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INSECT, DODDER AND SEED TRANSMISSIONS OF CITRUS VEIN PHLOEM DEGENERATION (CVPD)

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Abstract. **Further inoculation trials with *Diaphorina citri* Kuw. proved that this insect alone is able to transmit CVPD. It does not need to be accompanied by *Toxoptera citricida* Kirk. to produce the disease symptoms as stated in earlier reports. Therefore, CVPD is probably a severe strain of greening and not a result of dual infection by tristeza virus and the greening organism, although both disease agents may be present together in several citrus areas of Java and Sumatra. Periwinkle (*Vinca rosea*) is a host plant which produces clear symptoms if infected by CVPD agents, and might be used for additional means in identification. Marble-size fruits from CVPD-affected trees contain many aborted seeds which might yield infected seedlings.**

Introduction

Diaphorina citri Kuw. was suspected by Tirtawidjaja[4] of transmitting CVPD in 1965. In later papers,[5,6] however, he stated that for symptom expression *D. citri* needs to be accompanied by *Toxoptera citricida* Kirk. Meanwhile, it was proved that *D. citri* is a vector of both the Philippine yellow mottle and the Indian greening.[2,1] It is, therefore of importance to determine exactly the role of this insect in establishing CVPD symptoms. This may eventually answer the question if CVPD is the same as greening.

Since inoculations with insects can be done readily on very young seedlings, this method has a good chance for success with species of plants that function as food sources. On the other hand, a species of plant may have a potential of being a host for the disease agent without being a food source for the insect. To solve this problem, dodder was used to discover additional hosts for identification of CVPD.

The question of seed transmission needs to be elucidated since small, infected budlings were frequently encountered in nurseries. Although infected buds taken from diseased trees were blamed for this, this answer is not always satisfactory, because occasionally unbudded rootstocks were found to be infected.

Materials and Methods

Diaphorina citri used in these trials was obtained from CVPD-affected citrus trees in the Garut area (West Java) and Lampung (South Sumatra). The two following types of trials were carried out: 1) inoculations with 1 to 3 insects per 2-week-old seedling grown in test tubes; 2) inoculations with 200 to 300 insects per 100 2-week-old seedlings grown in inoculation boxes. Included in the latter trials were some species of Rutaceae obtained from the National Biological Institute at Bogor[1] (Table 2). Seedlings suspected of being infected by the pathogen were transplanted to pots for further observation.

Seeds of *Cuscuta* sp. (probably *C. australis* R.BR) usually found on a species of plant with the local name "Baluntas" (*Pluchea indica* Less.) were treated with concentrated sulphuric acid for 3 minutes, washed with water, and germinated. The strands that grew from the seeds were planted on tender shoots of healthy *Amaranthus* seedlings to obtain stock cultures of the parasite.

Symptomatic seedlings of 'Jeruk Siam'. *Citrus reticulata* Blanco, previously graft-inoculated with scions from affected field trees and seedlings previously insect-inoculated with *D. citri* were used as CVPD sources. Healthy seedlings used as receptor plants are listed in Table 3.

When enough strands had formed in the stock cultures, some were transferred to the CVPD source plants. After the dodder had established itself and formed strands, the terminals were wound around tender shoots of the receptor seedlings to be inoculated. Receptor seedlings suspected of being infected were cleansed of dodder and saved for further observation.

Normal and marble-size, but mature fruits[6] were collected from affected trees in the field. Normal looking (not abortive) seeds from these fruits were harvested and planted. Chlorotic and stunted seedlings which grew from them were transplanted for further observation.

1) The auther expresses his thanks to Mrs. Sri Setiati Sastrapraja and Mrs. N. Wulijarni-Sutjipto for the valuable seeds.

Results and Discussion

Table 1 shows results of transmission trials using 1 to 3 insects per seedling, 7 months after inoculation. Of 207 seedlings belonging to 5 different species, only 20 showed typical external and internal CVPD symptoms.[5] This figure is, however, enough to show that *D. citri* alone is able to carry and transmit the causal agent of CVPD. The incubation period needed to express the symptoms varied from 2 to 6 months. The inoculation feeding period was the period in which the insect remained living on the receptor seedlings.

Table 2 shows the results of "bulk" transmission trials with *D. citri* 10 months after inoculation. Transmission was not consistent in the 3 boxes of 'Jeruk Siam' seedlings. There were 9, 6, and 12 seedlings, respectively, with typical CVPD symptoms and 10, 14, and 17 seedlings, respectively, with atypical symptoms. The latter grew normally for about 8 months and then suddenly formed narrow, mottled young leaves whose color later changed to green, but whose shape remained narrow. The rest of the 'Jeruk Siam' seedlings, i.e. 80, 74, and 67, respectively, remained normal and grew much faster than symptomatic seedlings.

Although the number of seedlings with the typical symptoms was less than 10% of the total inoculated, it is clear that *D. citri* alone is able to transmit CVPD. At this moment it appears that CVPD is a very severe strain of greening, which has wiped out whole areas of citrus in some areas of Java and Sumatra.

As with 'Jeruk Siam', the number of successful transmissions varied with *Murraya paniculata* (L.) Jack., i.e. 15 and 5 for the 2 boxes. Similar variation is to be expected with the other species in these trials, and the results in Tables 1 and 2 do not indicate anything about the relative susceptibility of the species tested.

Results of dodder transmissions from citrus to citrus were disappointing. Only one rough lemon seedling was infected by this means. No success was obtained with *Amaranthus* sp. and celery, *Apium* sp. On the other hand, periwinkle, *Vinca rosea*, exhibited distinctive symptoms after 3 months. A bright yellow-green mottle was seen first in some mature leaves. This increased until almost all leaves showed a yellow-green mottle. Graft transmission using scions from this plant to inoculate previously healthy periwinkle seedlings yielded plants with mottle symptoms again. Additional clues that the causal agent of CVPD was transmitted were: 1) transmission by dodder from an insect-inoculated citrus seedling to periwinkle, 2) transmission by dodder living on symptomatic periwinkle to small 'Jeruk Onde', *Citrus reticulata*, seedlings led to formations of typical CVPD symptoms on 3 of them (Table 3).

Attempts were made to inoculate periwinkles with *D. citri*, without positive results. This is probably due to the inability of the insect to use this species as a food source. These negative results conform to observations is the citrus areas of Lampung where periwinkle is abundant among infected nursery stocks and infected field trees.

Nhami *et al.*[7] lists and pictures symptoms produced on periwinkle by different kinds of mycoplasmalike organisms and spiroplasmas. None of these approximate the symptoms of CVPD. The simplicity of procedure and the distinctive symptoms produced on periwinkle may lead to some practical value for this method to identify CVPD.

No indications of CVPD were observed on seedlings obtained from normal-size fruits, although they had been taken from CVPD-affected trees. On the other hand, seeds derived from the smaller fruits produced some stunted, chlorotic seedlings. Three of these had the same narrow, mottled appearance of insect-inoculated seedlings listed in Table 2. Although further inoculations are needed to clarify these results, the symptoms indicate that the seedlings might be affected by CVPD transmitted through seeds obtained from small-size fruits of CVPD-affected trees.

Table 1. Transmission of citrus vein phloem degeneration disease to young citrus seedlings in test tubes by *Diaphorina citri*.

Receptor species inoculated	Total Seedlings inoculated[z]	Total seedlings showing symptoms[z]		Incubation period (months)	Inoculation period (days)
		Typical CVPD	Stunted[y] chlorosis		
Jeruk Siam, *Citrus reticulata* Blanco	20	4	4	3 – 4	8, 13, 27 and 30
Rough lemon, *C. jambhiri* Lush.	29	1	8	4 – 5	4, 5, and 6
Jeruk lemon, *C. amblycarpa* Ochse	35	3	1	2 – 6	7, 8, and 11
Jeruk nipis, *C. aurantifolia* (Christm.) Swing.	89	8	12	4 – 5	6, 11, 14, 15, 17, and 19
Japanese citron Rangpur lime, *C. limonia* Osbeck	34	5	–	4 – 5	8, 14, 16, 25, and 36
Total	207	20	27		

z: 1–3 insects used per receptor plant.
y: Results read 7 months after inoculation.

Table 2. Transmission of citrus vein phloem degeneration disease to young seedlings in inoculation boxes by *Diaphorina citri*.

Receptor species	Inoculated[y]	Typical CVPD symptoms[z]		Stunted	Mottled shoots	Normal
		External	Internal			
Jeruk Siam	100	9	9	1	10	80
Jeruk Siam	100	6	6	6	14	74
Murraya paniculata (L.) Jack	100	12	12	4	17	67
M. paniculata	100	15	15	–	10	75
Swinglea glutinosa (Blanco) Merr.	100	5	5	–	5	90
Atalantia missionis	15	3	3	–	–	82
Clausena indica (Dalz.) Oliv.	17	–	–	13	–	4

z: Ten months after inoculations with *D. citri*. y: 200–300 insects per 100 seedlings were used except for *Atalantia missionis* and *Clausena indica*.

Table 3. Transmission of citrus vein phloem degeneration disease with dodder (*Cuscuta australis*).

Source of CVPD	Receptor	Cuscuta transmission		Subtransmission (graft)[z]		Subtransmission (Cuscuta)[z]		Subtransmission (Cuscuta)[y]	
		Inoculated	Positive	Inoc.	Positive	Inoc.	Positive	Inoc.	Positive
Jeruk Siam, *C. reticulata*, graft inoculated from field trees.	*Amaranthus* sp.	5	–	–	–	–	–	–	–
	Jeruk Siam	4	–	–	–	–	–	–	–
	Rough lemon	4	1	–	–	–	–	–	–
	Vinca rosea	3	1	17	10	5	4	50	3
Jeruk Siam inoculated with *D. citri*	*Vinca rosea*	6	4	–	–	–	–	–	–

z: Vinca to Vinca.
y: Vinca to citrus.

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INTEGRATED APPROACH TO CONTROL CITRUS GREENING DISEASE IN INDIA

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Abstract. **Greening disease is one of the major causes of decline of citrus industry in India. The causal pathogen, recently shown to be a bacteria-like organism (BLO), is spread by propagation and also by the psyllid vector, *Diaphorina citri* Kuway, which is prevalent in almost all parts of the country. Recent research has been aimed at controlling the disease by various tetracycline antibiotics, heat therapy of budwood, insect vector control and exploiting the resistance of different species and cultivars of citrus. Among the tetracycline antibiotics tested, achromycin (500 ppm), ledermycin, terramycin and streptocycline at 1000 ppm have given promising results used as sprays, for trunk or shoot injection, or for bud-wood immersion. Heat treatment of budwood at 47°C for 4 hours or at 45°C for 6 hours (moist hot air) or treatment of young, budded greening-affected plants (1 to 1½ yr old) for 21 days in a hot chamber at 38-40°C has helped to inactivate the pathogen. Weekly spraying with 0.1% Rogor (Dimethoate), 0.1% Dimecron (phosphamidon), 0.05% metasystox (Methyl Demeton) and 0.05% Nuvacron (Monocrotophos) effectively controlled the vector and the residual effect of the insecticides lasted for 3–4 weeks. Of twenty species and cultivars tested for their reaction to greening, sweet lime was resistant alone or as a rootstock for mosambi orange. 'Italian', 'Eureka' and 'Lisbon' lemon were tolerant and showed mild symptoms. An integrated control schedule incorporating use of disease-free or heat-treated budwood, injection with tetracycline antibiotics, insecticide sprays and use of tolerant rootstocks can greatly reduce disease losses.**

Greening disease is one of the major causes of the decline of the citrus industry in India. It is associated with the die-back disease complex which is responsible for heavy losses to the citrus crop in the country.[14] The disease, once believed caused by a mycoplasma like agent was recently shown to be caused by a bacterialike organism (BLO).[11] It is spread by grafting or use of infected budwood and by the psyllid vector, *Diaphorina citri* Kuway which is prevalent in most parts of the country.[1,8] The disease and the vector have also been reported from other Asian countries such as Pakistan, Nepal, Thailand, Malaysia, Indonesia, the Philippines, Taiwan and south China.[2] The current research in India has been aimed at controlling the disease by various tetracycline antibiotics and other chemicals, heat therapy of budwood, insect vector control and exploiting the resistance of different species and cultivars of citrus separately or in scion-stock combinations. On the basis of the results achieved the following methods of control have been recommended.

Use of disease-free areas for raising citrus nurseries

An extensive survey of the citrus orchards in India, and indexing of budwood collected from various localities on indicator plants in insect-proof glass houses revealed that the greening disease is very widespread and occurs in almost all the citrus growing areas in northern, southern, western and eastern India including the Sikkim State.[4,6,7,9] However, certain areas in Assam surrounded by natural barriers i.e. hills, such as Digaru and Jhalawar in Rajasthan, were found to be free from the greening disease.[7] Such isolated pockets can serve as centers for multiplication and distribution of disease-free budwood or nucellar plants of different citrus cultivars. It is essential, therefore, that the citrus nurseries be located in such areas.

Use of healthy budwood

The most ideal way to control greening disease is the use of certified healthy budwood. For this purpose a long range budwood certification programme was recommended[5] and put in force under the All India co-ordinated Fruit Improvement Project by the Indian Council of Agricultural Research. An alternative to the certified disease-free budwood has been found recently in heat-treated budwood or budwood treated with tetracycline antibiotics. Budsticks from greening affected trees when heated to 45°C for six hours or 47°C for four hours (dry moist heat) yielded greening-free buds in a majority of cases.[12,13] Also infected budsticks immersed in solutions of terramycin, ledermycin, streptocycline and BP-101 at 1000 ppm for three hours were freed of the greening pathogen[3] and buds taken from treated budsticks were completely healthy when side-grafted on healthy 'mosambi' plants.

Use of tolerant rootstock and scion cultivars

About twenty different species and cultivars of citrus (1½-2 year old plants) grown inside the glasshouse were side-grafted with budwood obtained from a greening-affected tree to test their comparative tolerance or resistance to the disease. The greening pathogen was transmissible to all the species and cultivars of

Table 1. Reaction of different species and cultivars of citrus to greening pathogen.

Citrus species or cultivar inoculated	No. of plants inoculated	No. of plants infected	Intensity of disease symptoms
Bengal citron (*C. medica* L.)	4	4	++++
Carrizo citrange (*C. sinensis* × *P. trifoliata*)	4	2	+++
Dancy tangarine (*C. reticulata Blanco*)	4	4	++++
Eureka lemon (*C. limon* (L.) Burm. f.)	4	2	++
Grapefruit (*C. paradisi* Macf.)	4	4	++++
Kagzi lime (*C. aurantifolia* (Christ.) Swing.)	4	4	++++
Karunjamir (*C. aurantium* L.)	4	3	+++
Lisbon lemon (C. limon (L.))	4	2	++
Malta (*C. sinensis* (L.) Osbeck)	4	4	++++
Mosambi (*C. sinensis*)	4	4	++++
Pineapple (*C. sinensis*)	4	4	++++
Rangpur lime (*C. aurantifolia* Austera)	4	4	++++
Santra (*C. reticulata*)	4	4	++++
Sweet lime (*C. limetta* Risso)	4	0	–
Sour orange (*C. aurantium* L.)	4	2	+++
Troyer citrange (*C. sinensis* × *P. trifoliata*)	4	2	+++
Valencia (*C. sinensis*)	4	3	++++
Trifoliata (*P. trifoliata* Raf.)	4	2	+++
Italian lemon (*C. limon*)	4	2	++
Pink pummelo (*C. grandis* Osbeck)	4	3	+++