Transmission of Citrus Likubin by a Psyllid, *Diaphorina Citri*[1]

C. H. Huang, M. Y. Tsai and C. L. Wang[2]

Abstract : In transmission studies of citrus likubin made during the years of 1981-1982, the psyllid, *Diaphorina citri* Kuway., successfully transmitted the likubin pathogen from diseased citrus plants to healthy Ponkan seedlings ; however, the efficiency of transmission was extremely low. Of 380 plants tested, only 5 plants showed likubin symptoms 1-2 months after test feeding. Leaf discs from the suspected plants, when grafted onto Ponkan indicator plants, induced likubin symptoms 2-4 months after inoculation indicating that the suspected symptoms are indeed due to likubin infection. Our preliminary results showed that new flush from diseased scions was a favorable inoculum source for acquisition feeding in transmission tests. The acquisition feeding time, infection feeding time required for successful transmission and the latent period in vector have yet remained to be determined.

(Key words : Citrus Likubin, Mycoplasma-like Organism, Vector Transmission).

Introduction

Citrus likubin, occurring in Taiwan since 1913 [18], has remained to be the major threat to citrus industry in Taiwan. Most studies of the disease during the past decade dealt with electron microscopic demonstration of the causal agent [7,12,19,20] and the disease -curing effect of tetracyclines by tree transfusion [9,21].

Matsumoto and Su [15] reported the transmission of citrus likubin by using citrus aphid, *Toxoptera citricidus*, as vector. They concluded that the disease was possibly closely related to tristeza. This conclusion was generally accepted until the early 1970's when further investigations provided evidence that citrus likubin was not caused by a virus, but a Mycoplasma-like Organism (MLO) [7,20].

The citrus greening in South Africa and the stubborn in California are similar to citrus likubin in many respects [3,10,22]. However, the symptoms and electron microscopic studies showed that the likubin is more closely related to the greening [1,10]. Transmission of citrus greening was obtained in South Africa by using a psyllid, *Trioza erytreae*, as vector [16]. Similar diseases known as citrus die-back in India and leaf-mottling in

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the Philippines are transmitted by another species of psyllid, *Diaphorina citri* [14,17]. Chen et al.[8] found that there were numerous MLO bodies in mucous cells of salivary glands of adult psyllids of *D. citri* feeding for one month on likubin-diseased Ponkan plants. However, transmission of the disease by *D. citri* has yet to be substantiated.

This paper presents some experimental evidence of the transmission of citrus likubin from diseased Ponkan plants to healthy seedlings by *D. citri*. Parts of the results have been published elsewhere[13].

Materials and Methods

The transmission studies were conducted in 1981 and 1982. The citrus psyllids, *Diaphorina citri* Kuway., free of likubin pathogen, were reared on 2-yr-old Sunki seedlings in the greenhouse. The ability of the psyllid to transmit likubin was tested in the following experiment series :

(1) In the first experiment series, the source of likubin was collected from a Ponkan tree showing typical likubin symptoms in an orchard in Tung-shih, Taichung. Scions taken from the diseased tree were side-grafted onto 2-yr-old Ponkan plants on Sunki rootstock. Inoculated plants were kept in the greenhouse for one month before testing. A total of 10 inoculated plants in a cage of 18×18×30cm were used in this experiment.

Four groups of test insects, each comprising 2 to 3 hundred adults, were used in this experiment series. The first group was placed in the cage for one month starting about one month after the enclosed plants had been graft-inoculated with a likubin source. The test insects were then removed for transmission test. Upon the removal of the first group, the second group was introduced onto the same inoculated plants in the same cage. This process was repeated for the third and fourth groups.

After acquisition, the psyllids were allowed an infection feeding period of one month on test plants, 6 psyllids per plant. The psyllids were collected after the first infection feeding and transferred to new test plants for another month. After second infection feeding period, the psyllids that survived were collected and tested again.

(2) In the second series of experiments, the disease sources came from two locations : the Kuan-hsi Citrus Research Station in Hsin-chu and a Ponkan orchard in Tung-shih, Taichung. Scions were top-grafted onto Sunki rootstock. New flushes from diseased scions were used as inoculum sources for psyllids. Either nymphs or adults were used in the transmission tests depending on the maturity of the new flushes.

Four experiments were conducted in this series, i. e., PT-1, PT-2, PT-3 and PT-4, each using 8-10 diseased plants selected for same maturity of the new flushes to provide inoculum. The origins of inoculum sources for PT-1 and PT-2 were Ponkan and Liuchen trees from the Kuan-hsi Citrus Research Station, and for PT-3 and PT-4 were Ponkan trees from Tung-shih. The acquisition feeding and infection feeding were same as described in the first series of experiments.

(3) In the third experiment series, the psyllids naturally occurring on 2-yr-old diseased citrus plants outside the greenhouse were collected. About 15-20 nymphs or adults

were directly transferred to each test seedling and allowed to feed for 2 months.

All test plants were sprayed with insecticides after the psyllids had been removed. The test plants were then moved to greenhouse for observation of the symptom development. Plants showing likubin-like symptoms were indexed on Ponkan indicator plants by leaf-disc grafting [2]. Those plants without symptoms were cut back after 8 months to enhance new growth and observation for symptoms continued for another 6 months.

Results

Transmission by adult psyllids feeding on likubin infected plants at various growth periods: In this experiment series, four groups of psyllids were used. Psyllids in the first group were tested after acquisition on 10 Ponkan seedlings during the first infection feeding period. Those that survived were collected and tested on 2 new seedlings for the second infection feeding. The psyllids that again survived were collectively tested on 1 seedling. Using the second group of psyllids, 25, 2 and 2 seedlings were tested in the three infection feeding periods, respectively. The corresponding numbers of test seedlings were 25, 4, 1 and 25, 0, 0 for the third and fourth groups of psyllids, respectively. Thus a total of 97 seedlings were tested in this experiment series. However, none was found with likubin symptoms. It seemed that adult psyllids could not acquire the likubin pathogen from the source plants.

Transmission by nymphs and adults feeding on new flushes: The results are shown in Table 1. Of the 260 seedlings tested, four plants, all fed on by test psyllids during the first transfer, showed likubin symptoms suspected to result from transmission by psyllids. The infected plants exhibited stunting and leaf yellowing 1-2 months after the psyllids had made test feeding on them. The most pronounced symptoms in these plants were leaf yellowing, sometimes with green islands on the yellowish leaves, zinc difficiency patterns on young leaves, and vein corking on mature leaves (Fig. 1, A and B). These symptoms were typical of citrus likubin observed in the field.

On indexing of those abnormal seedlings on 6-mo-old Ponkan indicator plants by leaf-disc graft method [2], likubin symptoms were observed in the indicator plants (Fig. 1 : C, D, and E) 2-4 months after inoculation. A test on Mexican lime seedlings gave no tristeza virus reactions. These results clearly indicate that the likubin pathogen was successfully transmitted by the psyllids from disease citrus plants to healthy Ponkan seedlings. However, the efficiency of transmission was extremely low.

Transmission by psyllids collected from 2-yr-old likubin infected citrus plants: Because of the low rate of transmission obtained in the above test series, a further experiment was performed. Table 2 showed the experimental results. Even though the number of the psyllids was increased to 15 for each test plant in the transmission tests, the rate of transmission did not improve. The only one plant of successful transmission was obtained when the test psyllids made acquisition feeding at their nymphal stage.

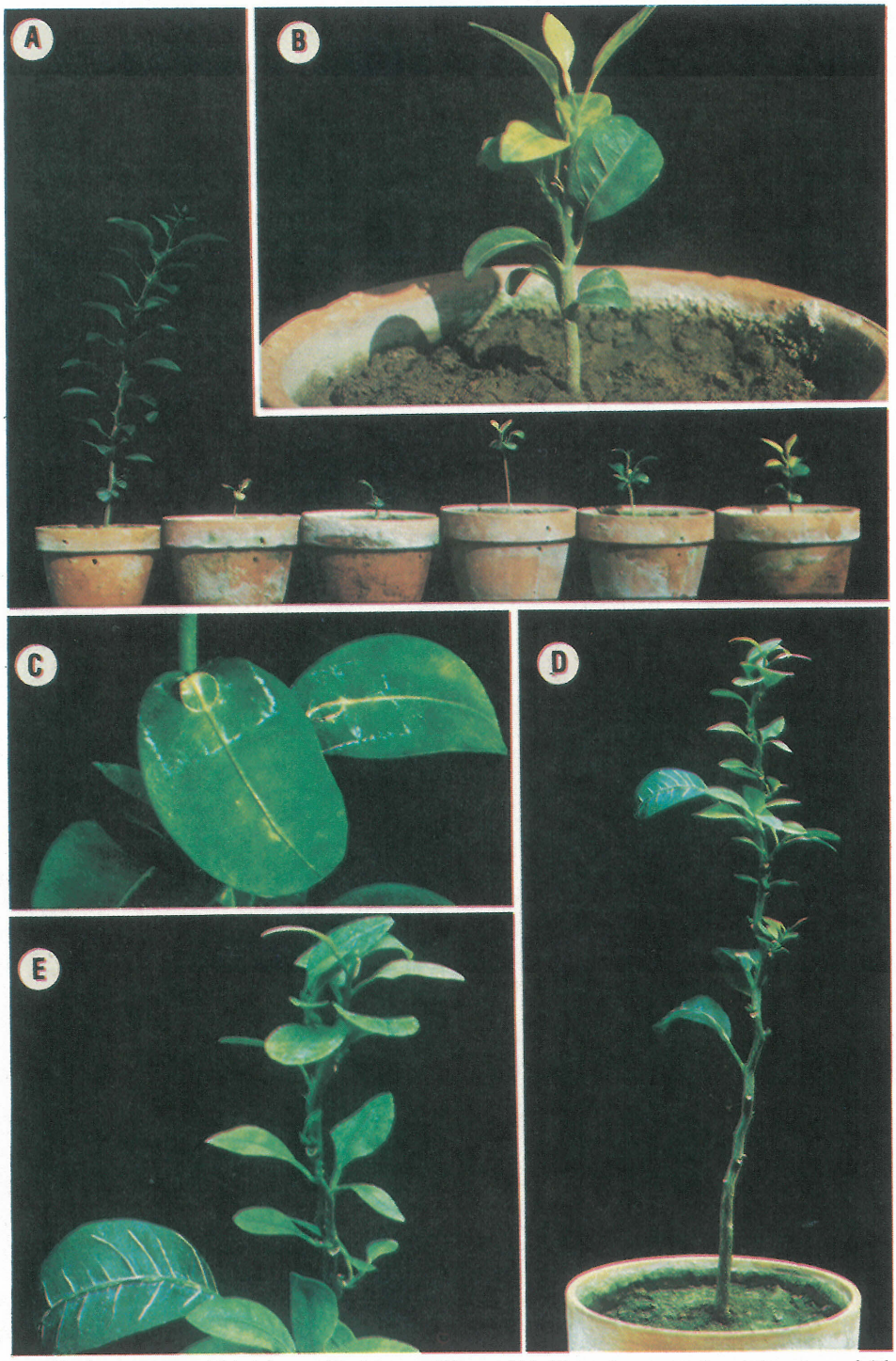


Figure 1. Symptoms of likubin on Ponkan seedlings. (A) Five plants to the right showed likubin symptoms 2-3 months after infection feeding by *D. citri*. The first plant from the left : healthy control. (B) Close-up of an infected seedling. (C) Ponkan indicator plant developed likubin symptoms 4 months after graft-inoculation with leaf tissues from psyllid-transmitted diseased Ponkan seedling. (D) Indicator plant showed typical likubin symptoms. (E) Close-up of the D plant. (Photographs of A, B and C were taken 4 months, and D and E taken 15 month after inoculation).

Table 1. Transmission of citrus likubin by *D. citri* that had previously fed for one month on new flushes from diseased scions

Exp.	Maturity of new flush	Developmental stage of psyllid	Infection feeding in serial transfers		
			1st transfer	2nd transfer	3rd transfer[a]
PT-1	1[b]	Nymph	1/12[c]	0/6	0/1
	5	Nymph	1/ 8	0/2	—[d]
	9	Adult	0/27	0/8	0/1
PT-2	3	Nymph & adult	1/15	0/4	0/1
	8	Adult	0/11	—	—
PT-3	1	Nymph	0/20	0/7	0/1
	5	Nymph	0/24	0/4	—
	9	Adult	0/25	—	—
PT-4	3	Nymph	1/17	0/7	0/2
	8	Adult	0/25	0/7	0/1
	12	Adult	0/24	—	—

a: The psyllids had an infection feeding period of one month on test seedlings after acquisition. They were then collected and transferred to new test seedlings for a second month of infection feeding. The survived psyllids were collected and tested again in the third transfer. During the infection feeding period, each test seedling was exposed to 6 psyllids.

b: Weeks after sprouting of new flush from diseased scions.

c: No. seedlings infected/total seedlings tested.

d: Not tested.

Table 2. Transmission of citrus likubin by *D. citri* collected from likubin-infected citrus plants[a]

Exp.	Developmental stage of psyllid	No. plants infected/total plants tested
1	Nymph	1/12
2	Adult	0/12

a: About 15 nymphs or adults were placed on each test seedling soon after they were collected from diseased plants outside the greenhouse. The test insects were allowed to remain on the test seedlings for two months.

Discussion

The spread of citrus likubin naturally in the field suggests that a vector must be involved. Su[19] stated that citrus likubin was probably transmitted by the oriental psyllid, but gave no details of the experiment. Tsai[23] and Yang, I. L. (Pers. Comm.)

obtained negative results in their studies on insect transmission of the disease. Therefore, the question about transmission of citrus likubin by a psyllid has remained unsettled. Recently, Chang[4] worked on 10 species of insects with sucking-mouth part to determine their possible role as likubin vector. These insects were directly collected from field and allowed a feeding on diseased plants for 5 to 30 days. Observing likubin symptoms on some of the test plants, he concluded that likubin was trasmitted by adult psyllids. However, Chang[4] did not index the suspected plants for verification. In the present study, we found that likubin could be transmitted by *D. citri* from diseased citrus plants to healthy Ponkan seedlings, although the efficiency of transmission was extremely low.

Capoor et al.[5] demonstrated that citrus greening in India was transmitted by *D. citri* with an acquisition feeding for 30 min or longer followed by an infection feeding for 1 hr or longer. Raychaudhuri et al.[17], and Catling and Atkinson[6] found that even a single psyllid was capable of acquiring the greening pathogen. Another psyllid, *T. erytreae*, vector of citrus greening in South Africa, became infective after 24 hr on diseased plant[5]. In contrast. the pathogen of likubin was very inefficiently transmitted by *D. citri* in Taiwan. Of the 380 seedlings exposed to the oriental psyllids that had made acquisition feeding, only 5 plants became infected. If likubin pathogen belongs to the greening-type, it may well be another strain of the greening pathogen which is more difficult to be transmitted by its vector than the greening pathogen found in India and South Africa. In addition, the uneven distribution of likubin pathogen in diseased plants[11] may possibly account in part for the low rate of transmission we observed.

Electron microscopic studies by Su and Leu[20], and by Huang et al.[12] showed that older leaves with vein corking symptoms had a higher concentration of likubin pathogen. In determining if the transmission is affected by the pathogen concentration in diseased plants, the plants used for acquisition feeding were divided according to growth periods after inoculation. However, our results did not show any positive correlation between pathogen build-up in inoculated plants and the ability of psyllid to acquire the likubin pathogen. In the first experiment series, adult psyllids had opportunities to feed on mature leaves of inoculated plants but they failed to acquire the pathogen.

It was reported that both nymph and adult of the psyllid were able to acquire the greening organism in India[5]. In South Africa, however, the nymph of *T. erytreae* seemed unable to transmit citrus greening[6]. Also, there was no evidence for transovarial transmission[5]. Because the acquisition feeding took longer time than it required for a nymph to become an adult in our experiments and because of the low rate of transmission obtained, the comparative efficiency of likubin by the psyllids at different developmental stages could not be determined although we observed all the five cases of successful transmission when the test insects were nymphs at the begining of the acquisition feeding.

The latent period of citrus greening in psyllids as determined in India was 8 to 12 days, and the psyllids retained infectivity throughout their life span[5]. In contrast, the vector became infective after one day on plants with greening symptoms in South Africa [5]. Our preliminary data suggest that *D. citir* is capable of transmitting the liku-

bin, and that the latent period was less than two months. Further work on likubin transmission is in progress.

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木蝨媒介傳播柑桔立枯病之研究[1]

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摘 要

室內飼養之四至五齡期木蝨若蟲或成蟲移至罹立枯病柑桔病穗之新生嫩葉上吸食一個月，再移出供傳播試驗。供試之三百八十餘棵柑桔苗中，有五棵呈現植株矮化，葉片黃化，老葉木栓化及新葉微量元素缺乏症。此種病徵與一般所稱之柑桔立枯病無異。病徵於木蝨吸食後約一至二個月呈現。自上述可疑柑苗切取葉片，以葉環組織接種法 (Leaf-disc graft method) 嫁接到椪柑指示植物，經二至四個月指示植物同樣產生立枯病病徵，證明立枯病病原由東方柑桔木蝨 (*Diaphorina citri* Kuway.) 蟲體傳到健全柑苗，唯木蝨傳播能力很低。其他有關研究，目前正進行中。

(關鍵字：柑桔立枯病、菌質病、媒介傳播)。

1.臺灣省農業試驗所 研究報告第1147號。
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