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## International Organization of Citrus Virologists Conference Proceedings (1957-2010)

### Title

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### Permalink

<https://escholarship.org/uc/item/2q42m488>

### Journal

International Organization of Citrus Virologists Conference Proceedings (1957-2010), 13(13)

### ISSN

2313-5123

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### Publication Date

1996

### DOI

10.5070/C52q42m488

Peer reviewed

# Wood Apple, *Limonia acidissima*: A New Host For the Huanglongbing (Greening) Vector, *Diaphorina citri*

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**ABSTRACT.** A study was conducted in Thailand to determine the host range of psyllid, *Diaphorina citri*, and the huanglongbing (HLB) (greening) pathogen it transmits. Approximately six-month-old seedlings of 15 Rutaceae plants including three citrus cultivars were exposed to *D. citri* that had fed on HLB-infected citrus plants collected from Thailand. Long-term survival of the psylla of more than 7 wk was observed on the following plants: *Balsamocitrus dawei*, *Murraya paniculata*, *M. koenigii*, *Limonia acidissima* (wood apple), *Atalantia* sp., *Severinia buxifolia*, *Poncirus trifoliata* and Som-pan and Som-keo-wan mandarins. Among them, marked multiplication of psylla was noted on *M. paniculata*, *Atalantia* sp. and *L. acidissima*. The former two did not develop any symptoms, but the *L. acidissima* developed leaf mottling and yellowing. An electron microscope study failed to show conclusive evidence of HLB organisms in sieve cells of infected *L. acidissima*. These results indicate that wood apple is a new host for *D. citri* and warrants further investigation as a possible host of the HLB agent.

*Index words.* Citrus huanglongbing disease, host range, *Limonia acidissima*, vector.

Citrus Huanglongbing (HLB) (greening) disease is a major factor limiting citrus production in tropical and subtropical Asia. An integrated management program which includes the propagation of disease-free plants, eradication of infected plants, and the effective control of the vector is recommended (1, 2). Identification of host plants for HLB and its vector is important to achieve this goal.

Until now, the pathogen has only been shown to infect certain Rutaceae, namely *Citrus* species, *Poncirus trifoliata*, and its hybrids. Other genera among the Rutaceae have not been confirmed as hosts (5). On the other hand, the psyllid vector of HLB, *Diaphorina citri* Kuwayama, naturally lives on citrus cultivars and *Murraya paniculata*, although some other plants support its multiplication (1). These experiments employed only a few rutaceous species. In addition, possible presence of ecotypes or races of *D. citri* is suggested (1). This study was carried out in Thailand to determine the host range of *D. citri* and the HLB pathogen, under a cooperative project between the Japan International Research Center for Agricul-

tural Science (JIRCAS) and the Thailand Department of Agriculture (DOA). We found the build up of large population of *D. citri* on the wood apple, *Limonia acidissima* L. (= *Feronia limonia*).

## MATERIALS AND METHODS

**Inoculum source.** The following four potted HLB-infected plants were used: Special Mandarin from Ratchaburi district, rough lemon and Avon Ever Bearing (Calamondin) from Nan Horticultural Experiment Station, and Madam Vinous sweet orange inoculated with a pumelo source from ChaiNat. These plants showed typical HLB symptoms, namely, leaf mottling in all plants and severe yellowing, stunting, and vein corking of Madam Vinous. HLB organisms were confirmed in Special Mandarin and Madam Vinous sweet orange by electron microscopy. They were kept in an incubator at 26-28°C (night and day) with artificial illumination at 10,000 lux for 16 hr per day.

**Vector.** About three hundred adults of *D. citri* were collected from *Murraya paniculata* trees planted in the yard of Department of Agricul-

ture, Bangkok and reared for propagation on one-year-old seedlings of *M. paniculata* in the incubator. The psylla laid eggs and increased abundantly. The seedlings were replaced every 2 to 3 mo with vigorous ones. Young psylla adults were transferred to the HLB-source plants and maintained for at least 1 wk in a plastic cage to acquire the HLB organism. The minimum acquisition period of psylla being 1 to 2 days (9).

**Seedlings used for the experiment.** Seeds of the rutaceous plants were obtained from USDA-National Clonal Germplasm Repository, Riverside, California in December 1992, except for *Poncirus trifoliata*, *M. paniculata* and *L. acidissima*. Trifoliolate orange was from Japan and the others were from Thailand. Of the 20 species of the Rutaceae acquired, seeds of 12 species germinated. Each plant was individually potted and grown in an insect-proof greenhouse. Insecticide application was stopped 1 mo before vector transmission.

**Vector transmission and population trends of psylla.** Fifteen psylla were transferred from the donors to each receptor plant which was covered with a net cage in an incubator. The number of adults on the plants were counted at weekly intervals for 5 to 7 wk. After killing the psylla by insecticide spray, the plants were transferred to a greenhouse for further observation of symptoms. The experiments were done from August to October 1993 with three repetitions. As a control, two healthy seedlings were used. The plants were cut back in December and subsequent growth and symptom development were observed until March 1994.

**Electron microscopy.** Electron microscopy was conducted on *L. acidissima* used as a rootstock in the field of Pichit Horticultural Research Center. Approximately 5-yr-old sweet orange, Som-tra, on the *L. acidissima* showed typical HLB

symptoms and was severely stunted. The rootstock developed suckers that showed leaf mottling and yellowing, and partial die-back; and the budunion was swollen. Midveins of the young leaves of the *L. acidissima* that showed HLB-like symptoms were collected in March 1995. They were fixed in glutaraldehyde, post-fixed with osmium and then embedded into epoxy resin. Ultrathin sections were made with a Sorvall MT2-B ultramicrotome using a diamond knife and observed with an Hitachi H-300 electron microscope after double staining with uranyl acetate and lead nitrate.

## RESULTS

**Population trends of *D. citri* on Rutaceae plants.** The population trends of psylla on *Clausena lansium*, *M. paniculata*, local mandarin Som-keo-wan and *L. acidissima* varied markedly in the three repetitions. Some instances of decrease were caused by fungal infection because of high humidity. Large numbers of psylla were alive for the first week after transfer. Marked increase was noted 4 wk after transfer on the following plants: *M. paniculata*, *Atalantia* sp., and *L. acidissima* (Table 1). On those plants, the psylla laid eggs which gave rise to abundant development of nymphs and adults.

Small increases in the number of adults were observed on *Murraya koenigii* and Som-keo-wan. No increase, but a long term survival of more than 7 wk, was noted on *C. lansium* (one out of three), *Severinia buxifolia*, *Poncirus trifoliata*, *Balsamocitrus dawei* and Som-pan mandarin. The survival of more than 5 wk was noted on *Triphasia trifolia*, *Microcitrus australasica*, *Aeglopsis chevalieri* and Ladu mandarin. In contrast, a rapid death of psylla was observed on *C. lansium* (two out of three) and *Eremocitrus glauca* (Table 1).

TABLE 1  
SURVIVAL AND MULTIPLICATION OF DIAPHORINA CITRI ON RUTACEAE PLANTS MAINTAINED IN A CAGE AND DEVELOPMENT OF DISEASE SYMPTOMS

Plant used for rearing psylla	No. adult psylla															Symptoms			
	Exp. 1 (wk)				Exp. 2 (wk)						Exp. 3 (wk)					Leaf-mottle	Stunting <sup>c</sup>		
	1	3	4	5	1	3	4	5	6	7	1	3	4	5	6			7	
<i>Clausena lansium</i>	8	1	0	0	11	0	0	0	0	0	11	11	8	8	5	3	Questionable	-	
<i>Murraya paniculata</i>	12	9	48 <sup>b</sup>	51	14	9	11 <sup>b</sup>	10	9	6 <sup>c</sup>							None	-	
<i>M. koenigii</i>	14	12	12	14 <sup>b</sup>	14	14	13	13	10	3 <sup>c</sup>	9	9	8	10 <sup>b</sup>	5	3 <sup>c</sup>	None	-	
<i>Triphasia trifolia</i>	10	5	2	2	14	4	1	1	0	0							None	++	
<i>Severinina buxifolia</i>	12	12	7	6	12	9	9	6	1	0	11	13	13	13	12	8	None	+	
<i>Atalantia</i> sp.											12	13	48 <sup>b</sup>	52	37	37	None	-	
<i>Eremocitrus glauca</i>	8	1	0	0													Death		
<i>Microcitrus australasica</i>	12	9	8	8													None	+	
<i>Poncirus trifoliata</i>	15	12	12	12	14	13	13	8	6	5	15	15	15	14	14	10	Questionable	-	
<i>Citrus reticulata</i>																			
"ladu"	14	8	5	4	13	2	2	1	0	0	15	7	4	4	0	0	Positive	-	
"Som-pan"					14	7	6	5	3	1	14	13	3	1	1	0	Death		
"Som-keo-wan"	14	6	5	18 <sup>b</sup>	13	14	5	4	3	3 <sup>c</sup>	13	13	15 <sup>b</sup>	6	3	3	Questionable	-	
<i>Balsamocitrus dawei</i>	14	12	9	3	14	14	13	10	10	10	10	10	7	3	2	0	Questionable	-	
<i>Aeglopsis chevalieri</i>	14	10	10	9													— <sup>w</sup>		
<i>Limonia acidisimma</i> (= <i>Ferronia limonia</i> )	12	8	4	7 <sup>b</sup>	14	12	39 <sup>b</sup>	42	53	46	15	16	30 <sup>b</sup>	39	43	41	Positive	-	

Fifteen adult psyllids were transferred to each plant. The cage was kept at 28/26°C (day/night) with 16 hr illumination in an incubator.

<sup>a</sup>Killed by fungal infection

<sup>b</sup>Increase in psylla

<sup>c</sup>Yellowing of leaf followed by defoliation

<sup>w</sup>No data because of no leaves

<sup>+</sup>++ = severe stunting; + = moderate stunting; - = no stunting.

**Symptom development after vector transmission.** Typical symptoms such as leaf mottle were observed in seedlings of Ladu mandarin and *L. acidissima* (Table 1). *C. lansium*, *P. trifoliata*, Som-keo-wan and *B. dawei* showed mild mottling but still questionable as HLB symptoms. Trifoliolate orange and Som-pan mandarin died, but it was not determined whether this was caused by HLB. Stunting was noted on *T. trifolia*, *S. buxifolia* and *M. australasica*. This data were obtained in mid March, after which high temperatures caused plant injury. Further observation was therefore not possible.

**Electron microscopy.** Prokaryotic and spherical bodies with 0.5 to 1.0 µm in diameter were observed in cytoplasm of sieve parenchyma cells. The envelope consisted of two membrane layers about 25 nm in thick. The bodies were not identical with HLB organisms observed by Garnier et al. (3) because of it was present in cytoplasm but not in sieve tubes. Therefore, this does not provide conclusive evidence of the presence of HLB organisms in *L. acidissima*.

## DISCUSSION

Xu et al. (10) observed that *D. citri* can survive and propagate normally on *Clausena lansium* and *Atalantia buxifolia*. Aubert (2) classified host plants of *D. citri* into four categories: 1) *M. paniculata* is the preferred host; 2) *C. aurantifolia* and *M. koenigii* are good hosts; 3) the common host on which feeding, egg laying and nymph development can occur including many citrus cultivars such as *M. australasia*, *C. lansium* and *C. excavata*; 4) occasional hosts on which occasional feeding, egg laying and nymph development can occur which includes *Triphasia trifolia*, *Fortunella* sp., *P. trifoliata*, and *Clausena anisum-olen*. Our experiments showed a marked increase of *D. citri* numbers on *M.*

*paniculata*, *L. acidissima* and *Atalantia* sp. In terms of feeding, egg laying, and nymph and adult development, there was no difference between *L. acidissima* and *M. paniculata*. Because *L. acidissima* is a popular local fruit in Thailand, further observation in the fields is needed to assess its role in HLB epidemiology.

Some population increase of *D. citri* was observed on *M. koenigii*. In West Malaysia, this was found to be a common alternate host of *D. citri* (6). We did not observe psylla multiplication on *Clausena lansium* or *Microcitrus australasica*. These different results do not necessarily mean the presence of different ecotypes of psylla, since high humidity or insufficient young leaves in the experiment may have been unfavorable for psylla.

Tirtawidjaja (8) reported positive symptoms in *M. paniculata*, *Swinglea glutinosa*, *Atalantia missionis* and *Clausena indica* after vector transmission with Indonesian greening disease. Electron microscopy did not confirm the presence of HLB organisms in inoculated *M. paniculata* (4). We observed typical leaf mottle on *L. acidissima* and suspicious symptoms on *Clausena lansium* and *Balsamocitrus dawei*. Marked stunting was also noted on *Triphasia trifolia*. Unfortunately, those plants declined and died because of high temperatures. Therefore, we could not carry out electron microscopy on them.

We observed symptomatic *L. acidissima* in the field where it was used as a rootstock for Som-tra sweet orange that developed HLB symptoms. The sucker shoots of *L. acidissima* also showed similar symptoms and partial die-back. Other trees of *L. acidissima* that had been grafted with Som-tra also developed yellow shoots. Electron microscopy did not provide conclusive proof of the presence of HLB organisms. Indexing with plants or

PCR techniques was not done, but the presence of symptoms suggests that *L. acidissima* may be a natural host of HLB. Further research is warranted, because leaf mottle may develop not only due to HLB but also

other agents. Recently, Su et al. (7) confirmed the multiplication of the HLB organisms in *Severinia buxifolia* and *L. acidissima* by DNA-hybridization with a specific probe.

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