Presence of "*Candidatus* Liberibacter africanus" in the Western Cape Province of South Africa

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ABSTRACT. For many years, in South Africa, Huanglongbing (HLB) was restricted to the Eastern part of the country (Rustenburg, Mpumalanga, KwaZulu-Natal) and absent from the Eastern (EC) and Western Cape (WC) provinces. In November 1994, suspicious symptoms were observed on clementine trees in the Stellenbosch area (WC). In 1996 and 1997, leaf samples from WC orchards were sent to Bordeaux for analysis and found to be infected by "*Candidatus* Liberibacter africanus". To confirm these results, 82 samples were collected by some of us in April 1998, and tested by PCR. The presence of "*Candidatus* L. africanus" in Stellenbosch/Paarl areas of WC was confirmed in clementine, grapefruit, and lemon. Mottled leaves of Cape chestnut (*Calodendrum capense*), a wild rutaceous tree also gave positive PCR tests.

Symptoms of Huanglongbing (HLB) were first reported in South Africa in the Rustenburg region in the late 1920s. The disease was known to be present in the citrus-growing regions of the North West Province, Mpumalanga and KwaZulu-Natal. For many years HLB was thought to be absent from the Eastern (EC) and Western Cape (WC) provinces. In November 1994, suspicious symptoms were observed on clementine trees in the Stellenbosch area (WC). In 1996 and 1997, leaf samples from WC orchards were sent to Bordeaux and tested by PCR using primers OI1/OA1/OI2c for amplification of the 16S rDNA (3). A DNA band could be amplified from some samples and upon digestion with *Xba*I, the characteristic profile of "*Candidatus* Liberibacter africanus" was evidenced. To confirm these results, 82 samples were collected by some of us (J. M. B., M. G., H. F. L.) in April 1998: 31 samples (mainly sweet orange) came from the Rustenburg and Nelspruit areas; five from EC; and 36 from WC. Sampling was quite difficult to achieve in the WC compared to the Nelspruit area. Indeed many of the clementine trees had strong mottling symptoms induced by the staples used to fix ant bands to the trunks. In addition, WC is the first area in the world where Clementine trees have been affected by HLB and, in this area, a Mediterranean type climate is encountered, however, HLB generally occurs under tropical climate. Previous observations that we had made in 1996, indicated that HLB-infected Clementine trees show stunting and pale green leaves without mottle (4).

Each sample used for PCR detection of the HLB liberibacter was tested with two sets of primers: (i) primers for 16S rDNA amplification (PCR16S) (3); and (ii) primers for amplification of the ß operon (PCR *rpl*) (2). In addition, most samples were subdivided into two halves. One half was analyzed by M. G. and J. M. B. (Bordeaux) the other by C. P. R. C. (South Africa). Results are presented in Table 1 for the samples from the Rustenburg and Nelspruit areas; and in Tables 2, 3 and 4 for those from EC and WC. There was very good agreement between the results obtained by the two groups of investigators, even though the Bordeaux team was using a quick DNA purification procedure on wizard columns (3) while C.P.R.C. used a much longer DNA purification procedure with chloroform/isoamyl alcohol. As seen on the tables, PCR *rpl* based on the amplification of the ß operon is more sensitive than PCR 16S as was already mentioned (2). Only "*Candidatus* L.

TABLE 1
PCR TESTING FOR "*CANDIDATUS* LIBERIBACTER AFRICANUS" FROM CITRUS SAMPLES TAKEN FROM RUSTENBURG AND NELSPRUIT AREAS

Sample	Cultivar[z]	Age (yr)	Symptoms	PCR 16S[y]	PCR RPL[y]
1	Amanzi/TR	22	Mottle	+	+
2	Amanzi/TR	22	Mottle	+	+
3	Eureka/RL	22	Mottle	—	f
4	Val/RL	10	Dieback	—	—
5	Eureka/Volka	5	Yellowing	—	—
6	Eureka/Volka	5	Yellowing	—	—
7	Growweskil	0.5	Yellowing	—	—
8	Eureka/RL	1.5	Yellowing	—	—
9	Bahianina/SW	1.5	Yellowing	—	-—
10	Eureka/RL	14	Mottle	+	+
11	Eureka/RL	14	Mottle	f	+
12	Val Late/RL	20	Mottle	+	+
13	Val Late/RL (= 12)	20	Mottle	+	+
14	Delta/SW89	10	Mottle	+	+
15	Sample lost		Mottle	nd	nd
16	Val Late/RL	15	Zn-like def	—	-—
17	Nova/Carrizo	6	Mottle	+	+
18	Nulles/Volka	10	Yellow	—	—
19	Nulles/Volka	10	Small leaves	—	+
20	Delta/Val/Yuma	13	Mottle	+	+
21	Eureka	8	Mottle	f	+
22	Val/RL	5	Mottle	+	+
23	Bahianina/Mandarin	5	Mottle	+	+
24	Bahianina/Mandarin	5	Symptomless	—	—
25	Palmer Nav/Mandarin	25	Mottle	+	+
26	Val/RL	30	Mottle	+	+
27	Old Clone Val/RL	6	Mottle	—	—
28	Val/RL	5	Mottle	f	+
29	Val/RL	5	Mottle	+	+
30	Val/RL	20-30	Mottle	+	+
31	Val/RL	20-30	Mottle	nd	+

[z]Cl = Clementine, Nav = navel, RL = rough lemon, TR = Troyer citrange, Trif = Trifoliate orange, Val = Valencia, Volka = Volkamer lemon.
[y]nd = not done, f = faint.

africanus" was found to be present in the samples collected, including those from Malelane (380 m altitude). The presence of HLB in Stellenbosch/Paarl area of WC was confirmed in Clementine, grapefruit, and lemon trees. Many of the Clementine trees that we have sam-

TABLE 2
PCR TESTING FOR "*CANDIDATUS* LIBERIBACTER AFRICANUS" FROM CITRUS SAMPLES TAKEN FROM EASTERN CAPE: UITENHAGE FOUNDATION BLOCK

Sample	Cultivar	Age (yr)	Symptoms	PCR 16S	PCR RPL
32	Mexican lime	12	Mottle	—	—
33	Novel/Volka R1	12	Stunt	—	—
34	Fino L/Volka X8	12	Bumps	—	—
35	Fino L/Volka W8	12	Mottle, bumps	—	—
36	Nova/Swingle	6	Bumps	—	—

TABLE 3
PCR TESTING FOR "*CANDIDATUS* LIBERIBACTER AFRICANUS" FROM CITRUS SAMPLES TAKEN FROM WESTERN CAPE: PAARL, WEMMERSHOEK AREAS

Sample	Cultivar[z]	Age (yr)	Area/Farm	Symptoms	PCR 16S	PCR RPL[y]
37	Nules Cl/Carrizo	5	Paarl/D.Clift farm	Lopsided fruit	+	+
38	Nules Cl/Carrizo	5	Paarl/D.Clift farm	Lopsided fruit	—	+f
39	Eureka L./Volka	25	Wemmershoek/Deltamer farm	Mottle	+	+
40	Eureka L./Volka	25	Wemmershoek/Deltamer farm	Mottle	—	+f
41	Eureka L./Volka	25	Wemmershoek/Deltamer farm	Mottle	—	+f
42	Nules Cl/Troyer		Wemmershoek/Deltamer farm	Stunt	—	—

[z]Cl = Clementine.
[y]f = faint.

pled were negative, corresponding to those with mottle symptoms induced by staples. Compared to the HLB-induced mottle which is observed on leaves of reduced size, this staple-induced mottle was seen on well expanded leaves. "*Candidatus* L. africanus" was also detected in

TABLE 4
PCR TESTING FOR "*CANDIDATUS* LIBERIBACTER AFRICANUS" FROM CITRUS SAMPLES TAKEN FROM WESTERN CAPE: STELLENBOSCH AREA

Sample	Cultivar[z]	Age (yr)	Area/Farm	Symptoms	PCR 16S[y]	PCR RPL[y]
43	*Calodendrum capense*	6-8	Dombeya	Mottle	+	+
44	Nules Cl./Troyer		"	Mottle	—	—
45	*Calodendrum capense*	8	"	Mottle	+	+
46	Nules Cl./Troyer		"	Mottle	—	—
47	Nules Cl./Troyer		"	Yellow shoot	—	—
48	Nules Cl./Troyer		"	Yellow shoot	—	—
49	Nules Cl./Troyer		"	Yellow vein	—	—
50	Sat. Mihowasi/Trif	7	"	Stunt, mottle	—	—
51	Eureka L./RL	15	"	Mottle	—	+f
52	Eureka L./RL		"	Mottle	—	+f
53	Eureka L./RL		"	Mottle	—	—
75	Nules Cl./Troyer		"	Yellow shoot	—	—
76	Nules Cl./Troyer		"	Yellow shoot	—	—
77	Nules Cl./Troyer		"	Small leaves	—	—
78	Nules Cl./Troyer		"	Yellow shoot	—	—
54	Nules Cl./Troyer		Blyheck	Mottle	+	+
55	Nules Cl./Troyer		"	Yellow shoot	—	—
56	Nules Cl./Troyer		"	Mottle, -Zn	+f	+
57	Nules Cl./SRA 88	12	Roos	Mottle, -Zn	+f	+
63	Nules Cl./Troyer		Hunting	Yellow shoot	—	—
64	Clem SRA/Troyer		Schoongesicht	Yellow shoot	—	—
65	Clem SRA/Troyer		"	Yellow shoot	—	—
66	Clem SRA/Troyer		"	Yellow shoot	—	—
67	Clem SRA/Troyer		"	Mottle	+	+
68	*Calodendrum capense*	30	"	Mottle	+	+
69	Nules Cl./Troyer		"	Mottle	—	+
70	Nules Cl./Troyer		"	Mottle	—	+
71	Clem SRA/Troyer		"	Mottle	+	+

[z]Cl = Clementine, Trif = Trifoliata orange.
[y]f = faint.

Cape chestnut, a wild rutaceous tree showing leaf mottle symptoms. Leaves from a symptomless Cape chestnut tree growing in the botanical garden of Cape Town tested negative by PCR. Recently, we have further characterized the liberibacter present in Cape chestnut and found it to be different from the two liberibacter species infecting citrus. Whether it represents a new species or subspecies is under study. With a reliable PCR technique now available to test for liberibacter in South Africa, problems in the WC, such as extent of HLB, symptomatology of HLB in Clementine and satsuma trees, and feasibility of HLB eradication, can be studied.

Note added in proof: This new liberibacter has been assigned a subspecies designation "*Candidatus* L. africanus subsp. capensis" and specific primers have been designed for its detection (1).

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