decline did not progress following the years after the first observation. The symptoms were more noticeable in trees on intermediate rootstocks than on the trees on vigorous rootstocks.

Trees budded on vigorous rootstocks were always the most productive each. The trees on Orlando tangelo and on the local and Red rough lemon were the most productive in the 1992 and 1997 and also when considering the cumulative yield per tree for a 13-yr period. Valencia sweet orange on Rangpur lime behaved similarly to the trees on Volkamer lemon in production. The trees on Orlando tangelo began to produce commercial yields later than other rootstocks. Conversely, the trees on Volkamer lemon were the most productive in the early years, but their yield became lower after the trees were about 12-yr-old.

In the intermediate group, the Troyer citrange, C-35 and C-84 had the greatest production with more the 1,300 kg/tree for the 13 yr cumulative yield (Table 2). The trees on

TABLE 2
CANOPY VOLUME AND YIELD IN 1992 AND 1997 AND CUMULATIVE 13 YR YIELD IN VALENCIA SWEET ORANGE TREES ON DIFFERENT ROOTSTOCKS CLASSIFIED BY SIZE AFTER 19 YR OF FIELD EXPOSURE TO DECLINAMIENTO IN MISIONES, ARGENTINA

Rootstocks and category	Volume canopy (m^3)	Average yield per tree (Kg)		
		1992	1997	Cumulative 13 yr
Vigorous				
Orlando Tangelo	39.3	273.8	145.7	1,577.2
Red rough lemon	32.8	229.5	115.1	1,589.4
Cleopatra mandarin	32.4	189.8	75.0	1,214.6
Rangpur lime	31.3	182.3	92.8	1,483.4
Rough lemon	30.7	232.1	119.8	1,577.7
Volkamer lemon	30.5	194.3	106.1	1,481.8
Intermediate				
C. taiwanica	25.4	178.0	67.3	1,130.6
C. amblicarpa	24.5	166.5	53.3	1,101.9
C-84 citrange	22.7	186.8	137.4	1,383.4
Comun sweet orange	22.5	169.0	42.9	1,166.4
Troyer citrange	21.9	156.4	112.1	1,319.7
C-35 citrange	20.2	149.6	102.6	1,337.4
Koethen sweet orange	19.9	159.4	66.5	961.4
Rubidoux trifoliate 35	18.9	142.7	93.6	1,281.6
Koethen sweet orange13	16.9	153.6	63.2	961.9
Koethen sweet orange34	16.9	139.5	64.8	927.8
Decline				
C-32 citrange	10.5	70.3	12.9	620.2
Carrizo citrange	9.5	58.5	30.6	732.9
Barnes trifoliate orange	6.4	24.0	4.1	213.3
Swingle citrumelo	6.3	26.3	12.2	349.6
Rubidoux trifoliate15	5.6	15.2	2.8	229.8
Yuma citrange	3.7	29.3	7.9	228.1
Pomeroy trifoliate	3.3	12.0	2.1	169.9
Sacaton citrumelo	3.0	21.8	15.8	240.6
Beneke trifoliate orange	2.9	3.0	0.9	135.6
Local trifoliate orange	2.8	6.2	2.8	159.9

C-84 grew poorly during the early years in the field, and, of course, their production was low at that time. They became more productive and larger seven yr after planting with very good yields in the latter years. Trees on Rubidoux trifoliate orange 35 produced well each year and ranked second in this group for cumulative 13 yr production. The trees on *C. amblicarpa* and *C. taiwanica* rootstocks as well as any of the sweet oranges were intermediate in productivity.

In the decline group, production per tree was very low. In those trees where the decline was less severe such as C-32 and Carrizo citrange rootstocks, the total yield was 620.2 to 732.9 kg compared to 169.9 to 349.6 kg for remaining trees on several other trifoliate orange selections or hybrids, citrumelos, or citrange (Table 2).

In addition to low yields in the declining trees, these trees had the highest percentage of fruit with size smaller than 6 cm in diameter which is the minimum commercial diameter (Table 3). The fruits produced by the trees budded on vigorous rootstocks were generally of medium size, between a diameter 6 and 7 cm, with the exception of the

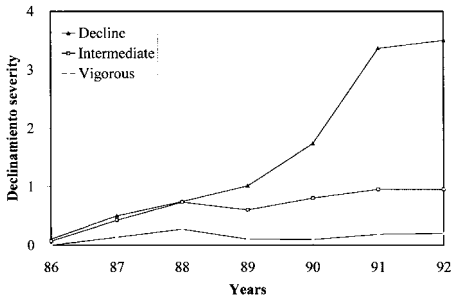

Fig. 2. Average of the composite declinamiento severity over a 6-yr period of Valencia on different rootstocks classified according to their growth pattern in relation to declinamiento as vigorous, intermediate, and declining in Misiones, Argentina. Resistance measured on a scale from 0 = healthy through 4 = complete declining trees.

fruit on Red rough lemon which had 62% of its fruit larger than the 7 cm. In contrast to the declining trees, the fruit produced by trees on intermediate rootstocks had the highest percentage, 60 and 70%, of the fruit of larger sizes.

Western blot analysis of all declining trees in the test of trees on trifoliate orange and some of their hybrids resulted in consistently positive tests for the presence the 12-Kd protein for Florida blight (Table 4). However, detection of this protein from the more tolerant rootstocks was more variable with a greater number of negative results. Although most trees from blocks of Orlando tangelo, Volkamer lemon, local rough lemon, and C-84 citrange were positive for the 12 Kd protein, in no case were all trees always positive for the protein.

DISCUSSION

Of the 26 different rootstocks tested, at least 10 were tolerant to declinamiento and resulted in good yields after 16 yr of field exposure. The most vigorous rootstocks such as lemon, tangerines, and tangerine hybrids were the best in declinamiento tolerance, production, and canopy volume. The intermediate group includes Rubidoux trifoliate orange 35 and the trifoliate hybrids, Troyer, C-35 and C-84 citranges. The remaining trifoliate orange and some of their hybrids were highly susceptible to declinamiento. To further test the tolerance of the Rubidoux trifoliate orange 35, Valencia on this rootstock were planted between declining trees in at least three locations where old and declining trees of Calderon sweet orange on local trifoli-

TABLE 3
FRUIT SIZE OF VALENCIAS IN 1995 AND YIELD CLASSIFIED IN THREE SIZE CATEGORIES IN A ROOTSTOCK TRIAL FOR CITRUS DECLINAMIENTO IN MISIONES, ARGENTINA

		% fruit per diameter classification		
Rootstocks	Category[z]	< 6 cm.	6-7 cm.	> 7 cm.
Volkamer lemon	Vigorous	7	59	34
Orlando Tangelo	"	4	53	43
Rangpur lime	"	3	42	56
Rough lemon	"	4	41	54
Cleopatra mandarin	"	5	59	37
Red rough lemon	"	3	34	63
C-84 citrange	Intermediate	1	26	73
C-35 citrange	"	3	34	63
Troyer citrange	"	3	33	64
Rubidoux trifoliate 35	"	1	33	66
Local trifoliate orange	Decline	29	63	8

[z]Rootstock category in relation to susceptibility to declinamiento.

TABLE 4
PRESENCE OF THE 12 KD BLIGHT-ASSOCIATED PROTEIN IN LEAVES OF VALENCIA ON DIFFERENT ROOTSTOCKS FROM AN EXPERIMENTAL TRIAL FOR TOLERANCE TO DECLINAMIENTO IN MONTECARLO, MISIONES, ARGENTINA

Rootstocks	Category[z]	Presence of 12Kd blight protein per block			
		Block 1	Block 2	Block 3	Block 4
Volkamer lemon	Vigorous	-[y]	+[w]	++[v]	+
Orlando Tangelo	"	++	+	+	++
Rangpur lime	"	-	+	+	+
Rough lemon	"	+	+ -[x]	+	++
C-84 citrange	Intermediate	-	+	+	++
C-35 citrange	"	-	+	+ -	+
Troyer citrange	"	+ -	+ -	+ -	+
Rubidoux trifoliate 35	"	-	+	+	+
Local trifoliate orange	Decline	++	++	++	++

[z]Rootstock category in relation to susceptibility to declinamiento.
[y]All the four trees in the block were negative.
[x]One tree out of four trees in the block were positive.
[w]Two or three of four trees in the block were positive.
[v]The four trees in the block were positive.

ate orange still survive. After 6 yr, all the planted test trees remain apparently healthy.

Beside differences in rootstock susceptibility between Florida blight and declinio of Brazil, other differences in declinamiento have been noted (9, 14). Response to the classical diagnostic tests such as water up-take, zinc concentration in the wood and number of amorphous plugs in the xylem were not consistent with declinamiento in Misiones (9, 14). Timmer and Agostini (10) categorized tolerant rootstocks as low vigor trees because they had reduced water uptake due to the more amorphous plugs in their xylem but these trees did not show other decline symptoms.

Fruit size is the best criterion to determine the tolerance of different rootstocks to declinamiento. Considering production was low in all declining trees on trifoliate orange, it might be expected that any fruit on an affected tree would be of a larger size. However, almost 30% of the fruit in these trees was not marketable because of its small size. In our experiment, Swingle citrumelo, which has been cited as having a low incidence of blight in Florida (2), were highly susceptible to declinamiento and suggests that differences between declinamiento and blight exist.

In the present report, the 12 Kd protein from declining trees on trifoliate orange were strongly detected from leaves, thus supporting the notion that the cause of this declinamiento is related to blight. However, when the western blot test was applied to other tolerant rootstocks, detection of the protein was not consistent. Asymptomatic trees which were commercially productive were not positive for the 12 Kd protein.

Hopkins et al. (5) has suggested the possible role of *Xylella fastidiosa* in Florida citrus blight etiology. Citrus variegated chlorosis (CVC) is now known to be caused by a different pathovar of *X. fastidiosa.* CVC-affected trees also produce small fruit size and *X. fastidiosa* has been detected since 1983 in Misiones (1). Considering that blight in Florida is transmitted by root piece grafting (11), and that the 12-Kd protein can be detected from non-symptomatic trees, it may now be possible to follow the development of blight and CVC in the same tree seedling by using specific antiserum for each disease.

The disease progress curve of declinamiento is different from that observed by Chelemi et al. (3) for blight of Florida, and by Tubelis et al. (12) for declinio of Brazil. Blight development fits a linear model and declinamiento fits a non linear pattern. A non-linear disease progress curve may indicate an infectious agent is involved which produces a compound interest incidence pattern which is different for blight which has a more simple interest disease incidence pattern. Thus, different causal agents may be involved.

In conclusion, the results of our experiment provides useful information to the citrus industry in Misiones. Rootstocks tolerant to declinamiento were identified. If the grower wants to plant sweet orange on a rootstock of intermediate growth, it can be planted at greater density than the more vigorous rootstock with an expected final production per ha to be similar.

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