Search for a Dwarfing isolate of *Citrus viroid III* for High Density Plantings and the Possible Association of CVd-III with Gum Pocket Disease in South Africa

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ABSTRACT. Four citrus viroid (CVd) isolates (CD 6, CD 12, CD 22, CD 47), as well as a *Citrus tristeza virus* (CTV) isolate (CD 4), were evaluated as dwarfing agents and compared to the normal pre-immunizing CTV isolate (LMS 6) which served as a control. The isolates were inoculated in virus-free Midknight Valencia sweet orange on 13 rootstocks. Nine of the rootstocks were trifoliate orange selections (five large flower and four small flower types) while four were trifoliate orange hybrids. Biological indexing of the CVd isolates induced typical CVd-III symptoms on Etrog citron but the intensity of the symptoms differed among the isolates. Clones of each isolate were sequenced and the sequences, with small variations, were similar to that of CVd-III. After 8 yr in the field the CVd isolates had less effect on the trifoliate orange hybrids than on the trifoliate orange selections regarding growth and production. Trees with the CTV pre-immunizing isolate were significantly larger and produced more fruit than trees with the various CVd isolates. The trifoliate orange hybrids and the Rubidoux selection of trifoliate orange showed no gum pocket symptoms. The CD 12 and CD 47 isolates induced gum pocket symptoms on more trees than the other two CVd isolates.

Index words. Tree size control, trifoliate orange, citrus viroids.

Tree size control is essential when citrus trees are planted at high densities. Since a CVd-III isolate is successfully used for dwarfing in Australia (3) and another isolate of the same viroid was selected in California that does not induce disease symptoms (13), attempts were made in South Africa to find an isolate with similar characteristics.

Gum pocket disease in South Africa affects the trifoliate orange rootstock used with sweet orange trees and was reported in 1969 (12). With the use of certified budwood, the disease "disappeared" until citrus viroids (CVd) were experimentally used to reduce tree size for high-density plantings (15, 16). An apparently similar disease, also associated with CVd dwarfing, was described in Australia as Gummy Pitting (8). Similar disease symptoms were later found in Italy and Turkey (1, 5). Marais et al. (10) associated CVd-III with gum pocket disease in South Africa. However, because the CVd was not fully characterized, the association of CVd-III was questioned (7).

This paper describes the search for a suitable dwarfing CVd isolate and the possible association of CVd-III with gum pocket disease in South Africa.

MATERIALS AND METHODS

Citrus dwarfing (CD) isolates. *CVds:* Four CVd-III isolates identified by sPAGE nucleic acid analysis and silver staining (CD 6, CD 12, CD 22, CD 47) were selected from a collection, maintained in sweet orange on Rough lemon rootstock, and obtained in commercial orchards from trees on various rootstocks. The viroid isolates were transmitted to healthy Etrog citron (Arizona 861-S-1) by slash inoculation, a method used to prevent co-

transmission of *Citrus tristeza virus* (CTV) present in the isolates. The inoculated plants were kept in a greenhouse at 28-32°C and served as source plants for the research project. Symptom expression on Etrog citron was compared to that of CVd-III isolates obtained from California and Spain (obtained respectively from J. S. Semancik and N. Duran-Vila), but which were not used in the field evaluation.

CTV: Two CTV isolates were included in the trial. CD 4 was collected from a dwarfed Valencia tree on trifoliate orange rootstock and was previously evaluated (18). Since CTV is endemic in South Africa, the control trees were inoculated with the CTV isolate LMS 6, the cross-protection isolate used to pre-immunize all virus-free commercial sweet orange material before it is released to the industry.

Isolation of double-stranded RNA (dsRNA) from infected tissue. Pooled samples of bark and midrib tissue (4 g) were frozen with liquid nitrogen and pulverized, and nucleic acids were extracted by the phenol-detergent method of Dodds et al. (6) with minor modifications. The aqueous phase containing nucleic acids was adjusted to 35% ethanol, incubated at -20°C for 1 h and then centrifuged at 8000 *g* for 20 min to remove impurities. Nucleic acid preparations enriched with viroids and dsRNAs were obtained by non-ionic cellulose column chromatography (CF-11; Whatman International, Maidstone, England). Cellulose contamination was prevented by an additional phenol extraction step. Finally the RNAs were concentrated by ethanol precipitation and resuspended in 50 µl sterile distilled water.

Reverse transcription-polymerase chain reactions (RT-PCR). Reverse transcription-polymerase chain reaction (RT-PCR) using CVd-III specific primers confirmed the presence of CVd-III in the CVd isolates. Complementary DNA was synthesized from dsRNA by reverse transcription and PCR amplification using the Titan One Tube RT-PCR System (Roche Diagnostics, GmbH) and primers based on the sequence of CVd-IIIb (11). Primer CV-IIIL contained an *Eco*RI restriction site (5-GGCGGAATTCACTCTCCGTCTTTACTCCA-3), while CV-IIIR had a *Hin*dIII restriction site (5-TATAAAGCTTCTCCGCTAGTCGGAAAGACTCCGC-3).

Two microliters of the RNA preparation was denatured in a boiling water bath in the presence of 0.4 µM of each primer for 5 min, chilled on ice for 5 min and primers were allowed to anneal at room temperature for 30 min. One-step RT-PCR was performed in a 25 µl reaction mixture containing 1× RT-PCR reaction buffer (with 1.5 mM $MgCl_2$; Roche Diagnostics, GmbH), 0.2 mM of each dNTP, 5mM DTT, and 0.5 µl enzyme mix (AMV reverse transcriptase and Expand High Fidelity enzyme mix; Roche Diagnostics, GmbH) in addition to the RNA-primer mix. Thermocycling conditions were: 30 min at 50°C for RT, 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, 20 s at 60°C and 60 s at 68°C. In all cases, a final extension of 5 min at 68°C was used. RT-PCR products were visualized in 1% agarose gels stained with ethidium bromide.

SSCP analysis. For single-strand conformational polymorphism (SSCP) analysis, a modified procedure described by Yap and McGee (19) was followed. One microliter of the RT-PCR product was mixed with 9 µl dH_2O and 1 µl denaturing solution (500mM NaOH, 10 mM EDTA pH 8.0). The mixture was heated for 10 min at 42°C and 1 µl loading dye added (0.5% xylene [w/v] and 0.5% bromophenol blue [w/v] in deionized formamide). Denatured DNA of the CVd-III RT-PCR products were separated by electrophoresis in a non-denaturing 12% polyacrylamide minigel (without glycerol) using 0.5× TBE buffer (44.5 mM Tris-Borate, 1 mM EDTA, pH 8.0) for electrophore-

sis at 200 V for 4 h at 8°C. Gels were stained with silver nitrate (2).

Cloning. RT-PCR products with different SSCP profiles were separated on a 1% agarose gel and eluted by using the Qiaex gel extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The recovered full-length cDNA was digested with both *Eco*RI and *Hin*dIII, cleaned and ligated to *Eco*RI/*Bam*HI restricted pGEM-3Z vector (Promega). Transformation of *Escherichia coli* JM109 (Promega, Wisconsin, USA) cells and plasmid isolation were done according to standard procedures (9).

Sequencing. Sequencing of both strands was performed using dye terminator sequencing kits from ABI and an ABI 310 genetic analyzer.

Preparation of plants for field evaluation. Virus- and viroid-free Midknight seedless Valencia sweet orange was budded on nine trifoliate orange selections (five large flower types and four small flower types (14), and four trifoliate orange hybrids (Table 1). After the scions had grown to approximately 30 cm, four CVd isolates and the two CTV isolates were bud-inoculated in the scions. Six months were allowed for the agents to become established in the plants, where after they were planted in the field during 1997 in the Nelspruit area according to a split plot design with four replications.

Data collection. *Biological indexing:* The symptoms induced by the four dwarfing isolates were compared with the symptoms induced by the isolates from California and Spain. The two CTV isolates had been previously indexed for the presence of CVds and were CVd-free. No indexing of the field trees were done for CTV and with the abundance of the brown citrus aphid, *Toxoptera citricida*, it was assumed that all the trees became infected with various strains of CTV during the 7-yr period. The control trees were protected by the LMS 6 CTV isolate.

Tree size: Canopy volumes were determined according to Burger et al. (4), where the canopy was calculated as a cylinder and half sphere.

Yield: The fruit were picked annually and weighed.

Tree abnormalities: The trees were inspected annually for disease symptoms and growth abnormalities.

RESULTS AND DISCUSSION

Isolate characteristics. With the exception of CD 47, the symptoms induced in Etrog citron by the

TABLE 1
TRIFOLIATE ORANGE SELECTIONS AND HYBRIDS USED TO ASSESS THE EFFECT OF CD ISOLATES ON TREE SIZE AND PRODUCTION OF MIDKNIGHT SEEDLESS VALENCIA SWEET ORANGE

Rootstock	Type description
Rich	Trifoliate orange, large flower.
Argentina	Trifoliate orange, large flower.
Kryder 55-1	Trifoliate orange, large flower.
Christian	Trifoliate orange, large flower.
Yamaguari	Trifoliate orange, large flower.
Rich 22-2	Trifoliate orange, small flower.
English	Trifoliate orange, small flower.
Rubidoux	Trifoliate orange, small flower.
Jacobsen	Trifoliate orange, small flower.
Benton citrange	Trifoliate orange × sweet orange hybrid.
Troyer citrange	Trifoliate orange × sweet orange hybrid.
Yuma citrange	Trifoliate orange × sweet orange hybrid.
Swingle citrumelo	Trifoliate orange × grapefruit hybrid.

CD isolates were similar to that of the two CVd-III isolates from California and Spain, with minor differences. The main symptoms were leaf curling, slight midvein and secondary vein browning, and petiole wrinkling and browning. No stunting occurred. The isolate from Spain appeared to give the mildest symptoms, while the plants that were inoculated with CD 47 displayed severe vein browning symptoms as well as leaf chlorosis.

RT-PCR using CVd-III specific primers confirmed that the four CD isolates contained CVd-III. Different electrophoretic profiles were obtained when the RT-PCR products were subjected to SSCP analysis (data not shown), indicating potential nucleotide differences in their sequences. The sequences of several clones of each isolate were compared with the type sequence of CVd-IIIb (11), and results are shown in Table 2. The degree of sequence homology varied only slightly or not at all, ranging from 0-5 nucleotide changes. Differences among the sequence variants are summarized in Table 3, and these changes indicate that all sequences are highly homologous to CVd-IIIb.

Effect on tree size. The average tree size for each isolate is presented in Table 4. Overall, trees on the trifoliate orange selections were significantly smaller than those on the trifoliate hybrids, except Yuma citrange which is affected by the endemic CTV (17). There were no size differences between trees sizes on large flower and small flower trifoliate orange types but the trees on hybrid rootstocks were significantly larger. The CD isolates also reduced tree size significantly in comparison with the control (LMS 6). Among the CVd isolates, trees with CD 6 were significantly smaller than those inoculated with CD 22. Tree sizes of the latter did not differ significantly from those with the CTV dwarfing isolate (CD 4), which were marginally larger, and from those with CD 12 and CD 47. However, trees with CD 4 were significantly larger than those with CD 12 and CD 47.

Effect of yield. The cumulative yield over 4 yr and the yield efficiency of the last year are presented in Tables 5 and 6 respectively. In general, trees on trifoliate orange rootstocks produced less fruit than those on the hybrid rootstocks, although the yield efficiency (kg/m^3) of trees on the trifoliate orange rootstocks was 13% higher (Table 6). Three CD isolates (CD 6, CD 12, CD 47) enhanced the productivity of the trees with a 25-33% increase in yield. The increase in yield confirms a previous report (13). The yield of CD 22 was similar to that of the two CTV isolates.

Symptoms. No disease symptoms other than those of Huanglongbing (HLB) were observed on the scions. Not all the trees presented HLB symptoms that were sectored on small branches. The rootstocks, however, displayed several symptoms. The main symptom was similar to that of Gum Pocket disease (10, 15, 16) (Fig. 1A), with gum pockets that developed on a flat area on the trunk. The flat area was the first symptom to be observed and it appeared as if growth ceased in that area. Lesions containing soft brownish gum developed and later became hard with necrotic tissue with star-like cracks. At this stage, the bark did not peel from the wood.

A pitting symptom, similar to that reported by Duran-Vila et al. (7), was also observed (Fig. 1B). Sometimes the two symptom types occurred together on the rootstock (Fig. 1C). The occurrence of symptoms at this stage is summarized in Table 7 and may increase over time. When both types of symptoms occurred on the rootstock, it was regarded as Gum Pocket positive and the pitting symptom was disregarded. All the trifoliate orange selections showed Gum Pocket and/or pitting symptoms. The Argentina,

TABLE 2
DIFFERENCES IN SINGLE POINT MUTATIONS AMONG CLONES OF FOUR CVD-III ISOLATES IN COMPARISON WITH THE CVD-IIIB SEQUENCE[z]

	Nucleotides														
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
CVd-III	ggaggaaacu	ccgugugguu	ccugugggge	acacccccuu	gccgaaaaua	aaacgcagag	agggaaaggg	aaacuuaccu	gucgucgucg	acgaaggcag	cuaaguuggu	gacgccgagu	ggaguaaaga	cggagagucu	ccgcuagucg
CD 6/1	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 6/6	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 6/8	————	————	————	————	————	————	————	————	————	————	————	————	————	————	———u——
CD 6/9	————	————	————	————	————	————	————	————	—u————	————	————	————	————	————	————
CD 12/1	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 12/5	————	————	————	————	————	————	————	————	——a———	————	————	————	————	————	————
CD 12/10	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 22/1	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 22/4	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 22/7	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 47/1	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 47/3	————	————	————	————c——a———	————	g———	—x———	————	————	————	————	————	————	————	————
CD 47/5	————	————	————	————	————	————	————	————	————	————	————	————	————	————	————
CD 47/6	————	————	————	————	————	————	————	————	————	————g————	————	————	————	————	————
CD 47/9	————	————	————a—	————	————	————	————	————	————	————	————	————	————	————	————

[z]Rakowski et al. (11) - = identical; x = missing nucleotides.

TABLE 2 (CONTINUED)
DIFFERENCES IN SINGLE POINT MUTATIONS AMONG CLONES OF FOUR CVD-III ISOLATES IN COMPARISON WITH THE CVD-IIIB SEQUENCE[z]

	Nucleotides														
	160	170	180	190	200	210	220	230	240	250	260	270	280	290	
	gaaagacucc	gcauccuccg	gcagacccuu	cuagcucccg	cuagucgagc	ggacaacuga	gugaguuguc	ccaauccuaa	ucuguuuuua	ucuaggcuag	aaggggauug	ggccuccagg	guaaaacacg	auugguguuu	cccc
CD 6/1									u						
CD 6/6									c		u				
CD 6/8															
CD 6/9											a				
CD 12/1															
CD 12/5	u														
CD 12/10											u				
CD 22/1										g	t				
CD 22/4															
CD 22/7						c									
CD 47/1															
CD 47/3											a				
CD 47/5									c						
CD 47/6															
CD 47/9															

[z]Rakowski et al. (11) - = identical; x = missing nucleotides.

TABLE 3
NUMBER OF NUCLEOTIDE DIFFERENCES AMONG CVD-III SEQUENCE VARIANTS

	CD 6/1	CD 6/6	CD 6/8	CD 6/9	CD 12/1	CD 12/5	CD 12/10	CD 22/1	CD 22/4	CD 22/7	CD 47/1	CD 47/3	CD 47/5	CD 47/6	CD 47/9
CD 6/1	—	1	2	3	1	3	1	3	1	2	1	6	2	2	2
CD 6/6		—	3	5	2	4	2	4	2	3	2	7	3	3	3
CD 6/8			—	3	1	3	3	3	1	2	1	6	2	2	2
CD 6/9				—	2	4	4	4	2	3	2	7	2	3	3
CD 12/1					—	2	2	2	0	1	0	5	1	1	1
CD 12/5						—	4	4	2	4	2	7	3	3	3
CD 12/10							—	4	2	3	2	7	3	3	3
CD 22/1								—	2	3	2	6	3	3	3
CD 22/4									—	1	0	5	1	1	1
CD 22/7										—	1	6	2	2	2
CD 47/1											—	5	1	1	1
CD 47/3												—	6	6	6
CD 47/5													—	2	2
CD 47/6														—	2
CD 47/9															—
CVd-IIIb[z]	99.7	99.3	99.7	99.3	100	99.3	99.3	99.7	99.7	99.7	100	98.3	99.7	99.7	99.7

[z]Percentage similarity to CVd-IIIb (Rakowski *et al.* 1994).

TABLE 4
EFFECT OF DIFFERENT GRAFT TRANSMISSIBLE ISOLATES, ON TREE SIZE (CANOPY VOLUME M^3) OF 7-YEAR-OLD MIDKNIGHT VALENCIA SWEET ORANGE TREES GRAFTED ON DIFFERENT ROOTSTOCKS*

	Graft transmissible isolate						
Rootstock	LMS 6 CTV	CD 4 CTV	CD 6 CVd-III	CD 12 CVd-III	CD 22 CVd-III	CD 47 CVd-III	Mean
Rich	4.3	3.7	2.1	2.6	3.4	1.9	3.0 z
Argentina	4.7	4.5	3.0	2.0	2.4	2.5	3.2 yz
Kryder 55-1	4.5	4.8	1.8	2.4	3.1	2.6	3.2 yz
Christian	4.4	3.6	1.1	1.9	3.7	2.4	2.8 z
Yamaguari	3.2	4.4	1.8	1.7	2.5	1.8	2.6 z
Rich 22-2	5.1	3.4	2.5	1.7	3.8	1.3	3.0 z
English	4.3	3.8	1.5	1.9	3.7	1.6	2.8 z
Rubidoux	3.0	5.0	2.3	2.1	4.0	2.1	3.1 z
Jacobsen	4.3	3.7	1.6	1.2	3.3	2.0	2.7 z
Benton citrange	6.1	5.2	2.9	4.5	4.7	4.0	4.6 y
Troyer citrange	9.6	7.2	5.9	7.0	7.6	5.8	7.2 x
Yuma citrange	5.2	2.4	2.8	2.9	4.1	5.0	3.7 yz
Swingle citrumelo	9.1	5.9	5.6	6.0	5.2	5.7	6.2 x
Mean	5.2 w	4.4 wx	2.7 z	2.9 yz	4.0 xy	3.0 yz	

*Rootstock and treatment means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Kryder 55-1, Rich 22-2 and Jacobsen selections showed the most Gum Pocket symptoms, while Jacobsen also showed the most pitting symptoms. No Gum Pocket symptoms developed on the Rubidoux selection, even though such symptoms were reported in a previous study

TABLE 5
EFFECT OF DIFFERENT GRAFT TRANSMISSIBLE ISOLATES, ON THE CUMULATIVE YIELD (KG/TREE) OF 7-YEAR-OLD MIDKNIGHT VALENCIA SWEET ORANGE TREES GRAFTED ON DIFFERENT ROOTSTOCKS*

	Graft transmissible isolate						
Rootstock	LMS 6 CTV	CD 4 CTV	CD 6 CVd-III	CD 12 CVd-III	CD 22 CVd-III	CD 47 CVd-III	Mean
Rich	97.8	82.6	58.6	98.8	113.0	73.0	87.3 bc
Argentina	129.5	114.3	95.8	86.8	62.0	98.5	97.8 b
Kryder 55-1	118.0	99.7	56.7	87.1	110.4	97.2	94.9 b
Christian	126.1	70.5	45.9	60.4	94.4	74.0	78.5 c
Yamaguari	76.2	70.5	45.0	53.5	59.2	63.3	61.3 d
Rich 22-2	121.9	74.2	54.1	52.1	87.2	57.6	74.5 cd
English	134.9	118.9	49.2	77.5	113.2	68.3	93.7 b
Rubidoux	68.2	91.7	65.4	57.2	110.9	50.1	73.9 cd
Jacobsen	98.8	91.0	58.6	39.0	96.9	52.3	72.7 cd
Benton citrange	182.2	148.2	82.6	140.0	174.0	121.9	141.5 a
Troyer citrange	155.8	143.7	75.2	161.0	128.7	111.5	129.3 a
Yuma citrange	144.4	55.5	87.8	85.2	111.1	126.7	101.8 b
Swingle citrumelo	167.0	134.5	140.9	155.1	101.9	124.6	137.3 a
Mean	124.7 a	99.6 b	70.5 d	88.7 c	104.8 b	86.1 c	

*LSD: Rootstocks, 15.0; Graft Transmissible agents, 10.2; Rootstocks/Graft Transmissible agents, 36.8. Rootstock and treatment means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

TABLE 6
EFFECT OF DIFFERENT GRAFT TRANSMISSIBLE ISOLATES, ON YIELD EFFICIENCY (KG/M^3) OF 7-YEAR-OLD MIDKNIGHT VALENCIA SWEET ORANGE TREES GRAFTED ON DIFFERENT ROOTSTOCKS*

	Graft transmissible isolate						
Rootstock	LMS 6 CTV	CD 4 CTV	CD 6 CVd-III	CD 12 CVd-III	CD 22 CVd-III	CD 47 CVd-III	Mean
Rich	10.1	8.3	9.8	14.4	9.7	10.7	10.5 bcd
Argentina	10.7	9.4	12.9	15.8	6.0	11.3	11.0 bcd
Kryder 55-1	10.9	9.9	12.8	12.2	10.8	11.2	11.3 bc
Christian	10.2	9.4	15.1	14.0	10.5	12.3	11.9 ab
Yamaguari	8.4	7.9	12.1	14.8	9.5	11.1	10.6 bcd
Rich 22-2	9.4	8.6	7.2	13.2	9.8	15.0	10.5 bcd
English	11.1	11.9	17.7	14.8	10.7	16.9	13.9 a
Rubidoux	6.1	8.8	9.7	8.0	10.1	10.2	8.8 d
Jacobsen	8.9	9.6	14.5	10.9	11.0	9.3	10.7 bcd
Benton citrange	7.3	10.7	10.6	9.7	12.0	11.3	10.3 bcd
Troyer citrange	8.5	8.8	9.0	10.1	8.5	9.9	9.1 cd
Yuma citrange	10.0	12.6	9.5	10.8	11.0	9.1	10.5 bcd
Swingle citrumelo	6.6	7.4	10.4	8.7	9.5	9.6	8.7 d
Mean	9.1 b	9.5 b	11.6 ab	12.0 a	9.9 ab	11.4 ab	

*Rootstock and treatment means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

performed in a hotter climate (15, 16). Three trees, however, also showed pitting symptoms. Yamaguari and English selections showed Gum Pocket symptoms on only two trees. None of the trifoliate hybrids displayed any symptoms. However, in a previous study gum-pocket symptoms were noted on Swingle citrumelo (15).

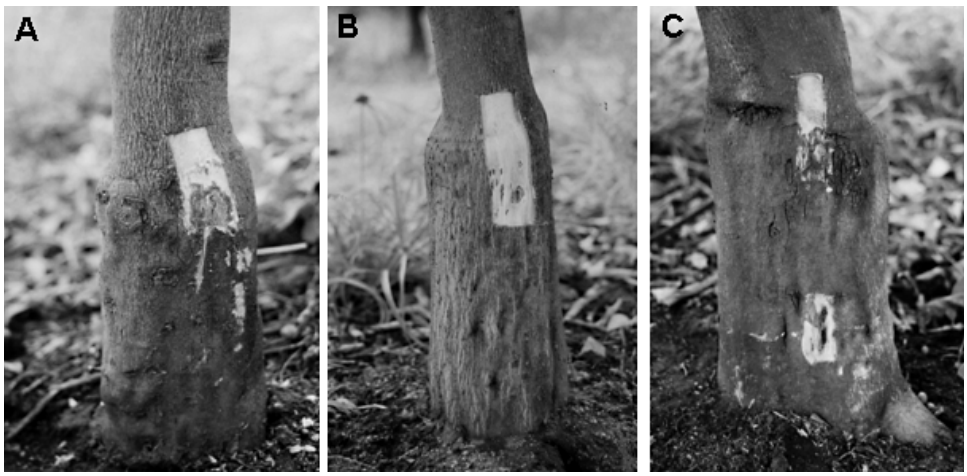

Fig. 1. Symptoms observed in trifoliate orange rootstocks seven years after inoculation with CVd-III isolates. (A) Typical Gum Pocket symptoms with dead bark patches observed in Jacobsen trifoliate orange inoculated with isolate CD 47. (B) Pitting symptoms showing no dead bark but necrosis at the cambium observed in Christian trifoliate orange inoculated with isolate CD 6. (C) Gum Pocket and pitting symptoms observed in trifoliate orange Kryder 55-1 inoculated with isolate CD 6. Note the girdling effect at the bud union.

TABLE 7
PRESENCE OF GUM POCKET (GP) AND PITTING (P) SYMPTOMS ON DIFFERENT TRIFOLIATE ROOTSTOCKS INOCULATED WITH DIFFERENT GRAFT TRANSMISSIBLE ISOLATES

	Graft transmissible isolate													
	LMS 6 CTV		CD 4 CTV		CD 6 CVd-III		CD 12 CVd-III		CD 22 CVd-III		CD 47 CVd-III		TOTAL	
Rootstock	GP	P	GP	P	GP	P	GP	P	GP	P	GP	P	GP	P
Rich	0*	0	0	0	0	2	2	0	0	0	2	0	4	2
Argentina	0	0	0	0	0	1	3	1	0	1	3	0	6	3
Kryder 55-1	0	0	0	0	1	1	2	1	1	2	2	1	6	5
Christian	0	0	0	0	2	0	1	2	0	2	0	1	3	5
Yamaguari	0	0	0	0	1	0	1	0	0	1	0	0	2	1
Rich 22-2	0	0	0	0	0	0	2	2	0	1	4	0	6	3
English	0	0	0	0	0	0	0	2	0	0	2	0	2	2
Rubidoux	0	0	0	0	0	2	0	1	0	0	0	0	0	3
Jacobsen	0	0	0	0	0	3	4	0	0	4	3	1	7	8
Benton citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Troyer citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yuma citrange	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swingle citrumelo	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total (+/65)	0	0	0	0	4	9	15	9	1	11	16	3		

*Figures indicate the number of trees showing Gum Pocket (GP) and Pitting (P) symptoms divided by the number of trees.

None of the control trees with LMS 6 or those with CD 4 (CTV) showed disease symptoms. All CVd isolates induced Gum Pocket and/or pitting symptoms, with CD 12 and CD 47 inducing Gum Pocket symptoms on the greatest number of trees.

CONCLUSION

- The CVd isolates contained CVd-IIIb (sequence size and homology) and induced Gum-pocket disease on the trifoliate orange rootstock.
- The CVd-IIIb isolates, although showing only minor sequence variations, differed significantly in their effect on tree size and fruit yield as well as in inducing Gum-pocket disease symptoms.
- Trifoliate orange selections differed in the number of trees showing Gum-pocket disease symptoms, but there was no difference between large flower and small flower types.
- None of the trifoliate orange hybrids showed gum-pocket disease symptoms and the CVd-III isolates were less efficient in controlling tree size.
- None of the CVd-IIIb isolates that were evaluated is suitable for tree size control in high-density plantings on a commercial scale in South Africa.

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