Huanglongbing in Cambodia, Laos and Myanmar

M. Garnier and J. M. Bové

ABSTRACT. Surveys conducted in Cambodia, Laos, and Myanmar have shown that several citrus species were showing HLB-like symptoms. PCR analysis of leaf midrib samples indicated that "Candidatus Liberibacter asiaticus" infects various citrus cultivars in all the sites visited. Diaphorina citri, the Asian psyllid vector of HLB, was also seen in Cambodia, Laos and Myanmar.

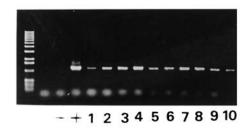
Huanglongbing (HLB) has been shown previously to be present in several south and south-east Asian countries, namely Bangladesh, India, China, Vietnam, Thailand, and Malaysia (1) We have now obtained evidence for the occurrence of HLB in three additional countries in South East (SE) Asia. Collection of citrus leaf samples showing HLB-like symptoms such as mottle were carried out in Cambodia in 1995, in Myanmar (formerly Burma) in 1996

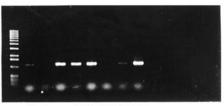
and in Laos in 1997. The samples were brought back to Bordeaux and tested by DNA/DNA hybridization (4) for the samples from Cambodia and by PCR with primers OI1/OI2C/OA1 (2, 3) for the samples from Laos and Myanmar. Examples of the PCR results are shown on Fig. 1 (top) for Myanmar samples. PCR results are presented on Tables 1 and 2. Pummelo near Phnom Penh and Siem Reap in Cambodia were found positive by DNA/DNA hybridization

TABLE 1
PCR-DETECTION OF "CANDIDATUS LIBERIBACTER ASIATICUS" IN MYANMAR

Region	Cultivar	Sample Number	PCR
MANDALAY			
Moemeik	Lime	1	+
Myitngde	Lime	2	2+
KALAW	Mandarin seedling	3	2+
PINDAYA/ZAYDAN	Mandarin/RL	4	3+
"	Rough lemon	5	2+
"	Lime	6	2+
"	Mandarin	7	3+
"	Mandarin	8	2+
ű	Mandarin	9	+
AUNGBAN	Navel	10	+
"		11	+
"		12	_
INLE			
Kyasar	Rough lemon	13	3+
"	Mandarin	14	3+
Inle	Lime	15	3+
PEGU/SARLAY	Pummelo	16	_
"	Pummelo	17	+
u	Lime	18	3+

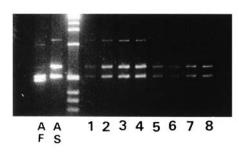
PCR DETECTION OF CANDIDATUS LIBEROBACTER ASIATICUM IN CITRUS FROM MYANMAR

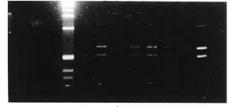




11 12 13 14 15 16 17 18

Xba1 DIGESTION OF PCR AMPLICONS





9 10 11 13 14 15 17 18

Fig. 1. PCR detection of "Candidatus Liberibacter asiaticus" in citrus from Myanmar.

with probe In 2.6 specific for "Candidatus Liberibacter asiaticus". Pummelo, lemon and lime near Vientiane and Louangprabang in Laos, and lemon, lime, mandarin, pummelo, rough lemon and sweet orange near Mandalay, Pindaya, Kalaw, Inle and Pegu in Myanmar gave positive reactions by PCR.

After digestion of the amplified DNAs with restriction enzyme XbaI, all the DNA profiles corresponded to "Candidatus L. asiaticus" as illustrated in Fig. 1 (bottom) for Myanmar samples. $Diaphorina\ citri$, the Asian psyllid vector of HLB, was found in all places visited.

TABLE 2 PCR-DETECTION OF "CANDIDATUS LIBERIBACTER ASIATICUS" IN LAOS

Region	Cultivar	PCR
Vientiane	Pummelo	2+
"	Pummelo	3+
«	Lime	+
u	Pummelo	+
Louang Prabang	Pummelo	+
"	Murraya paniculata	_
"	Lemon	2+
u	Pummelo	_

The presence of HLB and *D. citri* was expected in the above three countries as they are surrounded by countries where the disease and its vector are known to occur. As HLB was found at all places visited, its

geographical distribution must be large and probably includes areas that could not be surveyed. This is the first report of the presence of HLB and of its psyllid vector in these three countries.

LITERATURE CITED

- 1. Garnier M. and J. M. Bové
 - 1996. Distribution of the huanglongbing (greening) liberobacter species in fifteen African and Asian countries. In: *Proc. 13th Conf. IOCV*, 388-391. IOCV, Riverside, CA.
- 2. Jagoueix S., J. M. Bové, and M. Garnier
 - 1996. PCR detection of the two liberobacter species associated with greening disease of citrus. Mol. Cell. Probes 10: 43-50.
- 3. Jagoueix S., J. M. Bové, and M. Garnier
 - 1996. Techniques for the specific detection of the two huanglongbing (greening) liberobacter species: DNA/DNA hybridization and DNA amplification by PCR. In: *Proc. 13th Conf. IOCV*, 381-384. IOCV, Riverside, CA.
- 4. Villechanoux S., M. Garnier, J. Renaudin, and J. M. Bové
 - 1992. Detection of several strains of the bacterium-like organism of citrus greening disease by DNA probes. Curr. Microbiol. 24: 89-95.