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Low Incidence of Huanglongbing Fruit Symptoms in Valencia Sweet Orange Trees in the Presence of a Population of Citrus Tristeza Virus

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ABSTRACT. Huanglongbing (causal agent is *"Candidatus* Liberibacter africanus") (HLB) remains a limiting factor for citrus production in the cooler areas of Southern Africa notwithstanding present control measures. These include the establishment of new orchards with certified plant material, chemical control of the insect vector, *Trioza erytreae*, and the removal of infected plant material. Delta Valencia on Yuma citrange rootstock was planted in 1985 with GXI [greening cross-protecting isolate, formerly citrus dwarfing isolate 4 (CD 4)] as one of the treatments for dwarfing. HLB was first observed in 1988 and by 1990 fruit symptoms had increased to 100% in some trees which forced the termination of the dwarfing trial. Infected branches were removed by pruning and HLB continued to be monitored in 1991, 1993, 1996 and 1998 in the control trees and those with GXI. The percentage HLB fruit symptoms remained at a low level in the trees with GXI. Several attempts were made to identify the agent(s) that is present in GXI. Biological indexing was employed for citrus viroids, citrus tristeza, citrus psorosis, citrus impietratura and citrus tatter leaf. Only citrus tristeza virus (CTV) was found to be present in this isolate. Mass and single aphids were used to transfer the CTV component from GXI. The mass-aphid as well as the single-aphid sub-isolates reacted differently on Mexican lime, suggesting different strains within the GXI isolate. Differentiation between sub-isolates was also shown by amplifying the coat protein gene by the reverse transcriptase polymerase chain reaction (RT-PCR) followed by restriction fragment length polymorphism (RFLP) and single-strand conformational polymorphism (SSCP) techniques.

Huanglongbing (causal agent is *"Candidatus* Liberibacter africanus") (HLB) (greening) remains a limiting factor for citrus production in the cooler areas of Southern Africa notwithstanding present control measures. These include the establishment of new orchards with certified plant material, chemical control of the insect vector, *Trioza erytreae*, and the removal of infected plant material (2).

A transmissible agent was obtained in the early 1980's from a 25-yr-old Valencia tree on *Poncirus trifoliata* rootstock in a row of navels planted as a guard row of an experimental block at the research institute at Nelspruit. The exact scion and rootstock cultivars are unknown. The tree was small for its age, approximately two meters high and it was thought to carry a transmissible dwarfing factor, which was the original reason for its selection. No symptoms were observed on the rootstock that would indicate the presence of viroids. The canopy appeared healthy, with little sign of HLB infection, although the adjacent navel trees showed severe decline due to HLB infection.

In the mid 1980's this isolate was incorporated in a dwarfing trial as a possible dwarfing agent. Delta Valencia on Yuma citrange rootstock was planted in 1985 with GXI [greening cross protecting isolate, formerly citrus dwarfing isolate 4 (CD 4)] as one of the treatments for dwarfing. HLB was first observed in 1988 and by 1990 fruit symptoms increased to 100% in some trees of the other treatments of dwarfing isolates, that forced the termination of the dwarfing trial. Infected material was removed by pruning and HLB continued to be monitored in 1991, 1993, 1996 and 1998 in the control trees and those with GXI (Table 1). The percentage HLB fruit symptoms remained at a low level in the trees with GXI.

TABLE 1
THE PERCENTAGE FRUIT WITH HUANGLONGBING (HLB) SYMPTOMS OF DELTA VALENCIA TREES INOCULATED WITH DIFFERENT DWARFING ISOLATES DURING 1990 AND THE OCCURRENCE OF THE DISEASE IN FOLLOW UP YEARS IN CONTROL TREES AND GXI CITRUS TRISTEZA VIRUS ISOLATE INOCULATED TREES

	Dwarfing isolates				
Year	Control	GXI(CD4)	CD8	CD9	CD10
1990	4[z]	0	35	29	14
1991	19	2	—[y]	—	—
1993	27	1	—	—	—
1996	46	1	—	—	—
1998	26	11	—	—	—

[z]Infected branches were removed each year after HLB assessment.
[y]Trees removed due to high HLB infection.

Glasshouse work was done in an attempt to identify the agent(s) that is present in GXI. Biological indexing was employed for citrus viroids, citrus tristeza, citrus psorosis, citrus impietratura and citrus tatter leaf (10). Except for citrus tristeza virus (CTV), none of the other agents was found to be present in this isolate.

Mass and single aphids were used to transfer the CTV component from GXI. The mass-aphid as well as the single-aphid sub-isolates reacted differently on Mexican lime and grapefruit, suggesting different strains within the GXI isolate (Table 2). Differentiation between sub-isolates was also shown by amplifying the coat protein gene by the reverse transcriptase polymerase chain reaction (RT-PCR) (9) followed by restriction fragment length polymorphism (RFLP) (6) and single-strand conformational polymorphism (SSCP) tech-

TABLE 2
THE EFFECT OF SUB-ISOLATES OF GXI CITRUS TRISTEZA VIRUS (CTV) ISOLATE ON THE GROWTH OF MEXICAN LIME AND MARSH GRAPEFRUIT SEEDLINGS AND THE COMPARISON OF THE ISOLATES BY MOLECULAR CHARACTERIZATION[z]

Isolate or subisolate	Seedling growth (mm)				Molecular characterization[y]	
	Mexican lime[z]		Marsh grapefruit[z]		RFLP	SSCP
GXI-original	385	bc	105[x]	bc	– 2 3 4 5	1 2 3 4 5 6 7 8 9 10
GXI-CTV1[w]	185	a	203	ab	– 2 3 4 5	1 2 3 4 5 6 7 8 9 10
GXI-CTV2[w]	403	c	196	ab	– 2 3 – –	– – – – 5 6 – – 9 10
GXI-CTV3[w]	120	abc	155[x]	abc	– 2 3 4 5	1 2 – – 5 6 7 8 9 10
GXI-CTV4[w]	265	a	97	c	– 2 – 4 –	1 2 – – – – – 8 9 –
GXI-CTV5[v]	365	a	209	a	– 2 3 – –	– – – – 5 6 – – 9 10
GXI-CTV6[v]	368	abc	97	c	– 2 3 – 5	1 2 – – 5 6 – 8 9 10
GXI-CTV7[v]	282	ab	194	abc	– 2 3 – –	– – – – 5 6 – – 9 10
GXI-CTV8[v]	313	ab	189	abc	– 2 3 4 –	– – 3 4 5 6 – – 9 –
GXI-CTV9[v]	353	ab	173[x]	abc	1 – – 4 –	– – 3 4 – – – – 9 10
GXI-CTV10[v]	340	a	126	abc	– 2 3 4 –	– – 3 4 5 – – – 9 10

[z]Figures in each column which are followed by the same letter do not differ significantly at the 5% level (LSD).
[y]Similar RFLP (Fig. 1) and SSCP (Fig. 2) bands for each isolate are indicated by the same number.
[x]Positive seedling yellows reaction.
[w]Mass aphid transfers.
[v]Single aphid transfers.

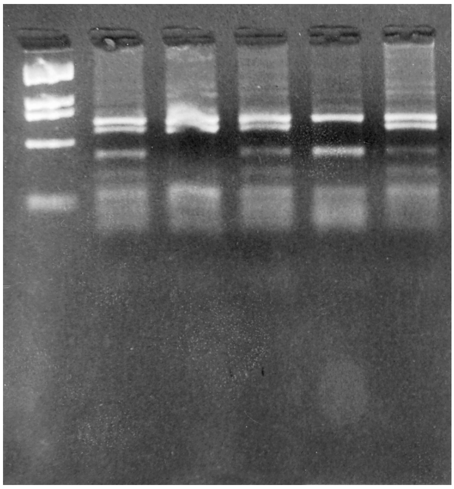

Fig. 1A

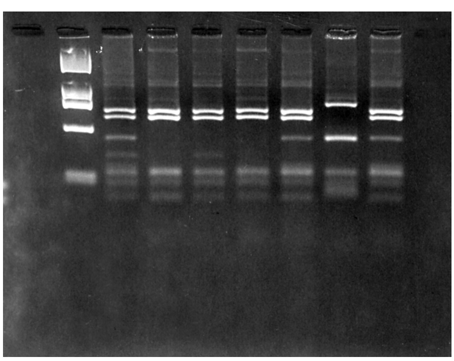

Fig. 1B.

Fig. 1. RFLP groups of mass aphid transmissions (A) and single aphid transmissions of the GXI-CTV coat protein gene defined by HinfI digestion. A. Mass aphid transmissions are in lane 1: pUC19 digested with HinfI; lane 2: GXI-CTV1; lane 3: GXI-CTV2; lane 4: GXI-CTV3; lane 5: GXI-CTV4; lane 6: GXI original. B. Single aphid transmissions are in lane 1: pUC19 (HinfI) marker; lane 2: GXI original; lane 3: GXI-CTV5; lane 4: GXI-CTV6; lane 5: GXI-CTV7; lane 6: GXI-CTV8; lane 7: GXI-CTV9; lane 8: GXI-CTV10. Marker bands ranging in size from 1419, 517, 396, 214, 75 and 65 base pairs. Restriction digests were separated on 4% agarose gels.

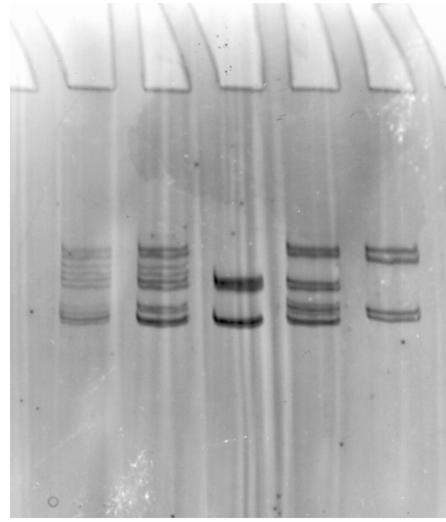

Fig. 2A

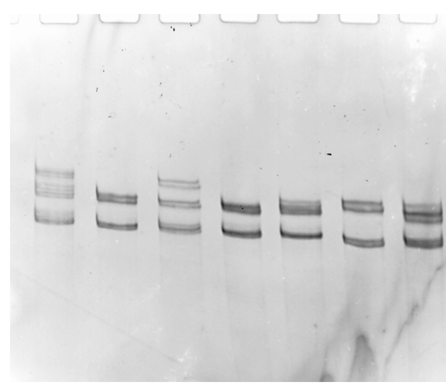

Fig. 2B.

Fig. 2. SSCP patterns of mass aphid transmissions (A) and single aphid transmissions (B) of the GXI-CTV coat protein gene. A. Mass aphid transmissions are lane 1: GXI original; lane 2: GXI-CTV1; lane 3: GXI-CTV2; lane 4: GXI-CTV3; lane 5: GXI-CTV4. B. Single aphid transmissions are lane 1: GXI original; lane 2: GXI-CTV5; lane 3: GXI-CTV6; lane 4: GXI-CTV7; lane 5: GXI-CTV8; lane 6: GXI-CTV9; lane 7: GXI-CTV10. Electrophoresis under non-denaturing conditions was performed at room temperature at 300V for 2h in 8% acrylamide gels with 5% glycerol. Gels were stained with silver nitrate.

niques (12). Biological differences were detected among sub-isolates and between the original isolate and sub-isolates transmitted by mass aphids. Molecular characterization (Table 2, Figs. 1 and 2) revealed even greater variation among the sub-isolates. It appears that at least five groups exist among the single aphid sub-isolates and their abilities to protect against HLB, singly or in combination, will be investigated.

Apart from cross protection by mild virus isolates of the same virus, several cases have been reported where protection was afforded by non-related viruses as well as non-related pathogens. Koizumi and Sasaki (8) reported cross protection against CTV, a closterovirus (1), by citrus vein enation virus, a luteovirus (4). Apple mosaic virus in plum trees was suppressed by the presence of plum dwarf virus (5). In the first cross protection studies to control tomato mosaic virus, a mild strain of tobacco mosaic virus was used (7). The inoculation of citrus viroids in sweet orange induced greater resistance to *Phytophthora* infection (11).

The presence of a virus or biological control agent may protect against fungal infection (13, 15) or may promote infection (14). Chen et al. (3) rarely found the HLB bacterium and CTV in the same plant cell. The reasons for these phenomena are unknown but may be the result of chemical changes in the plant cell, the production of protective substances on the surface of the host or the production of substances in the plant tissue which prohibit entrance or multiplication of the pathogen (2, 11, 15).

Several formal and commercial trials have been initiated for further evaluations of GXI as a protector against HLB as well as against severe CTV. Investigations into the characteristics of the sub-isolates are also continuing.

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