Citrus Greening Disease – A major cause of citrus decline in the world – A Review

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ABSTRACT: Citrus Greening Disease (CGD) was critically reviewed with respect to its history, origin, symptomatology, host range, pathogenic association, identification and detection, transmission and management. It is evident that the citrus disease has emerged as a potential serious threat to citrus production in all the citrus growing countries of the world including Pakistan. It is has been proved that in Pakistan the greening disease is the major cause of citrus decline; hence it is necessary to formulate definite planning and strategies.

Keywords: citrus greening disease; citrus decline; symptomatology; protection

Citrus greening disease probably originates in China at the end of the 19th century; it was called "yellow shoot disease". According to Lin (1956), the first epiphytotic conditions of CGD were noticed in Chaoshan and Yuenchung districts of Fukien province in 1925. The disease was later reported in South Africa in 1929 as "yellow branch disease", and later called "greening", which refers to the green colour of fruits at 2nd harvest (Su 1998). Since the discovery of this disease, it has been called different names in different countries (Graca 1991), yet "greening" is the most frequent name. A bacterium-like organism was found to be associated with CGD, and the incitant was established by Lafleche and Bove (1970). The disease is graft-transmissible; in nature, it is transmitted by psyllid vectors identified as *Trioza erytreae* (Del Guerico) in Africa and *Diaphorina citri* (Kuwayama) in Asian countries. The disease is associated with a decline of citrus trees and is probably widespread in all the citrus growing countries of the world. It possesses a great potential to affect citrus trees of all cultivars and cause great damages to the citrus industry by shortening the productive life span of trees. It is suspected that greening has caused colossal losses in many parts of Asia and Africa.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Citrus greening disease was presumed to originate in China during the 1890's as a "yellow shoot disease" (Graca 1991). In India, shoot die-back was attributed to poor drainage (Raychaudry et al. 1969; Graca 1991). Similarly, a nematode-associated problem was identified in Taiwan (Otake 1990). CGD was also confused with mineral toxicity in South Africa in 1937 (Smith et al. 1988) and was linked to the stubborn disease and zinc deficiency in Philippine and California (Lee 1921). African greening was first identified in 1929 (Oberholzer et al. 1965; Roistacher 1991).

The disease is widely distributed in Asia and Africa. The heat-sensitive form (*Liberibacter africanum*) prevails in Saudi Arabia, Yemen, Burundi, Cameron, Central African Republic, Comoros, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Reunion, Rwanda, Somalia, South Africa, St. Helena (unconfirmed), Swaziland, Tanzania and Zimbabwe. *T. erytreae* occurs in Saudi Arabia, in the eastern and highland areas of the country with favorable climate, whereas *D. citri* is widespread in the western, more equable coastal areas (Aubert et al. 1988; EPPO/CABI 1996b; Frank 1998). A more heat-tolerant form (*Liberibacter asiaticum*) is spread in the Asian countries, namely Bangladesh, China, India, Pakistan, Bhutan, Indonesia, Japan, Malaysia, Nepal, Philippines, Saudi Arabia, Taiwan, Thailand, and Vietnam. In Africa, this form prevails in Mauritius and Reunion (EPPO/CABI 1996b; Wooler et al. 1974).

SYMPTOMS

On trees. Greening disease has different names according to their symptom expression in different areas of the citrus growing regions of the world. "Yellow shoot" (Huanglongbing) and "yellow dragon" are the names, describing the symptoms of leaf yellowing that may appear on a single shoot or branch (Chung, Brlansky 2005). This disease is called "decline" (likubin) in Taiwan, "dieback" in India, "leaf mottle" in Philippines, "vein phloem degeneration" in Indonesia and "yellow branch", "blotchy mottle" or "greening" in South Africa, based on the symptoms (Vichitrananda 1998).

Under certain conditions, symptoms are displayed on different parts of the plant. In general, the greening-affected trees show the open growth, stunting, twig dieback, sparse yellow foliage, or severe fruit drop (EPPO/CABI 1996a; Catara et al. 1988; Khan 1989). In some cases, green colour develops on fruit at the peduncular rather than the styler end, as in normal case. This is known as "colour inversion" or "red nose" (Akhtar, Ahmad 1999). Symptoms of these two types are similar. Although the Asian form of greening causes extensive dieback, the African form is temperature sensitive with symptom suppression at temperature above 27°C (Bove et al. 1974; Zhao 1981). The symptoms of CGD appear both on fruit and on leaves. With regard to the rootstock, studies show four variable results. In some cases, the rootstocks can affect the expression of symptoms; 5 of 23 rough lemon rootstock selections in India induced a degree of tolerance in sweet orange scion in green house. In another study, 100% of trees of rough lemon were infected compared to only 25% of trees of blood red on sweet orange rootstock (Kapur et al. 1984; Graca 1991). However, sweet orange is not commonly used as rootstock.

On leaves. Characteristic symptoms are mottling and chlorosis of leaves. Mottling resembles zinc deficiency symptoms, which are characteristic for the CGD (Elizabeth et al. 2005). A citrus life is on average five to eight years in the Asian region where the disease prevails in the endemic form and displays its symptoms in warm temperature (Knapp et al. 2004). Root systems are poorly developed in both forms of greening. Roots start decaying from the rootlets (Graca 1991). Other problems associated with CGD are the attacks of citrus longhorned beetle and *Phytophthora* fungi on trees (Halbert, Manjunath 2004). Moreover, citrus is often affected by more than one pathogen and displays symptoms such as tristeza, yellowing of seedlings in severe cases, chlorosis etc. (Graca 1991).

On fruits. Some fruits are under-developed, lopsided and poorly coloured. Grayish-white waxy marks appear on the rind surface when pressure is exerted with a finger. In the case of African greening, fruits remain immature and green and seeds are aborted and stained (EPPO/CABI 1996a; Garnier, Bove 1983).

PATHOGEN

Citrus greening pathogen, now known as Huanglongbing is a phloem-limited, uncultivable gram-negative bacterium (Subandiyah et al. 2000; Weinert et al. 2004). Initial reports suggested it was a virus (Hofmeyr, Oberholzer 1948; Akhtar, Ahmad 1999). Lafleche and Bove (1970) considered it a mycoplasma organism. This pathogen was defined as a gram-negative bacterium in 1984 by Garnier et al. (1984) and was further characterized by Jagoueix et al. (1996). The genus is *Liberibacter* from Asia and Africa and two geographically distinct *Candidatus* species, *L. candidatus asiaticum* and *L. candidatus africanum,* are widely distributed (Garnier et al. 1984; Jagoueix et al. 1996; Akhtar, Ahmad 1999). The size of this bacterium ranges from 350–550 × 600–1,500 nm and its thickness is 20–25 nm. They are generally rigid rod-like, and are pleomorphic bodies during growth (Su 1998).

Strains. Greening pathogen occurs in two forms, Asian form and African form. They can be clearly distinguished according to their temperature sensitivity. Symptoms of African greening are more pronounced in cooler areas; Asian greening is more severe than African (Graca 1991). Genus *Liberibacter* was described as a greening pathogen, and considered a member of alpha subdivision of proteobacteria based on the PCR studies (Jagoueix et al. 1994). Two forms were named *L. asiaticum* and *L. africanum;* they can be distinguished as separate species according to the sequence homology.

The new species recently found in Brazil has been referred to as *L. americans*. African greening manifests symptoms primarily under cool conditions whereas Asian greening appears under hot conditions (Chung, Brlansky 2005).

African greening does not show symptoms above 27°C under greenhouse conditions. In South Africa, the greening symptoms are more pronounced in winter than in summer. Similarly, the African citrus greening symptoms are severe in higher elevations, whereas they are absent in lower hot areas. On the contrary, Indian greening does well in hot conditions, above 25°C. Asian citrus greening symptoms are however less pronounced and disappear above 1,500 m, possibly because the vector is absent (Aubert, Bove 1980). In a laboratory study, symptoms of African citrus greening were moderate to severe at 22–24°C and disappeared at 27–32°C, whereas symptoms of Asian citrus greening from India and Philippines were displayed strongly at both temperature regimes (Halbert, Manjunath 2004).

The symptoms of these two types are similar, however, the Asian form causes more extensive dieback than the African form (French et al. 2001).

Detection. Citrus greening is detected through different techniques. Indicator seedlings were the first diagnostic test developed (Schwarz 1968a). Yellow shoots, yellow veins, mottling and zinc-like deficiency symptoms are partially diagnostic; when combined with the greening of fruit and a presence of vector, they are highly diagnostic. Graft transmission is variable; African greening is more difficult to transmit through grafting than Asian greening (Roistacher 1991). Grafting sticks are grafted on different citrus spp. (indicator plants) in different areas, symptoms usually appear in 3 to 4 months.

In quick laboratory indexing, Schwarz (1968b) introduced the paper on thin chromatography using n-butanol: acetic acid: water (5:1:1) to detect the biochemical changes in trees infected with greening. The presence of a specific florescent marker, gentisoyl glucoside, in infected tissues was used for detection (Feldman, Hanks 1969; Hooker et al. 1993). Polymerase chain reaction (PCR) detected the pathogen in samples collected from greening-infected trees in Bhutan (Doe et al. 2003).

Electron microscopy, monoclonal antibodies and polymerase chain reaction (PCR) assays were used for detection of the disease (Knapp et al. 2004). Monoclonal antibodies successfully detected the Indian form of citrus greening bacterium. In addition, electron microscopy 7 can be used to detect the pathogen (Chung, Brlansky 2005). Other possible techniques are immonuflorescence and ELISA (Garnier et al. 1987). Recently, DNA probes have been used to detect the two forms of greening (Villechanoux 1992).

HOST RANGE

Greening affects almost all citrus cultivars; relatives like sweet orange, tangelo and mandarin are the most susceptible, while lime, pummelo and trifoliate orange are the least susceptible (Knapp et al. 2004). Infected lemons, grapefruits, pummelos and sour oranges remained non-productive, whereas Mexican limes, trifoliate oranges and some trifoliate orange hybrids showed only leaf symptoms (Chung, Brlansky 2005). Symptoms have also been observed in *Microcitrus australasica, Swinglea glutinosa, Atalantia missionis, Clausena indica, Limonia acidisimma, Balsamocitrus dawei, Aeglopsis chevalieria, Severinia buxifolia, Murraya paniculata. Catharanthus roseus* (Periwinkle) and *Nicotiana xanthii* (Tobacco) are the only reported non-Rutaceous hosts. Both of the latter hosts were infected only under laboratory conditions and acted as indicator plants (Knapp et al. 2004). The positive presence of pathogen needs to be investigated for the information on host range.

Some species (*Citrus indica* Tan and *Citrus macroptera* Montr.) remained symptom-free under heavy inoculum pressure (Bhagabati 1993). Citrus limetta remained symptom-free after a laboratory inoculation (Nariani 1981).

In some cases, the rootstock can affect the occurrence of symptoms. In South Africa the percentage of greening in Valencia oranges was higher on trifoliate orange rootstock than on Empress mandarin or Troyer citrange. The trifoliate rootstock might cause an extension of the growth flesh period and thus extend the feeding time for insect vector. However, no differences were found in a Chinese study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (Lin 1963).

There are many observations about preferred hosts of *D. citri*. In laboratory studies, *Murraya paniculata* L. (orange Jasmine), *C. jambhiri* Lush. (Rough lemon), *C. aurantium* L. (Sour orange) and *C. paradise* Macf. (Grapefruit) were screened against CGD. Grapefruit was the best host, followed by the other species (Halbert, Manjunath 2004).

TRANSMISSION

Two species of citrus psyllid, *Diaphorina citri* Kuwayama (Asiatic psylla) and *Trioza erytreae* Del Guerico, (African psylla) can transmit the greening pathogen. Acquisition feeding period is 30 min or longer. The pathogen remains latent for 3–20 days. Inoculation feeding period is one hour or more (Huang et al. 1990). In 1943, yellow shoot was suggested to be a viral disease (Chen 1943). Soon afterwards, similar opinions were put forward in South Africa, strengthened by the results of grafting trials showing that greening was inconsistently transmitted to healthy plants. In China, Lin (1963) confirmed that yellow shoot was graft transmissible. Graft transmissibility of African greening was confirmed in 1965 by Oberholzer (Graca 1991).

Variability in graft transmission of *Candidatus liberibacter* spp. depends upon the plant part used for grafting, the amount of tissue used, and the pathogen isolate with single buds. Graft transmission of African greening varied from 0–50%, depending upon the isolate used. Side grafts with twigs were even more efficient at transmitting the pathogen. In an experiment, seven months after grafting diseased buds on healthy rootstocks, 58% of grafts survived, and out of those, 20% showed citrus greening symptoms. In another experiment, 10–16% of grafts with buds from asymptomatic branches on diseased trees developed symptoms, while 40% of grafts from symptomatic branches developed symptoms of citrus greening in 3–9 months (Halbert, Manjunath 2004).

It is generally accepted that this pathogen is not seed-transmitted. Tirtawidjaja (1981) collected normal and greening-affected (very small) fruits and harvested normal-looking seeds from each. No symptoms were observed on seedlings from seed taken from normal fruit, even when they were collected from greening-infected plants; however, seeds derived from smaller, greening affected fruits produced some stunted chlorotic seedlings. Three of the seedlings had the same appearance as the insect-inoculated seedlings (Halbert, Manjunath 2004).

Two species of psyllids that transmit CGD are widely distributed. One species, residing in the Indian Ocean islands, is associated with the spread of the African Huanglongbing (HLB); the other vector is the Asian citrus psyllid, which is adapted to warm humid areas and has widespread throughout Asia, the Indian subcontinent, Saudi Arabia, Reunion and Mauritius. *D. citri* has also been reported in South and Central America, Brazil, and is now well established in Florida. *Trioza erytreae* is sensitive to heat whereas *D. citri* is more resistant to extremes of temperature but more sensitive to high rainfall and humidity (Morvan, Blowers 1967; Catling 1972; Aubert 1987; Graca 1991). The HLB bacterium can multiply in both of them. Once infected, the psyllids remain capable of transmitting HLB for their entire lives, but progeny of infected psyllids are free of the bacterium (Huang et al. 1990; Halbert, Manjunath 2004; Chung, Brlansky 2005). The 5th or 6th-instar nymphs, as well as the adults born from these nymphs, are capable of transmitting the greening agent to citrus. This is probably the way by which the Asian forms of citrus greening bacterium were introduced into Saudi Arabia. The rutaceous plant *Murraya paniculata*, frequently used as an ornamental bush or hedge, is one of the best hosts of *D. citri*. This plant can carry eggs or nymphs of the vector and therefore its introduction into disease vector-free regions could be dangerous (EPPO/CABI 1996a,b).

Transmission of CGD happens in a persistent manner, i.e. bacteria multiply in the psyllids. Transmission rates up to 100% have been reported. The greening bacterium can be found in the haemolymph of the vectors. Under laboratory conditions, Asian citrus psylla can transmit both Asian and African forms of citrus greening bacteria simultaneously (Graca 1991; Knapp et al. 2004).

The acquisition latent period is 24 hours for African psyllid. Transmission is thought to occur via salivary secretions. Serial transfer experiments suggest that young nymphs of *T. erytreae* can acquire the bacteria even though they do not transmit them. There are conflicting reports as to whether *Candidatus liberibacter* spp. are transmitted transovarially. The most extensive studies on transovarial transmission of citrus greening pathogens were done with *T. erytreae* (Halbert, Manjunath 2004). In different experiments transovarial transmission varied up to 1–21%.

The only other means of transmission is by Dodder (*Cuscuta* spp.). *Cuscuta reflexa* was used to transmit the pathogen from citrus to citrus; *Cuscuta campestris* transmitted the citrus greening disease from periwinkle to Sweet orange and within 3 months yellowing symptoms appeared on sweet orange (Graca 1991). Transmission of greening organism from affected citrus to periwinkle was achieved by graft inoculation (Garnier, Bove 1983).

STATUS OF GREENING DISEASE IN PAKISTAN

Pakistan is one of the major citrus growing countries of the world, and citrus greening disease (CGD) is one of the most important and severe diseases of citrus in Punjab and N.W.F.P. During 1991 surveys, its incidence was 22% in Kinnow, 25–40% in sweet orange, 15% in grapefruit, 10% in sweet lime, and 2% lemon. Variability in disease severity and incidence was found at different locations (Akhtar, Ahmad 1999). Diagnostic tests using light microscopy of thin sections stained with phenolic thionin and orange combination showed the abnormality in infected tissues. Bacteria like organisms (BLO) were observed in infected tissues (Akhtar, Ahmad 1999). Extensive studies are needed to investigate the greening disease in Pakistan, as it has emerged as a potential threat to the citrus industry. Only limited work is available on this disease. The first research was started late in 1980's, in which Catara et al. (1988) surveyed and collected the samples of greening and observed the infestation of *D. citri*. Bacterium associated with greening was detected in sieve tubes of the columella and in leaf midribs (Girmaldi, Catara 1989). Similarly, electron microscopic observations revealed the presence of greening organism in phloem cells of samples collected from throughout the province. *D. citri* was also found widespread in the province (Catara et al. 1991).

CONTROL

Different management strategies are needed to avoid a potential threat of greening disease.

Legislative control. Legislative control has been made to prevent the spread of pathogen. Following legislative control is needed to be established (Kawano 1998).

(1) Regulatory plants: Rutaceae. *Poncirus trifoliata*, *Fortunella* spp., and living plants of citrus genus (excluding fruits and seeds).
(2) Regulatory pathogen and insect: citrus greening bacteria and citrus Psylla.
(3) Protection of mother stock: Mother plants should be regularly checked for citrus greening and should be kept under isolation to prevent the plants from insect.

Thermotherapy. Another approach found to be very effective in the control of citrus greening. Graftwood is heated to 48–50°C for several minutes (Graca 1991; Baniqued 1998). Elimination of yellow shoot disease was reported by Lin in 1964, giving a saturated hot air treatment to graftwood at 48–58°C.

Chemotherapy. Tetracycline hydrochloride showed good results in reducing leaf symptoms (Martinez et al. 1970). Penicillin carbendazin gave complete control (Cheema et al. 1986). Schwarz and Van Vuuren (1970) showed the best results through the injections of tetracycline hydrochloride. The best time for injection is in the spring season (Martinez et al. 1970; Schwarz et al. 1974; Chiu et al. 1979; Aubert, Bove 1980; Graca 1991).

Eradication and replacement. Young trees (less than four-year old) and those not bearing fruits and showing symptoms should be eradicated and replaced, whereas trees with fruit should be pruned. Trees infected up to 50–70% should be eradicated (Baniqued 1998).

Control of vector. Attempts have been made to control the vectors through different approaches e.g. by means of cultural, chemical or biological measures. The parasite (*Tamarixia radiata*) was found effective against the disease (Baniqued 1998). Systemic insecticides like dimethoate and monocrotophos controlled *D. citri* (Graca 1991). Methomyl or melathion sprays on citrus trees at 10–12-day intervals from March to May were not effective against citrus greening. Sometimes 44% Dimethoate EC, 50% melathion EC and 40.64% carbofuran FP showed economically good control of the *Psylla* (Chiou-Nan Chen 1998).

CONCLUSION

Greening is one of the major causes of citrus decline throughout the citrus growing areas of the world as well as in Pakistan. The Asiatic form is prevalent in Pakistan, which may be a threat for the citrus industry of Pakistan as well as for the neighboring countries. Citrus production can be increased through nurseries run on a scientific and professional basis. Certified citrus nurseries are needed to solve the problem of citrus greening. Pathogen-vector relationship should be further investigated. Certified budwood programs might be the best way to establish disease-free citrus orchards. In addition, an IPM program should be launched for the control of this disease.

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Choroba zelenání citrusů – hlavní příčina úhynu citrusů ve světě – Přehledová studie

ABSTRAKT: Choroba tzv. zelenání citrusů (Citrus Greening Disease) byla podrobena odbornému zkoumání z různých hledisek, jako je např. původ, vývoj, symptomatologie, nositelé, patogenická spojení, identifikace a detekce, přenos a ochrana. Je zřejmé, že se tato choroba stává potenciálně vážnou hrozbou pro produkci citrusů ve všech pěstitelských zemích světa včetně Pákistánu. Existují důkazy, že zelenání je v Pákistánu hlavní příčinou úhynu citrusů; je tedy nezbytné, aby byla ustanovena jasná opatření a strategie, kterými by se tomuto problému dalo zabránit.

Klíčová slova: choroba zelenání citrusů; úhyn citrusů; symptomatologie; ochrana

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