CHAPTER 5

Stubborn, Greening, and Related Diseases

A Review of Stubborn and Greening Diseases of Citrus

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History and Distribution

STUBBORN.—This disease, now present in many citrus areas of the world, was recognized as a problem over 50 years ago by growers near Redlands, California (6, 35). Topworking with budwood from thrifty trees failed to improve diseased trees, so the propagator called them "stubborn" (34). Stubborn was first described and its transmissibility indicated in 1944 (35). Fawcett (32) reported its virus nature in 1946. Names often considered synonymous with stubborn include acorn, blue nose, blue albedo, pink nose, stylar-end greening, and puny leaf.

By 1946, stubborn disease was relatively common in navel oranges [*Citrus sinensis* (L.) Osb.] and grapefruit (*C. paradisi* Macf.) in Southern California and Arizona. Research was accelerated on stubborn in the mid-1950's. On the basis of fruit or branch symptoms, Carpenter (12) tentatively identified stubborn in some locations in Florida and Texas and throughout the citrus areas of California and Arizona. In the latter two states, high percentages of trees were affected in some orchards. By 1965, the number of stubborn trees in California was estimated at 200,000 and may have been larger (6). Childs (27) recently reported evidence that stubborn occurs in Florida.

Stubborn disease has been reported from many citrus producing areas around the Mediterranean Basin (19, 20, 21, 22, 28, 55, 56) and is suspected in others (4, 18, 45, 81, 82). The extensive occurrence of stubborn in several Mediterranean countries suggests that it probably is not a new disease there and that it has been introduced into all citrus areas in that part of the world. In recent years, stubborn has become increasingly important in Morocco (20, 23, 28, 61) and in California, where it is now the worst virus disease problem in some citrus orchards (6).

General symptoms of stubborn disease were noted in Brazil, Argentina, and Peru by Vogel and Bové (83), and fruit symptoms were reported in Brazil by Cintra and Nakadaira (30). Some diseased trees in Peru were reportedly from California sources (3).

GREENING.—This disease, first called yellow branch, is similar to stubborn and has been known since 1929 in South Africa. It caused severe crop losses in the years 1932-1936 and 1939-1946 in two areas of the eastern Transvaal, but did not do much damage elsewhere in South Africa. Since 1958, however, greening has been severe in western and northern Transvaal, and has appeared in eastern Cape Province (1, 59, 72). Oberholzer *et al.* (59) estimated that greening has rendered 100,000 trees unprofitable in the Transvaal and that it is present in many South African regions previously considered free of the disease.

That greening might be of virus origin was recognized in 1948 (41), and some evidence of its transmission by grafting was reported in 1955 (58).

LITTLE LEAF.—This disease of Palestine citrus, described by Reichert and Perlberger (66) in 1931, is similar or identical to stubborn (19, 20, 26, 34, 43, 44, 60, 65). Although its possible identity with xyloporosis has been suggested (64), indexing results from California (7, 13) and Morocco (17), plus data from hundreds of inoculations with xyloporosis, cachexia, and stubborn, convince the reviewer that xyloporosis and stubborn are distinct, although sometimes concurrent, diseases.

Host Range and Symptoms

HOST RANGE.—Stubborn disease apparently can affect most, or all, commercial varieties of citrus grown in California and Morocco. In the United States, Carpenter (12) found symptoms on 26 varieties of grapefruit, 10 of orange, 4 of tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco), and 3 of shaddock [*C. grandis* (L.) Osb.]. Calavan and Christiansen (7) observed symptoms on young Tien Chieh mandarin (*C. reticulata* Blanco), Eureka lemon [*C. limon* (L.) Burm. f.], West Indian

lime [*C. aurantifolia* (Christm.) Swing.], calamondin (*C. mitis* Blanco), Koethen, navel, Shamouti, and Valencia sweet oranges, and Satsuma mandarin trees inoculated from thoroughly indexed stubborn navel orange trees. Many varieties of citrus inoculated from 1959 to 1966 (unpublished) were sensitive to stubborn virus. Severe damage was incurred by all important commercial varieties grown in California and many less important ones, including Egyptian Blood, Hinckley, Limoneira, and Madam Vinous sweet oranges; Sexton and Thornton tangelos; Batangas, Cleopatra, and Laranja Cravo mandarin; Japansche Citroen mandarin-lime (*C. limonia* Osbeck); Dweet tangor [*C. reticulata* Blanco x *C. sinensis* (L.) Osb.]; [*C. pennivesiculata* (Lush.) Tanaka] (a lemon type); Rough lemon [*C. limon* (L.) Burm. f.]; and several varieties of citron (*C. medica* L.). Sour orange (*C. aurantium* L.) and Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.] were slightly to moderately affected. Although trifoliate orange [*Poncirus trifoliata* (L.) Raf.] and Troyer citrange [*P. trifoliata* (L.) Raf. x *C. sinensis* (L.) Osb.] seedlings were not severely damaged by stubborn, they imparted no tolerance to sweet orange and mandarin tops. In California, stubborn disease now occurs more or less commonly in Valencia orange, navel orange, grapefruit, and in several varieties of tangelo, but has been found infrequently in Clementine, Dancy, and Satsuma mandarins, and lemons.

In Morocco, acorn and other stubborn symptoms have been reported on mandarins, mandarin hybrids (Temple and Campeona), sweet oranges (Washington navel, Thomson navel, Surprise navel, Golden Buckeye, Hamlin, Jaffa, Tarocco, Grosse Sanguine, Salustiana, Petite Jaffa, Vernia, Valencia late, Magnum Bonum, and several local varieties), grapefruits, Bergamot orange, and the kumquats *Fortunella margarita* (Lour.) Swing. and *F. japonica* (Thunb.) Swing. (17, 20, 22, 24, 28). Safargali oranges in Egypt (20, 29, 56) and several varieties of citrus in Corsica (81, 82) also are affected.

In South Africa, greening is recognized as predominantly a disease of the sweet orange (52, 59) regardless of the rootstock, but Empress mandarin is also severely affected (53, 69), and other mandarins, Satsuma, Troyer citrange, lemon, and grapefruit evidently are sensitive to the disease (50). The sensitive host ranges of stubborn and greening appear to be very similar. Cintra and Nakadaira (30) reported in detail symptoms that resemble stubborn on Lima, Pera, Piralima, and Seleta oranges in Brazil.

SYMPTOMS OF STUBBORN DISEASE.—A wide variety of symptoms has

been attributed to stubborn disease (6, 25, 34, 35, 40). Most or all of the tree may be affected, but normal branches often occur in diseased trees, and shaded portions of trees often have milder symptoms than exposed portions. Carpenter (12) and Hilgeman (40) described different types of stubborn trees, and Calavan and Carpenter (6) have emphasized the variable symptomatology of stubborn.

Fruits on stubborn trees are often small and malformed; they may be lopsided with a curved columella or acorn-shaped, that is, elongated and having a thick peel on the stem end and thin peel on the stylar end. Other fruit symptoms include insipid or sour taste, irregular or inverse coloration (stylar-end greening), premature mummification, early drop, and white waxy appearance of the rind where pressure is applied. Stubborn trees tend to have growth flushes out of phase with those of normal trees and to bloom several times a year. Many stubborn trees, especially navel oranges, yield few fruits (6, 33). In 1944, Tidd (79) reported that stubborn decreased production of navel orange trees 30 to 50 per cent. Recent observations on naturally infected and inoculated navel trees indicate yield reductions of 90 per cent or more, in many infected trees. Substantially reduced yields were also noted in many stubborn Valencia trees. Fruit production of inoculated Lisbon lemon trees on sweet orange rootstock in California was 59 per cent below normal for the period 1962-1966.

Stubborn trees usually show an abnormal habit of growth with shortened intervals between leaves, bunchy upright growth with an excessive number of shoots, multiple buds, small cupped leaves, a variety of chlorotic or mottled leaf patterns resembling various nutritional disorders, and green or premature yellow banding of veins of some leaves. These leaf patterns occur irregularly and many leaves drop early. However, some leaves of many stubborn trees appear normal. In Arizona, thick leaves with prominent veins (40), blunted vein terminals, and relatively few calcium oxalate crystals (77) were reported on severely affected stubborn trees. Considerable twig dieback and foliar chlorosis occur in some severely affected trees giving them a rather open appearance, but many stubborn Valencia trees are compact, bushy, and green. Heliotropism occurs in stubborn trees in Morocco (25) and occasionally in California.

The validity of some alleged symptoms of stubborn remains in doubt, and in the orchard the diagnostic value of most symptoms is limited, except where severe foliar, shoot, and fruit symptoms are not attributable to other causes. As originally noted, acorn fruits do not occur every

year even on severely affected trees although some of the fruits are more likely to exhibit this symptom late in the season (35). Acorn and stubborn diseases of navel orange were considered to be probably identical by Fawcett *et al.* (35) and by Haas *et al.* (38) in 1944, but later Haas (37) questioned the cause of acorn-shaped grapefruits on vigorous trees. Few acorn fruits, if any, can be found on most stubborn citrus trees at Riverside, although a small number of such fruits have been found on many of the inoculated trees of several species. Also, stubborn has been transmitted from at least two navel orange trees that have a history of acorn fruit production. Yet, acorn fruits are frequently produced by vigorous, non-inoculated, nucellar-clone grapefruit trees from which we apparently have failed thus far to transmit any virus. Experiments to determine the relation of acorn fruit to stubborn are in progress.

Blue albedo or blue nose may be classed as a doubtful symptom since Carpenter and Hield (16) showed that this symptom is influenced by 2, 4, 5-T sprays (12). In some seasons, blue albedo is relatively common on some stubborn trees, and has occurred in inoculated trees of Marsh grapefruit, and navel and Valencia oranges. However, it is rare or absent in many stubborn trees, especially sweet oranges. Thus, blue albedo seems to be intensified by stubborn virus, but has little diagnostic value by itself.

Fawcett *et al.* (35) described an abnormal type of branching and foliage as the "most constant characteristic of the disease." However, these branch and leaf symptoms can be unreliable indications of stubborn under some conditions because they may result from a variety of causes (7).

Excessive seed abortion has been a valuable symptom of stubborn in Valencia and certain other seedy varieties (6, 10, 15). Although not present in every fruit on a stubborn tree, seed abortion has been severe in inoculated trees of several seedy varieties.

SYMPTOMS OF GREENING DISEASE.—The similarities of stubborn and greening diseases were noted by the reviewer (5) in 1959, by McClean and Oberholzer (50) in 1965, and by Schneider (67) in 1966. Greening disease (50, 59), like stubborn, has a long list of symptoms. It usually causes stunting, leaf and fruit drop, twig dieback, sparse foliation, out-of-season growth and blossoming, and poor crops of predominantly greened, worthless fruit. Sometimes, as with stubborn, only a portion of the tree is affected. A diversity of foliar chloroses, often more severe than stubborn chloroses, accompany greening, and a type of leaf mottle resembling zinc deficiency patterns often predominates (59). Young

leaves appear normal, but soon assume an upright position, become leathery, and develop prominent veins and a dull olive green color. Rather severe dieback, sometimes with multiple bud formation, characterizes severely diseased trees.

Fruit on greening-affected trees are often small, lopsided with a curved columella, and poorly colored. The side of the fruit exposed to direct sunlight usually develops full color, but the remainder of the fruit is usually a dull olive green. Blue albedo and acorn fruits occur occasionally in affected trees, but are not typical of greening disease (59). Greened fruits are low in juice and soluble solids, but fairly high in acid, so they are worthless as fresh fruit or for processing. Valencia and other sweet orange varieties develop some normal seeds, but most are small, poorly developed, and dark colored (59). Certain differences in symptoms exist between stubborn and greening (50, 67). Apparently, greening affects Valencia trees more severely than does stubborn, whereas stubborn may cause more damage than does greening in navel orange trees. Schneider (67) suggested that stubborn virus may be an attenuated form of the greening virus. Certainly the relation of stubborn to greening requires further study.

Recent Research Developments

Considerable progress has been made in the past few years on transmission and indexing, and on biochemical and vector studies of both stubborn and greening disease. The anatomical aspects of greening have been described by Schneider (67), and their value in diagnosis indicated. Nour-Eldin (57) reported a tumor-inducing agent associated with stubborn disease.

TRANSMISSION.—In California, transmission of stubborn virus to several citrus species by buds and grafts was reported at the Second Conference of the IOCV (7). Cassin (17) later reported virus transmission from stubborn trees of several sweet orange varieties in Morocco. Chapot and Delucchi (25) stated that several sources of stubborn caused a reaction on inoculated citron plants. In California (39), indexing was used to identify a topworking disorder as stubborn. In 1962, Calavan and Christiansen (8) summarized the effects of stubborn on several kinds of citrus trees infected by graft inoculations, and in 1965, they reported indexing of stubborn disease within two to eight months (9). Under suitable greenhouse conditions, the stubborn virus quickly caused a rather specific reaction in young indicator plants—general stunting of the shoots accompanied by small, mottled, cupped leaves. Mottling ap-

pears first around the margins of the distal half of expanding leaves and spreads until much of the blade is affected. At maturity, affected leaves often closely resemble those of stubborn trees in the field (6, 32). The technique of indexing on Madam Vinous sweet orange and other indicators has been improved and shortened. Through these methods, an irregular distribution of stubborn virus was found within the tree, and indications of mild forms of stubborn were noted (11). Irregular distribution of stubborn virus complicates indexing, but offers a plausible explanation for the normal trees reported among inoculated plants or among bud progeny of diseased trees (7, 12, 14, 17, 25, 40). Similarly, healthy propagations from greening-affected trees (50, 59, 67) suggest that distribution of greening virus in the host is also restricted or only partially systemic. A comparable situation occurs with the pear decline virus, which was transmitted by only a small percentage of grafts (75).

By bud propagation, Carpenter and Allen (14) obtained many normal trees from stubborn appearing Marsh grapefruit trees and many trees with stubborn symptoms from normal appearing parents. They seldom found shoot and fruit symptoms concurrent in the same trees or at the same time in different trees. Such results are difficult to interpret.

Schwarz (68) and McClean and Oberholzer (51) showed that in certain regions of South Africa greening virus is transmitted by the citrus psylla (*Trioza erythreae* del Guercio). Tirtawidjaja *et al.* (80) noted that another psyllid, *Diaphorina citri* Kuw., seemed to transmit citrus vein-phloem degeneration virus, which causes a disease resembling greening (67).

Likubin disease in Formosa and yellow shoot in mainland China are similar to greening and stubborn in many respects and are reported to be transmitted by the citrus aphid, *Toxoptera citricida* Kirk. (31, 54), but this has been questioned (47). *T. citricida* apparently does not transmit greening (50). Elimination of the yellow shoot virus by hot-air or hot-water treatment of budwood and hot-air treatment of trees has been reported (46, 48). In California, propagations from heat-treated stubborn budwood appear to be healthy.

Fraser and Singh (36) believe that greening may be contributing to the decline of citrus trees in India. Reddy (63) suggested earlier that the decline of trees on jamberi rootstock in India is probably due to the coexistence of some other viruses with tristeza.

Mechanical transmission to cucumber has been reported for stubborn virus by Storm and Streets (78) and for greening by Schwarz (73).

CHEMICAL STUDIES.—A fluorescent substance in the albedo of green-

ing-affected sweet oranges was reported by Schwarz (70), and his recent studies (71) indicate that chromatographic techniques for isolating the fluorescent substance have considerable merit for diagnosis.

A serological test for stubborn disease reported from Arizona (76, 77) may be based on a virus or on a protein by-product of disease (62, 77). Storm (76) reported that stubborn disease virus was inactivated between 40 and 50°C and below pH 5. He found no chromatographic differences in amino acid content between healthy and diseased plants. A temporary response to application of iron chelate was noted in some stubborn trees by Hilgeman (40).

Studies comparing the chemical constituents of stubborn (acorn, lopsided, or blue albedo) fruits with apparently normal fruits show that affected fruits differ chemically from normal fruits and are inferior to them. Bové *et al.* (2) found the stylar half of acorn fruits of Washington navel to have a higher content of citric acid, aspartic acid, arginine, and alanine than do stylar halves of normal fruits from the same tree. Huet (42) found a decrease in sugar content and acidity and an important difference in time of ripening in stylar and peduncular halves of acorn-shaped fruits of navel orange, but not in malformed Valencia orange fruits, thus confirming the earlier work of Schwob and Dupaigne (74). Long and Childs (49) determined that the solids-to-acid ratio of the stylar half and the calyx half of Temple tangor fruits with stylar-end greening was lower than that of corresponding halves of normal fruits, and the gradients were reversed.

Discussion and Conclusions

The concurrence of strong symptoms in the fruit and branches usually suffices to identify stubborn, but often neither fruit nor branch symptoms alone are specific enough in the field for positive identification. Consequently, diagnosis of stubborn often requires confirmation by graft transmission, and the new techniques now available should lead to clarification of both stubborn and greening syndromes.

Strong evidence indicates that stubborn is a virosis, although several strains of stubborn virus or a virus complex may be involved. Recent studies suggest that mild strains of virus exist in some stubborn-affected plants, but cross-protection and other experiments must be performed to clarify this matter. Stubborn or greening viruses may infect alone or concurrently with other viruses; such concurrence of stubborn with tristeza, exocortis, and cachexia viruses often hindered field diagnosis of stubborn in California.

The irregular and seasonal distribution of stubborn virus in its hosts suggests that the amount of virus may increase or decrease in response to changing host physiology or environment. Such characteristics indicate a rather labile virus and one that may be inactivated by heat treatment of excised budwood, or largely avoided in budwood of some varieties collected at certain seasons.

There is good evidence that both stubborn and greening viruses spread naturally in certain areas, but at present a vector has been identified only for greening virus. More information on natural spread is essential to better evaluation of the threat from stubborn and greening and formulation of better control programs. In most areas, neither virus appears to have spread rapidly, but high vector populations and abundant inoculum would favor natural spread and might make profitable citrus culture impossible in some areas. Production of nursery trees in such areas is inadvisable.

Reported similarities and differences between stubborn and greening viruses suggest that collections from diseased trees in different countries should be brought together in a safe place for vector studies, indexing, and direct comparison.

Recently developed techniques have greatly simplified diagnosis of stubborn and greening, and proper use of these methods should help to prevent further widespread dissemination of these diseases by man. These procedures also permit better future research on stubborn and greening diseases.

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