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DETECTION OF ‘CANDIDATUS LIBERIBACTER ASIATICUS’ IN CACOPSYLLA (PSYLLA) CITRISUGA (HEMIPTERA: PSYLLIDAE)

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ABSTRACT

Nymphs of the pomelo psyllid, Cacopsylla (Psylla) citrisuga Yang & Li, were collected from huanglongbing (HLB) symptomatic lemon trees, Citrus limon (L.) in Yunnan Province, China. DNA samples extracted from groups of 2-10 pomelo psyllid nymphs of all stages and from leaves of lemon plants were analyzed with nested-PCR in order to detect ‘Candidatus Liberibacter asiaticus’ (Las). The results showed that 24 out of the 49 lemon trees were Las-positive. Also psyllid nymphs collected from 10 out of the 24 Las-positive plants were Las-positive. DNA extracted from individual late stage nymphs (3rd to 5th instars) collected from Las-infected trees were then subjected to nested-PCR trials. Twelve out of the 29 nymphs proved to be Las positive. All nymphs collected from Las-negative lemon trees were also Las-negative. Most psyllid samples which showed positive in nested-PCR were also positive in conventional-PCR detection. The amplified fragment of the 16S rRNA gene of ‘Ca. Liberibacter spp.’ from positive psyllid samples was 99% similar to those of Las strain psy62 in GenBank. These results demonstrate that Cacopsylla (Psylla) citrisuga is another Las carrier insect. Transmission studies are underway to determine whether Las-positive psyllids can transmit Las to healthy citrus.

Key Words: pomelo psyllid, Huanglongbing, PCR, ‘Candidatus Liberibacter asiaticus’, detection

RESUMEN

Ninfas del psílido del pomelo, Cacopsylla (Psylla) citrisuga Yang & Li, fueron colectadas de árboles de limón, Citrus limon (L.), con síntomas de huanglongbing (HLB) en la provincia de Yunnan, China. Muestras de ADN extraídas de grupos de 2-10 ninfa de todos los estadios del psílido, y de hojas de limonero, fueron analizadas mediante PCR- anidada para detectar a ‘Candidatus Liberibacter asiaticus’ (Las). Los resultados mostraron que 24 de los 59 árboles fueron Las-positivos. Ninfas provenientes de 10 de los 24 árboles Las-positivos, también fueron Las-positivas. ADN extraído individualmente de ninfa de últimos estádios (3er a 5to instar) colectadas de árboles infectados con Las fueron sujetas a ensayos de PCR anidada. Doce de las veintinueve ninfa demostraron ser Las-positivas. Todas las ninfa colectadas de árboles de limón Las-positivos también fueron Las-negativas. La mayoría de muestras de psílidos que resultaron Las-positivas mediante el PCR anidado también fueron positivas en la detección mediante PCR convencional. El fragmento amplificado del gen 16S rRNA de ‘Ca. Liberibacter spp.’ de muestras de psílidos Las-positivos mostró un 99% de similitud con la cepa Las psy62 del GenBank. Estos resultados demuestran que Cacopsylla (Psylla) citrisuga es otro insecto portador de Las. Estudios están en curso para determinar si psílidos que son Las-positivos pueden transmitir Las a plantas de cítricos saludables.

Huanglongbing (HLB) is the most devastating disease of citrus (Sapindales: Rutaceae) in the world. It is associated with 3 species of Liberibacter, i.e., ‘Candidatus Liberibacter africanus’ (Laf) - the African form, ‘Ca. L. asiaticus’ (Las) - the Asian form, and ‘Ca. L. americanus’
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(Lam) - the American form (Planet et al. 1995; Jagoueix et al. 1996; Coletta-Filho et al. 2005; Teixeira et al. 2005). McClean et al. (1965) reported that the African citrus psyllid, Trioza erytreae Del Guercio, transmits Laf. Capoor et al. (1967) found that the Asian citrus psyllid, Diaphorina citri Kuwayama, vectors Las. Diaphorina citri also vectors Lam (Teixeira et al. 2005; Yamamoto et al. 2006). Laboratory studies indicated that T. erytreae can also transmit Las (Massonié et al. 1976), while D. citri can also transmit Lah (Lallemand et al. 1986). However, field studies and surveys have suggested that D. citri is as the sole insect vector of Las, and that T. erytreae is the sole insect vector of Lah (Bove 2006).

In addition to D. citri, 4 other species of citrus-feeding psyllids have been recorded in Yunnan Province, China, i.e., Cacopsylla (Psylla) citrisuga Yang & Li, C. (P.) citricola Yang & Li, Trioza citriimpura Yang & Li (Yang & Li 1984), and C. (P.) heterogena Li (Li 2011). The hosts of these 4 psyllid species include pomelo, Citrus grandis (L.) Osbeck, Citrus reticulata Blanco, Citrus medica L., and other Citrus species (Li 2011).

However, information about biology and ecology of the psyllid species above is very limited, and no study has been conducted on whether these citrus-feeding psyllids can vector Liberibacter. Among the 4 species, the above authors found that the pomelo psyllid, C. (P.) citrisuga (Fig. 1), is the most frequently encountered in Yunnan Province. The nymphs of C. (P.) citrisuga damage the young flush and cause changes in the foliar phenotype (Fig. 2 and Fig. 3). We therefore initiated this investigation to determine whether 'Ca. L. asiaticus' can be detected in life stages of C. (P.) citrisuga.

**MATERIALS AND METHODS**

Collection of Psyllids and Host Plant Materials, and Identification of Psyllid Species

Pomelo psyllids were collected from a 6-yr old severely HLB-infected orchard of ‘Eureka’ lemon, Citrus limon (L.) Burm.f. in Bangkong Village, Mengxiu Town (24.0712 N, 97.7999 E, 1200 m asl), Ruili City, Yunnan Province, China. Newly flushed leaves with nymphs were collected from...
Psyllid species were identified based on the genitalia of adult psyllid males according to Yang & Li (1984) and Li (2011).

PCR Analyses of DNA of Individual or Groups of Psyllids

The first batch of psyllids was collected on 17 Mar 2011 from 27 HLB-infected lemon trees. The DNA of all 1st to 5th instar nymphs collected from each single tree was extracted in order to determine whether Las DNA could be detected (Table 1). The number of psyllids in each group ranged from 2 to 63 individuals. The second batch was collected on 1 Apr 2011 from each of 22 lemon trees in the same orchard. The DNA of each group of 10 or less late stage-nymphs (3rd to 5th instars) collected from 1 tree was extracted and analyzed by nested-PCR (Table 2). Additionally, 29 psyllids from 3 trees from which the above PCR test had been positive for Las in groups of psyllids were subjected to further nested-PCR testing of individual psyllids (Table 2, Tree # 13, 14, and 20). Conventional PCR was then run to detect the psyllid samples that had been shown to be positive in the nested-PCR.

DNA Extraction from Psyllids and from Citrus limon Host Plants

The DNA of psyllid nymphs was extracted by TIANamp Genomic DNA Kit (provided by Tiangen Biotech (Beijing) Co., Ltd, Beijing, China). The DNA of lemon leaves was extracted using E.Z.N.A.™ Plant DNA Kit (provided by OMEGA Company, Norcross Georgia, USA).

Nested-PCR and Conventional PCR for Detection of Las DNA

Nested-PCR (Harakava et al. 2000) was used for Las detection in the lemon plants and psyllid nymphs. The primer, 1500R/27F (AAGGAGGTGATCCACCCGC/AGAGTTTGATCATGGCTCAG), was used for the first amplification, and OI1/OI2c (GCGCGTATGCAATACGAGCGGC/GCCTCGCGACTTCGCAACCCAT) was used for the second amplification (Jagoueix et al. 1994).

The first amplification system was carried out in a final volume of 25 μL. The mixture contained 17.6 μL of ddH2O, 2.5 μL of 10 × PCR buffer (Mg2+), 2.5 μL of dNTPs (2.5 mmol/L), 0.5 μL each of primers (10 μmol/L), 0.4 μL of Taq enzyme (2.5 U/μL), and 1 μL of Sample DNA. DNA amplification by PCR was performed as follows: reactions were preheated at 94 °C for 5 min; followed by 20 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 4 min. The second amplification system was also carried out in a final volume of 25 μL. The mixture contained 17.6 μL of ddH2O, 2.5 μL of 10 × PCR
buffer (Mg²⁺), 2.5 μL of dNTPs (2.5 mmol/L), 0.5 μL each of primers (10 μmol/L), 0.4 μL of Taq enzyme (2.5 U/μL), and 1 μL of PCR product of the first amplification. DNA amplification by PCR was performed as follows: reactions were preheated at 96 °C for 1 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 4 min.

Conventional PCR was then run to confirm the psyllid samples which had showed positive in nested-PCR. The method was the same as the second amplification of nested-PCR.

DNA Clone and Sequence Analysis

PCR amplification was subjected to electrophoresis on a 1% agarose gel, purified from the gel band according to the Gel Extraction Kit (BioTeke Corporation, Beijing, China) manual instructions, and ligated to the pEASY-T1 cloning vector (TransGen Biotech, Beijing, China). Four μL of the ligation mixture was used to transform E.coli Trans1-T1 competent cells (TransGen Biotech, Beijing, China). Cloned DNA was sent to Invitrogen Biology and Technology Company, Shanghai, China for sequencing. The DNA sequence was compared with the current GenBank database using the BLAST network service available in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

RESULTS

Among the first batch of 27 lemon tree samples, 10 trees proved to be Las-positive (Table 1). The number of psyllid nymphs collected from each of the lemon trees was also listed in Table 1. The result showed that 1 nymph sample collected from 1 of the 10 Las-infected trees (Tree No. 2) was Las-positive in nested-PCR (Table 1). In this case the psyllid DNA was extracted only from the 2 nymphs in the sample, and the band density was very low.

Among the second batch of 22 samples, 14 lemon trees proved to be Las-positive. Psyllids collected from 9 of these 14 trees also proved to be Las-positive by nested-PCR (Table 2). Psyllid samples collected from the negative trees also showed Las-negative result, the same as the result from the first batch detection.

Twenty-nine psyllids, 2 from tree No. 13, 16 from tree No. 14, and 11 from tree No. 20, were individually tested by nested-PCR after Las-
Table 2. Detection of *Candidatus Liberibacter asiaticus* (LAS) by PCR in DNA extracted from individual and from groups of Pomelo psyllid nymphs and from host plants, Citrus limon - second collection.

| Tree No. | Las detected in host plant by means of nested PCR** | Psyllid number | Nested PCR** | Conventional PCR*** | Tested individual | Positive individual | Positive individual in conventional PCR*** |
|----------|----------------------------------------------------|----------------|--------------|---------------------|-------------------|---------------------|------------------------------------------|
| 1        | +                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 2        | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 3        | —                                                   | 8              | —            | —                   | —                 | —                   | —                                        |
| 4        | +                                                   | 8              | +            | +                   | —                 | —                   | —                                        |
| 5        | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 6        | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 7        | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 8        | +                                                   | 3              | +            | +                   | —                 | —                   | —                                        |
| 9        | +                                                   | 9              | +            | +                   | —                 | —                   | —                                        |
| 10       | +                                                   | 3              | +            | +                   | —                 | —                   | —                                        |
| 11       | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 12       | +                                                   | 10             | +            | +                   | —                 | —                   | —                                        |
| 13       | +                                                   | 10             | +            | +                   | 2                 | 2                   | 2                                        |
| 14       | +                                                   | 10             | +            | +                   | 16                | 10                  | 8                                        |
| 15       | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 16       | +                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 17       | +                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 18       | +                                                   | 10             | +            | +                   | —                 | —                   | —                                        |
| 19       | +                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 20       | +                                                   | 10             | +            | —                   | 11                | 0                   | 0                                        |
| 21       | +                                                   | 10             | —            | —                   | —                 | —                   | —                                        |
| 22       | —                                                   | 10             | —            | —                   | —                 | —                   | —                                        |

*Psyllids used in this table were late-stage-nymphs collected from the same tree.

**+: positive; —: negative with respect to *Ca. Liberibacter asiaticus* (Las).

***Nested-PCR positive psyllid samples were amplified by the second set of primer of nested-PCR.
positive results obtained from the group test of the nymphs collected from these 3 trees (Table 2). Twelve of the 29 psyllids (2 from tree No. 13, and 10 from tree No. 14) were Las-positive, but the psyllids from tree No. 20 were not Las-positive (Table 2, Fig. 4). The band densities of all positive samples from the second batch of PCR amplifications were higher than those of the first batch.

Conventional PCR was then run to confirm the 22 psyllid samples (both in groups and individuals) which had been shown to be positive by nested-PCR. The results showed that 18 samples were positive (Tables 1 and 2). The positive sample of the first collection, the sample of tree No. 20 of the second collection, and 2 individual samples of tree No. 14 were negative, which may suggests that nested-PCR is more sensitive than conventional PCR for Las detection.

Sequence analysis showed the DNA sequence of the 16S rRNA gene of ‘Ca. Liberibacter spp.’ from positive psyllid samples was 99% similar to that of ‘Ca. L. africanus subsp. capensis’ (Accession NO. CP001677), 97% similar to that of ‘Ca. L. asiaticus’ (Accession NO. AF137368.), and 94% similar to that of ‘Ca. L. americanus’ (Accession NO. AY742824) in GenBank. This result demonstrates that pomelo psyllid, C. (P.) citrisuga, is a Las carrier.

DISCUSSION

A number of studies have been conducted to investigate potential vectors of ‘Ca. Liberibacter asiaticus’, such as the citrus aphid, Toxoptera citricidus (Kirkaldy), in South Africa (McClean et al. 1965), Toxoptera citricidus (Kirkaldy), Toxoptera aurantii (Boyer de Fonscolombe), citrus red mite Panonychus citri (McGregor) and yellow mite Eotetranychus kankitus Ehara (Guangxi HLB Research Group 1977), and many other insects with piercing mouthparts in China (Cen et al. 2011, unpublished data). The outcomes of these studies were all negative.

So far psyllids are the only group of insects known to transmit ‘Ca. Liberibacter spp.’. Hansen et al. (2008) reported that in addition to the 3 ‘Ca. Liberibacter’ species associated with HLB,
'Ca. Liberibacter psyllaurous', which infects tomato and potato, is vectored by the potato psyllid, *Bactericera cockerelli* (Sulc). Also 'Ca. Liberibacter europaeus', which apparently behaves as an endophyte rather than as a pathogen, was found to be transmitted by the pear psylla, *Cacopsylla pyri* (L.) (Raddadi et al. 2010).

According to Halbert & Manjunath (2004), 13 citrus-feeding psyllid species have been reported, and 7 of them belong to the genus of *Diaphorina*. These species are *D. citri* Kuwayama, *D. amoena* Capener, *D. auberti* Hollis, *D. communis* Mather, *D. murrayi* Kasandasamy, *D. punctulata* (Petey 1924), and *D. zebrana* Capener. The other 6 citrus-feeding psyllid species include *Mesosomotoma lutheri* (Enderlein 1918) (= *Udamostigma lutheri* Enderlein), *Psylla citricola* Yang & Li 1984, *Psylla citrisuga* Yang & Li 1984, *Psylla murrayi* Mathur 1975, *Triozia citrompusra* Yang & Li 1984, *Triozia erytreae* (del Guercio 1918) (= *Aleurodes erytreae* del Guercio 1918) (= *Triozia citri* Laing, = *Triozia meroe* Petey, = *Spanioza meroe* Petey), = *Spanioza erytreae* (del Guercio) (Hollis 1984), and *Triozia litseae* Bordage 1898 (= *Triozia eastopi* Orian 1972) (Halbert & Manjunath 2004). But there are uncertainties about the synonymy for some species (Halbert & Manjunath 2004). A new citrus-feeding psyllid species, *C. (P.) heterodena* Li, was reported in China recently (Li 2011). Among these psyllid species, *D. citri* and *T. erytreae* are the only known insect vectors of HLB (Halbert & Manjunath 2004, Nadarassah & Stavrinides 2011). Donovan et al. (2011) reported Las-positive some *D. communis* individuals. No other citrus-feeding psyllids have been reported to be Liberibacter carriers.

This study is the first to demonstrate that *C. (P.) citrisuga* carries Las. Transmission studies are underway to determine whether Las-positive *C. (P.) citrisuga* can transmit Las to citrus.

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References Cited

Bordage, E. 1898. Notes d'entomologie agricole tropicale. II. Un ennemi du Vanillier. Rivue Agricole de la Réunion 2: 524-525.

Bove, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

Capon, S. P., Rao, D. G., and Viswanath, S. M. 1967. *Diaphorina citri* Kuway., a vector of the greening disease of citrus in India. Indian J. Agric. Sci. 37: 572-576.

Coletta-Filho, H. D., Takita, M. A., Taboco, M. L. N., and Machado, M. A. 2005. Analysis of 16S rDNA sequences from citrus Huanglongbing bacteria reveal a different "Ca. Liberibacter" strain associated with citrus disease in Sao Paulo. Plant Disease 89: 848-852.

Del Guercio, G. 1918. Note ed osservazioni di entomologia agraria. Il ciclio delle foglie del limone ed il suo cicidozoio in Eritrea. Agricoltura Coloniale 167-169, 2 figs, 1 pl.

Donovan, N. J., Beattie, G. A. C., Chambers, G. A., Folford, P., Englehou, A., Hardy, S., Dorjee, Wangdi, P., Thinyal, and Om, N. 2011. First report of 'Candidatus Liberibacter asiaticus' in *Diaphorina communis*. Australasian Plant Dis. Notes. (Online 7 Oct 2011)

Enderlein, G. 1918. Psylldiologica V. Zoologische Jahrbücher Jena Abteilungen der Systematik Ökologie und Geographie de Tiere 41: 479-486.

Guangxi Huanglongbing Research Group. 1977. Citrus psyllids and citrus huanglongbing. Citrus Sci. Technol. Communication (3-4): 23-24. (In Chinese).

Halbert, S. E., and Manjunath, K. L. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomol. 87(3): 330-353.

Hansen, A. K., Tromble, J. T., Stouthamer, R., and Paine, T. D. 2008. A new Huanglongbing species, 'Candidatus Liberibacter psyllaurous," found to infect tomato and potato, is vectored by the Psyllid *Bactericera cockerelli* (Sulc). Appl. Environ. Microbiol. 74(18): 5862-5865.

Harakava, R., Marais, L. J., Ochanan, J., Manjunath, K. L., Bebrers, V. J., Lee, R. F., and Niblett, C. L. 2000. Improved sensitivity in the detection and differentiation of citrus huanglongbing bacteria from South Africa and the Philippines. pp. 195-199 In Proc. of the 14th Conference Int. Org. Citrus Virologists. Unier. California, Riverside, CA, USA.

Holle, D. 1984. Afrotropical jumping plant lice of the family Triozidae (Homoptera: Psyllodea). Bull. Brit. Mus. (Natural History) 49(1): 1-102.

Jagoueix, S., Bove, J. M., and Garnier, M. 1994. The phloem-limited bacterium of greening disease of citrus is a member of the α subdivision of the Proteobacteria. Int. J. Syst. Bacteriol. 44: 379-386.

Jagoueix, S., Bove, J. M., and Garnier, M. 1996. PCR detection of the two 'Candidatus' Liberibacter species associated with greening disease of citrus. Mol. Cell. Probes 10: 43-50.

Lallemand, J., Fos, A., and Bove, J. M. 1986. Transmission de la bactérie associée à la forme Africaine de la maladie du “greening” par le psyllide Asiatique *Diaphorina citri* Kuwayama (Transmission by the Asian vector *Diaphorina citri* of the bacterium as-
associated at the African form of the greening disease]. Fruits 41(5): 341-343. (In French.)
Li, F. S. 2011. Psyllid host plants. pp. 63-78 In F. S. Li [ed.], Psyllidomorpha in China (Insecta: Hemiptera) Volume I. Beijing: Science Press. (In Chinese.)
Massonie, G., Garnier, M., and Bove, J. M. 1976. Transmission of Indian citrus decline by Trioza erytreae (Del Guercio), the vector of South African greening. pp. 18-20 In E. C. Calavan [ed.], Proc. 7th Conf. Int. Org. Citrus Virologists, IOCV, Riverside, California.
Mathur, R. N. 1975. Psyllidae of the Indian Subcontinent. Indian Council of Agricultural Research, New Delhi. 429 pp.
McCLean, A. P. D., and Oberholzer, P. C. J. 1965. Citrus psylla, a vector of the greening disease of sweet orange. S. Afr. J. Agric. Sci. 8: 297-298.
Nadarasah, G., and Stavrinides, J. 2011. Insects as alternative hosts for phytopathogenic bacteria. FEMS Microbiol Review 35: 555-575.
Orian, A. J. E. 1972. The Psylloidea of Mauritius with a description of Trioza eastopi sp. nov. Fauna of Mauritius 1: 1-19.
Petrey, F. W. 1924. South African psyllids. Entomol. Mem. Dept. Agric. Union of South Africa 2: 21-30.
Planet, P., Jacqueix, S., Bove, J. M., and Garnier, M. 1995. Detection and characterization of the African citrus greening liberobacter by amplification, cloning and sequencing of the rplKAJL-rpOB operon. Curr. Microbiol. 30: 137-141.
Raddadi, N., Gonella, E., Camerota, C., Pizzinat, A., Tedeschi, R., Crotti, E., Mandrioli, M., Bianco, P. A., Daffonchio, D., and Alma, A. 2010. ‘Candidatus Liberibacter europaeus’ sp. nov. that is associated with and transmitted by the psyllid Cacopsylla pyri apparently behaves as an endophyte rather than a pathogen. Environ. Microbiol. 13(2): 414-426.
Teixeira, D., Do C., Danet, J. L., Eveillard, S., Martins, E. C., Jesus Junior, W. C. De, Yamamoto, P. T., Lopes, S. A., Bassanezi, R. B., Ayres, A. J., Saillard, C., and Bove, J. M. 2005. Citrus huanglongbing in Sao Paulo State, Brazil: PCR detection of the ‘Candidatus’ Liberibacter species associated with the disease. Mol. Cell. Probes 19: 173-179.
Yamamoto, P. T., Felippe, M. R., Garbim, L. F., Coelho, J. H. C., Ximenes, N. L., Martins, E. C., Leite, A. P. R., Sousa, M. C., Abrahao, D. P., and Braz, J. D. 2006. Diaphorina citri (Kuwayama) (Hemiptera: Psyllidae): vector of the bacterium Candidatus Liberibacter americanus, pp. 96-97 In Proc. Huanglongbing - Greening Int. Workshop, Ribeirao Preto, Sao Paulo, Brazil.
Yang, C. K., and Li, F. S. 1984. Nine new species and a new genus of psyllids from Yunnan. Entomotaxonomia 6(4): 251-266. (In Chinese.)