# Citrus Greening and Psylla Vectors of the Disease in the Arabian Peninsula

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ABSTRACT. Greening disease of citrus was discovered in the Arabian Peninsula in two surveys of virus and virus-like diseases for FAO. Identification of the disease was based on symptom expression and on electron microscopical detection of the greening organism. The disease was found in Saudi Arabia in the areas of Taif, Turabah, Korhma and Bisha. The insect vector, *Diaphorina citri*, reaches extremely high population levels at certain times of the year (November). The disease is very probably responsible for the decline and disappearance of sweet orange and mandarin trees in the affected areas. Today, Mexican lime is the only citrus species still grown. Since Mexican lime is relatively tolerant of greening and is also an excellent host for *D. citri*, efficient transmission of the greening organism is favored. The type of greening involved is the heat tolerant, Asian one, a more severe form of the disease.

Greening is also present in North Yemen. It occurs only at elevations above 1000 m or so (Taiz, Hamman Ali). It is not present along the coast. The vector is *Trioza erytreae* and the type of greening involved is the African one, a less severe form.

The presence of the two forms of greening and the two psylla vectors along the East coast of the Red Sea is a threat to the citrus growing areas of the Arabian Peninsula and to the citrus industry of the Mediterranean Basin and the Near East.

*Index words.* citrus, greening, psylla, Saudi Arabia, Yemen.

"Greening" is one of the most serious diseases of citrus and exists on the African continent south of the Sahara in citrus growing countries including Ethiopia. The disease is not present in the northern parts of Africa. It is transmitted in Africa by the "African" psylla, *Trioza erytreae* (Del Guercio) (11). This vector is sensitive to high temperatures and low humidity, and it is often confined to cool, elevated areas, as is the disease. For instance, greening affects the "highveld" areas of Transvaal and Swaziland but not the "lowveld" regions of Swaziland. Similarly, in the island of Madagascar, greening and *T. erytreae* are only present on the high plateau, not on the coastal areas. The same is true in Kenya where greening is severe in the citrus areas above 700 m but absent from the coastal regions. A Gram-negative bacterium-like organism is present in the sieve tube elements of affected plants (4). This "greening organism" (GO) is thought to be the causal agent of the disease. The GO of African greening is sensitive to heat, and no symptoms develop when plants grow at temperatures above 32°C. No GOs are found in such symptomless trees (3) and the African strain of the GO is probably unable to multiply at such temperatures. The heat sensitivity of the African GO and the African psylla vector probably explains the geographical distribution of greening disease in Africa.

A second form of greening is the Asian greening. It is a more severe and more widely distributed form of the disease. Affected citrus areas extend from China and southeast Asia to Nepal, India and Pakistan. In Asia, the GO is transmitted by the Asian psylla, *Diaphorina citri* (Kuwayama) (5, 6, 9) but we have shown that the African psylla can also transmit the Asian strains of the GO (10). The Asian psylla is a much sturdier insect than *T. erytreae*. It resists high temperatures and low humidities. Similarly, the Asian strain of the GO is heat tolerant (3). Hence, the geographical distribution of Asian greening occurs not only in relatively cool areas, but also in hot coastal zones.

The two forms of greening as

well as the two psylla species occur together on Reunion island and Mauritius island. In Reunion, *D. citri* is present from sea level up to an elevation of 600 m, and *T. erytreae* only above 600 m. Greening disease extends from sea-level up to 1000 m.

Greening is not present in the countries surrounding the Mediterranean sea, nor does it affect Jordan, Iraq and Iran. However in November 1981 and April 1982, while conducting a survey for virus and virus-like diseases of citrus on behalf of FAO, J. M. Bové discovered greening disease in Saudi Arabia and North Yemen.

## MATERIALS AND METHODS

Diagnosis of greening was based on symptom expression and electron microscopical detection of the GOs in the sieve tubes of leaves and/or fruits.

Leaves and fruits were kept in plastic bags for a few hours until they could be further processed. The leaf midrib was cut out with a razor blade and chopped into 2 to 4 mm long pieces. About five such pieces were fixed by immersion into a 5 ml screwcap tube filled with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Midrib pieces from about five to 10 leaves were placed in the same tube for each tree.

The peduncular end of the fruit axis is rich in phloem tissue and is the best material for GO detection. The columella tissue was dissected from the fruit with a razor blade, chopped into 2-4 mm long fragments and fixed.

The samples remained in 2% glutaraldehyde for 2 to 12 days until they reached the Bordeaux laboratory where they were post-fixed in 1% osmium tetroxide in the above buffer. After dehydration in ethanol, specimens were embedded in Epon and thin sectioned. Ultra-thin sections were examined in a Siemens Elmiscope 101 electron microscope.

## RESULTS AND DISCUSSION

**Asian form of greening disease and *D. citri* in Saudi Arabia.** Clear-cut symptoms of greening disease were observed on Mexican lime trees in the following plateau oases in the southwestern part of the country (fig. 1): Zaymah, Taif (1682 m), Turabah (1450 m), Khurmah, Bisha. An organism identical to the greening organism (GO) as known elsewhere was detected by electron microscopy (EM) in affected citrus material from all these areas (fig. 2 A, B, C). These EM results confirm that the symptoms observed are those of greening.

In the above areas, the only citrus species now grown is Mexican lime. According to citrus growers, sweet orange and mandarin trees used to do well, but have perished within the last ten years. Greening disease is very probably the cause of this destruction since severe symptoms of greening were observed in diseased sweet orange and mandarin trees at Taif, Turabah and Bisha and electron microscopy showed the GO to be present in these trees.

Mexican lime proved to be less susceptible to greening than sweet orange and mandarin. In the affected areas, lime trees show greening symptoms (leaf mottle), and electron microscopy indicates the presence of the GO, yet the trees reach a relatively good size. An additional reason for fairly good growth is that tristeza disease is not present. This is a remarkable situation since in Africa and Asia, greening is always accompanied by tristeza.

In the above areas, high percentages of trees are affected. This is correlated with high populations of the Asian vector of greening,

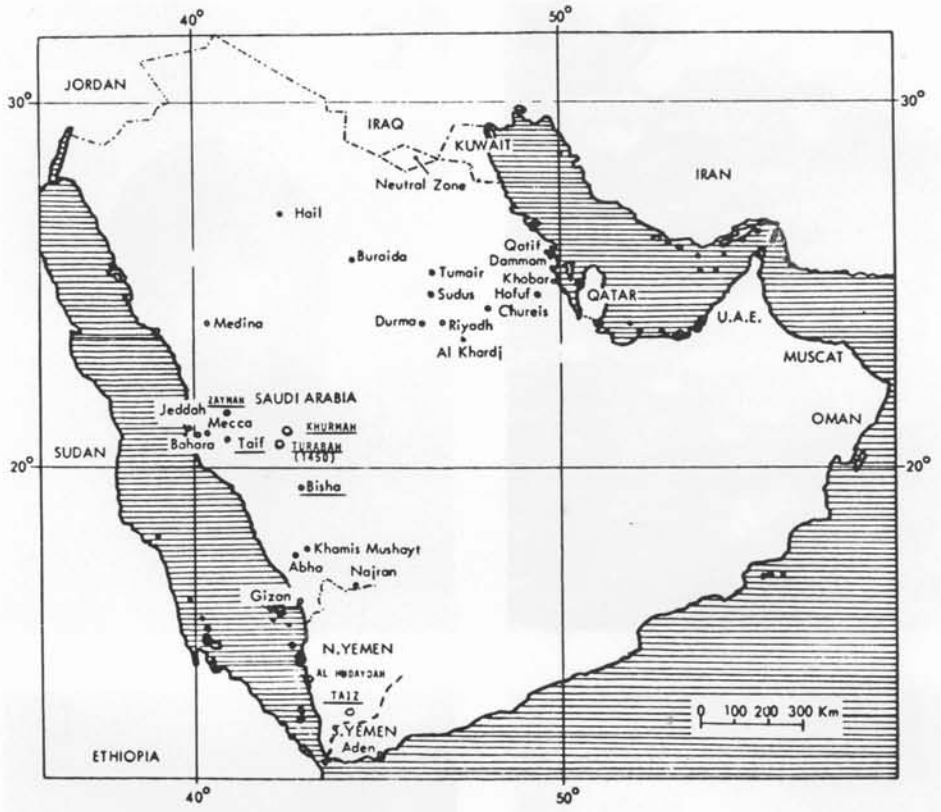


**Fig. 1. Arabian peninsula with some of the main towns. International boundaries are approximate or in some cases still to be settled. Names of towns where greening occurs are underlined.**

*D. citri*. The presence of this psylla was first observed in 1972 and reported in 1974 (12) on citrus in the Djeddah, Wadi Kaulais, Mecca and Hadat Ash Shan areas. However, even though it has been known since 1966 that *D. citri* is a vector of greening (5, 6, 9), no thought was given until 1981 in Saudi Arabia to the possibility that the greening might also be present in areas where the vector occurred.

In certain areas (Taif, Turabah) the psyllas have become a real pest and cause dieback and severe leaf dwarfing and curling. Insecticide treatments have been totally ineffective.

The form of greening present in Saudi Arabia is very probably the Asian form for the following reasons:

1) The vector is the Asian psylla, *D. citri*, and the disease was probably introduced with *D. citri* from an Asian country possibly by pilgrims;
2) The daily summer temperatures are well above 32°C even at the elevation of Taif and Asian greening could survive in this hot climate; and
3) The destruction of sweet orange and mandarin trees suggests that the disease must be the severe, Asian form.

We conclude that citrus other than Mexican lime has disappeared or is dying as a result of greening in the areas mentioned above. A high percentage of the Mexican lime trees are affected, but because of their relative tolerance to greening and the absence of tristeza, these trees achieve a certain development. These numerous rela-

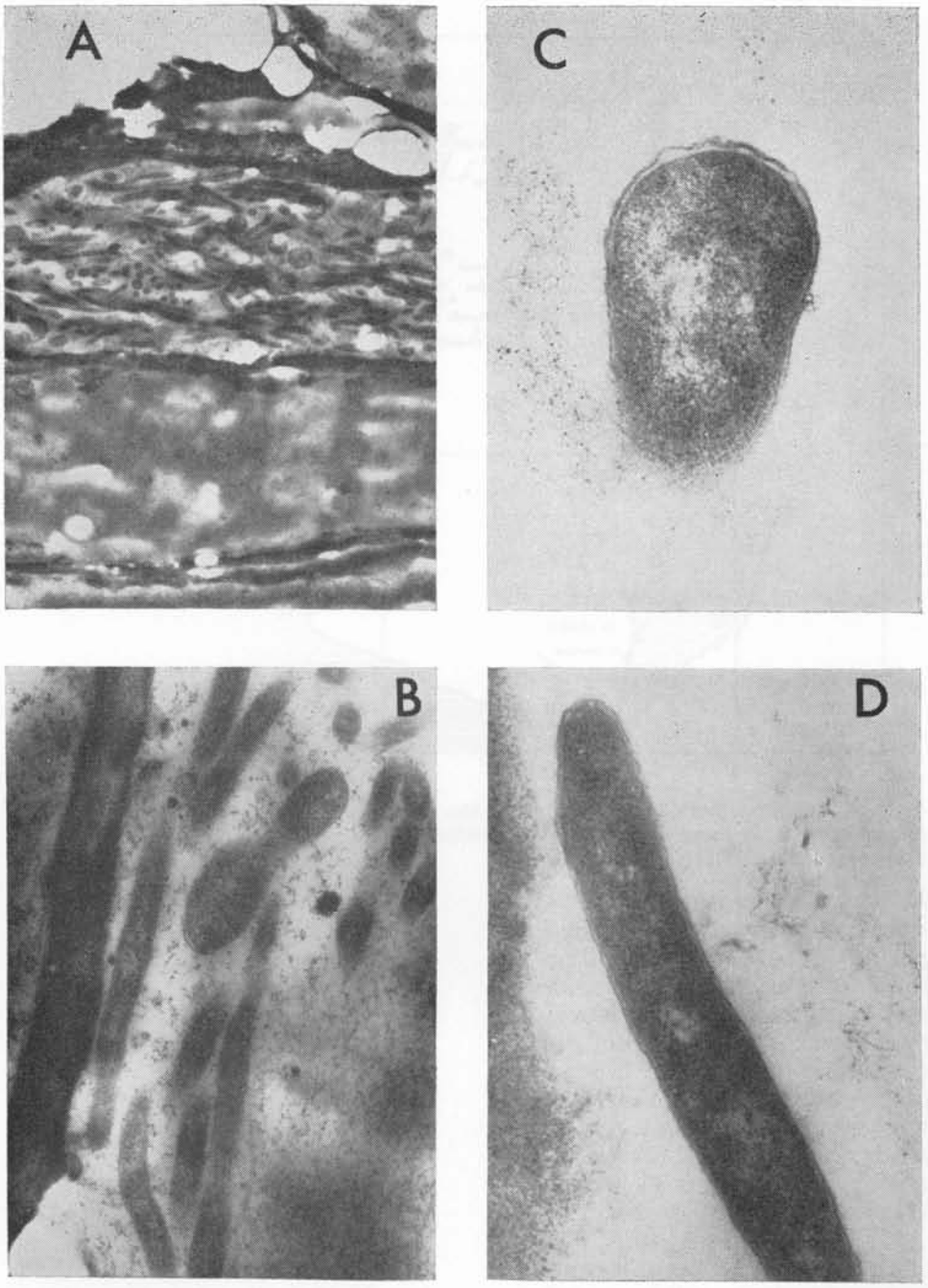


**Fig. 2. Electron micrographs of greening organisms (GO) in citrus from Saudi Arabia (A, B and C) and North Yemen (D). A and B) Sieve tubes of sour orange columella (Taif). Upper sieve tube is packed with GO. (A = X 5,000; B = X 20,000). C) GO in sieve tube of Mexican lime leaf (Turabah) (X 5,000). D) GO in sieve tube of sweet orange leaf (Tiaz) (X 55,000). The characteristic ultrastructure of the GO envelope is clearly apparent in C and D.**

tively well developed and GO-affected lime trees are vast reservoirs of GO inoculum and excellent hosts for the Asian psylla, *D. citri*. Ideal conditions for transmission and spread of greening thus exist (a large reservoir of inoculum and a large population of vectors).

**African form of greening disease and *T. erytreae* in North Yemen.** Advanced symptoms of greening disease were observed in April 1982 on many trees of the citrus collection at the Agricultural Research Station in Taif (Aussfera farm). The trees of this collection were planted in 1974. Leaf and fruit samples taken from badly affected sweet orange trees were examined by electron microscopy at the Bordeaux laboratory, and all were found to contain greening organisms (GO) (fig. 2 D). These observations indicate that the disease affecting these trees is greening. The trees tested negatively for tristeza virus by ELISA. Symptoms of greening (yellow branch) were also observed at Haman Ali in the Anis region.

The areas where greening was seen were all located at an elevation of 1000 m and more. No signs of the disease were found in the coastal Tihama areas examined (Garouba, Wadi Sardut, Jaraba). The geographical distribution of the disease reflects the distribution of the insect vector and in North Yemen the vector is the heat sensitive African psylla, *T. erytreae*. This psylla is known to exist in neighbouring Ethopia and has very probably been introduced with citrus from there into the southwestern part of the Arabian Peninsula.

The form of greening present is very probably the heat sensitive African form since the insect vector is the African psylla, the disease and the psylla vector only occur in cool, elevated areas, and susceptible species and varieties such as sweet orange are badly affected, but not killed.

**Greening, a potential danger for citrus in the Arabian Peninsula and the Mediterranean countries.** The presence of Asian greening and *D. citri* in Saudi Arabia within the oases of the triangle Djeddah-Khurmah-Bisha and perhaps beyond this triangle, and of African greening and *T. erytreae* in the elevated citrus areas of Yemen, is not only detrimental to citrus in these areas, but it is also a high potential danger to other citrus areas of the Arabian Peninsula, as well as to the large citrus growing regions around the Mediterranean sea.

The Najran oasis (fig. 1) of Saudi Arabia, immediately North of Yemen, seems well suited for citriculture, and projects for citrus development are underway. The area was still free of psyllas and greening as of November 1981, but both the disease and its Asian vector are present at Bisha only 300 km north of Najran. It is even possible that the disease is closer than that, since the area between Bisha and Najran has not yet been surveyed.

The Unayzah-Buraydah (fig. 1) area is also free of greening and psylla vectors. Uncontrolled movement of citrus could introduce both the citrus psyllas and the disease.

The coastal Tihama areas south of Al Hudaydah (the northern areas were not surveyed) in Yemen are free of greening and psylla vectors. Several important citrus projects are being developed at Garouba, Wadi Sardut, and Jaraba. The Asian psylla if introduced from Saudi Arabia could entirely ruin the efforts that are now being devoted to citrus in the Tihama.

The Djeddah-Mecca-Taif area is only 1000 km south of the eastern Mediterranean area and Egypt is just across the Red Sea. The Mediterranean basin is free of

greening and citrus psyllas and it is very probable that *T. erytreae* and especially *D. citri* would be able to multiply if introduced in the Mediterranean regions. It is highly likely that the disease would be introduced along with the vector. The introduction of greening and *D. citri* would be extremely detrimental to the Mediterranean citrus industry and must be avoided by all means.

The spread of greening within the Arabian Peninsula and towards the Mediterranean area can only be controlled by controlling the psylla vectors. Insecticide treatments have proven inadequate. It is therefore proposed that integrated control of the citrus psyllas be initiated in Saudi Arabia and Yemen along lines successfully conducted in Reunion Island (1, 2, 7, 8).

We have also found recently (December 1983) greening in the southwest part of Saudi Arabia (Abha, Khamis Mushayt). The two psyllas were present in this area indicating that *Trioza erytreae* has moved across the border up north into Saudi Arabia.

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