Huanglongbing (Greening) Detection in South Africa

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ABSTRACT. Thirty leaf samples collected from citrus orchards in South Africa in December 1993 were tested by DNA-DNA hybridization with probes In 2.6 and As 1.7 specific for Liberobacter asiaticum and L. africanum, respectively. All samples showing Huanglongbing (HLB) (greening) symptoms gave positive hybridizations with probe As 1.7. None reacted with In 2.6. Leaves of a citrus relative, *Toddalia lanceolata*, on which leaf distortions caused by larvae of the HLB vector, *Trioza erytreae*, were seen, also gave a positive hybridization with As 1.7. Four year-old Nules Clementine trees showing stunting and small yellow leaves but no mottle gave strong hybridization signals with As 1.7. The presence of the HLB Liberobacter in these trees was confirmed by electron microscopy. Forty-two samples collected in June 1994 were also analyzed by DNA-DNA hybridization and PCR. Among the 25 trees showing mottle or yellow vein symptoms, 20 were positive and five were negative with the two techniques. The 17 samples from symptomless trees gave negative results by PCR and hybridization.

In recent years, various serological and molecular reagents for the detection of "*Candidatus* Liberobacter africanum", the Huanglongbing (HLB) (greening) liberobacter species occurring in Africa (2), have been produced. These include monoclonal antibodies (1, 4), DNA probe

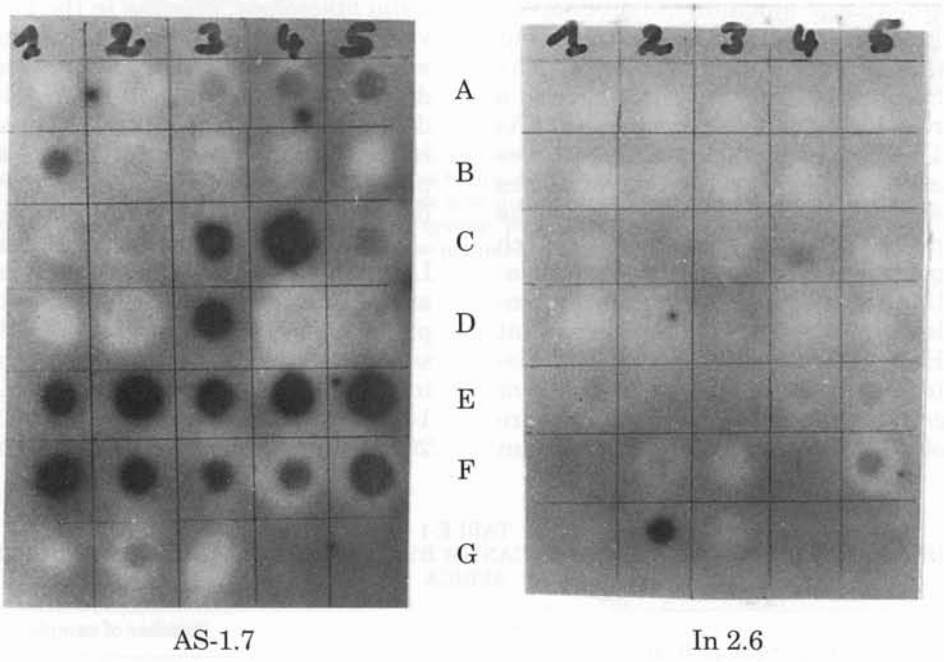

Fig. 1. Dot blot hybridization at high stringency, of probe As 1.7 (left) and In 2.6 (right) with the DNAs extracted from citrus leaves of field trees in South Africa (see text). G1: DNA from healthy, greenhouse grown citrus leaves; G2, left and G3 right: DNA extracted from greenhouse grown citrus leaves infected with L. africanum (South Africa); G3, left and G2, right: DNA extracted from greenhouse grown citrus leaves infected with L. asiaticum (India).

As 1.7 (6) and specific primers for DNA amplification by PCR (5). These reagents were used to detect L. africanum in citrus samples from various orchards in South Africa in the study reported here.

Thirty leaf samples were collected in December 1993 in citrus orchards in Northern Province (Letaba Estate), Mpumalanga (Nelspruit and Schoemans Kloof Valley) and Gauteng (Pretoria) and tested by DNA/DNA hybridization, with probe As 1.7, specific for L. africanum, and probe In 2.6, specific for L. asiaticum (7). None of the samples reacted with probe In 2.6, whereas all samples showing HLB symptoms reacted with probe As 1.7 (Fig. 1, Table 1). Because there is 74% homology between the two probes (3, 6), a faint cross-hybridization is noticed with probe In 2.6 when large amounts of heterologous L. africanum DNA were present (Fig. 1, In 2.6, C4, E4, E5, F2, F5). Samples A4, A5, B1, which showed a relatively faint signal on Fig. 1 (As 1.7), came from symptomless leaves collected in Letaba, on branches which were symptomatic during the previous winter. Sample C3, which gave a relatively strong hybridization signal, was also from symptomless Valencia sweet orange leaves (at Haffenden farm) on which leaf distortion induced by larvae of *Trioza erytreae* (Del Guercio) were observed. Sample C4 came from an adjacent tree with severe leaf mottle. All samples in row E which were collected at Hall and Sons' farm (Nelspruit) show strong hybridization signals. They had the characteristic symptoms of HLB. Samples F1, F2 and F3, were collected on 4 yr-old Nules Clementine trees grafted on *C. volkameriana*, at Bakgat farm, in Schoemans Kloof. The trees were severely stunted, with pale green leaves occasionally showing some zinc deficiency. No leaf mottling could be observed on these trees. As HLB has never been described in Clementines and as the symptoms were not characteristic, we performed electron microscopy on sample F1. Numerous liberobacters could be observed in the phloem, in agreement with the positive hybridization result. Finally, leaf sample F4 came from a citrus relative, *Toddalia lanceolata*, growing in the University of Pretoria garden and on which many *T. erytreae*-induced leaf deformations were observed. The deformations and the positive hybridization indicate that this plant is not only a host for the psyllid but also for the HLB liberobacter.

In 1994, 42 samples from the Letaba and Nelspruit areas were analyzed by both hybridization with probe As 1.7, and PCR (Table 1), 33 samples were also analyzed by immunofluorescence (IF) with MA 14A1 (Table 2). As shown in Table 1, 20 of 21 symptomatic samples were

TABLE 1
DETECTION OF LIBEROBACTER AFRICANUM BY HYBRIDIZATION AND PCR IN SOUTH AFRICA

Huanglongbing (HLB) or HLB-like symptoms	Hybridization		PCR	Number of samples	
	In 2.6	As 1.7		1993	1994
+	-	+	ND[z]	27	
+	-	+	+		20
+	-	-	-		1
+[y]	-	-	-		4
-	-	-	-	3	17

[z]ND = Not done
[y]Yellow vein but no mottle

TABLE 2
DETECTION OF LIBEROBACTER AFRICANUM BY HYBRIDIZATION, PCR AND IMMUNOFLUORESCENCE IN 1994 IN SOUTH AFRICA

Huanglongbing (HLB) or HLB-like symptom	Hybridization	PCR	Immunofluorescence[a]	No. samples
+	+	+	+	16
+	+	+	-	2
+	-	-	-	4
-	-	-	-	11

[a]MA 14A1

positive by hybridization, and they were also positive by PCR. Four samples from leaves showing yellow vein but no mottle, as well as 17 samples collected on symptomless trees, were negative with both techniques.

As shown on Table 2, 18 samples, that were positive by PCR and hybridization, were tested by IF. Sixteen of the samples gave a positive immunofluorescence in the phloem, and two were negative. There were also 15 samples that tested negative by both PCR and hybridization; they were similarly negative by the serological method.

CONCLUSION

DNA/DNA hybridization and PCR have been used successfully for the detection of L. africanum in various citrus orchards in South Africa, with a similar sensitivity. The presence of the bacterium was even detected in samples showing no symptoms at the time when they were collected, but coming from branches known to be affected by HLB. L. asiaticum was not detected.

Clementine trees have been shown for the first time to be infected by the HLB liberobacter; the trees were stunted, but showed no leaf mottling at the time. *Toddalia lanceolata,* a good host for *T. erytreae,* was found to be infected by the liberobacter and is therefore, a reservoir plant for the pathogen.

The results of IF show that at least two serotypes of L. africanum occur in South Africa, one reacting with MA 14A1, the other giving no reaction. The first seems to be predominant as MA 14A1 detected it in 16 out of 18 samples. Hence, IF with MA 14A1 can be helpful to identify some infected trees. However, it cannot be used as a diagnostic tool because it does not detect all serotypes present.

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