PRESENT STATUS OF SOME INVESTIGATIONS ON STUBBORN DISEASE OF CITRUS IN THE UNITED STATES

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SUMMARY

Stubborn disease in citrus is considered to be caused by a virus entity or complex. Mature acorn-shaped fruits provide the best single diagnostic symptom of stubborn disease. Blue albedo in fruits is believed to be a characteristic symptom, but its specificity has not been established. Poor tree condition in conjunction with other symptoms is a useful, but nonspecific indicator. Mummified fruits, off-bloom, and autumnal defoliation are secondary indicators of stubborn. No diagnostic foliage condition has been observed.

Stubborn has been identified tentatively in individual trees of 26 varieties of grapefruit, 10 of orange, 4 of tangelo, and 3 of shaddock. A suitable indicator plant for stubborn is being sought among 60 species and varieties of *Citrus*. About 40 trees with stubborn disease are being indexed to determine whether known viruses are associated consistently with this disease, and to find sources of stubborn virus free of other virus entities or complexes.

INTRODUCTION

Stubborn disease of citrus (*2, 3, 6, 7, 10, 11, 14*) is of increasing interest to growers and research personnel, for evidence is accumulating that it is a widespread and important disease of grapefruit and navel orange; some other sweet oranges, tangelos, and shaddocks are believed to be affected also. This disease has been classed with the virus diseases of citrus on the basis of bud-transmission studies (*5, 6, 8*) and a causal agent designated *Citrivir pertinaciae* Fawcett (*5*). Nevertheless, the cause of stubborn disease is still not clearly established. The strong suggestion that it is of a virus nature has influenced the course of the present studies. Investigations conducted in Arizona, California, Florida, and Texas during the past three years are reported here.

FIELD OBSERVATIONS

Expression and Diagnostic Value of Field Symptoms

At present, stubborn disease is identified by field symptoms because suitable indexing procedures are not known. Mature acorn-shaped fruits (called acorn fruits) provide the best single diagnostic symptom of stubborn disease, but these occur sporadically, being more common in some areas and in some years than in others. They are not common in all kinds of citrus suspected of having stubborn. Although stubborn-affected

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trees of Washington Navel orange probably produce more acorn fruits than any other variety, in some areas such as the Salt River Valley in Arizona, where stubborn is a major disease problem, very few acorn fruits are found on navel trees at harvest time. Thus, other and perhaps less certain symptoms must be used at times to identify this disease.

Evident variants of the symmetrical acorn fruits are referred to as malformed fruits. They have irregular areas of normal or thickened peel at the stem end, ranging from single knobs and longitudinal crests to broad areas involving more than a third of their surface. The remainder of the peel is abnormally thin.

Blue albedo[2] is a valuable indicator of stubborn, although it may be nonspecific (*1*), and has been used as a diagnostic symptom in these studies. The blue stain occurs in part or all of the albedo of affected citrus fruits, particularly grapefruit, and has a wide range of expression. Fruits representing one or more of the several stages of bluing may be found on a given tree. Affected fruits are commonly small and become classified as culls, but larger, marketable fruits may be affected. In mild cases the oil glands of the fruits are faintly dulled, sometimes without detectable bluing of the albedo. Dulled oil glands are not diagnostic, but a further search usually reveals fruits with blue albedo. Most of the affected fruits have dull-gray to slaty-blue oil glands and a visible blue stain in the albedo. Finally, some have both an internal and an external blue stain of the peel. Occasionally, strong blue albedo occurs without readily detectable external symptoms, particularly in large fruits. The albedo of affected fruits, especially that of the smaller ones, differs in consistency from typical albedo. It is more compact or dense and the veins are more prominent, sometimes giving it a fibrous appearance. In some very small fruits, the blue stain appears to be closely associated with the veins, perhaps because of the greatly reduced amount of parenchyma in the albedo.

Blue albedo has been associated with stubborn disease for many years (*6*, *14*), especially in grapefruit. It occurs either in typical fruits or in acorn and malformed ones on trees of all ages and conditions, particularly on obviously affected trees. Blue albedo has not been associated with any other disease or physiological disorder of citrus. There have been no chemical or horticultural studies of bluing except those reported in a recent brief note (*1*).

The term *stubborn fruits* is used here to indicate acorn-shaped, malformed, or blued fruits, especially when these occur in some combination on trees suspected of having stubborn disease.

The presence of mummified fruits is a useful secondary indicator of stubborn disease, especially on grapefruit trees in dry climates, suggesting that many small, unmarketable fruits have been present. This symptom is usually confirmed at harvest by abnormal fruits in the current crop. Fruits with conspicuous bluing may begin to mummify by early December at Indio, California.

Poor tree condition is a good indicator only in the more severe cases of stubborn, where the tree is dwarfed and twiggy without other obvious disorders. The dwarf condition results from an early onset of successive cycles of growth with short internodes and excessive development of axillary buds into short weak shoots. Dwarfed trees frequently produce off-bloom and undergo partial autumnal defoliation. Less than 3 per cent of the trees in most Marsh grapefruit plantings are of the dwarf type, but about 10 per cent of the trees in some Washington Navel orange plantings may be dwarfed. Acorn or blued fruits have been found on vigorous old-line trees, however, and thus suggest that stubborn is widespread in these varieties.

Wood and bark pitting of the stock and scion may occur in varying degrees on stubborn-affected trees but are not consistently associated with this disease. More than

[2] The terms *blue albedo, blued fruits,* and *bluing* all refer to the blue stain in the albedo of citrus fruits, whether or not it is visible externally in the peel.

30 stubborn-affected trees, both with and without pitting, were indexed for tristeza; all yielded negative results.

Abnormal foliage on obviously affected trees is not diagnostic for stubborn if the assumption that stubborn is generally present in many old-line citrus varieties is correct. If the vigorous trees of a variety commonly carry stubborn, and their foliage is the standard of comparison, then abnormal foliage on weak trees reflects an unhealthy condition of no special significance.

Distribution and Description of Stubborn Disease in Various Kinds of Citrus

Grapefruit. Stubborn disease has been tentatively identified in the principal grapefruit-producing areas of Arizona, California, Florida, and Texas. Acorn fruits, blue albedo, and other symptoms of stubborn have been seen frequently in Marsh, Redblush, Ruby, and Webber's Java Pink grapefruit. Blue albedo has been found in individual trees or orchards of the Duncan, Foster, Frost's nucellar Marsh, Thompson (Pink Marsh), and 18 other grapefruit varieties of more restricted distribution. The observations reported here were made mostly on Marsh grapefruit trees. This is the principal variety grown in Arizona and California, and many mature groves are available for study. In a planting of Marsh grapefruit of uniform age, mature trees affected with stubborn may be grouped into three categories.

The first group includes weak, dwarf trees that are twiggy, dense, and low, bearing mostly inferior fruits, of which many show stubborn symptoms. Off-bloom and partial autumnal defoliation are common on these.

In the second group the poorest trees approach the dwarf type described above, but there are all gradations up through those with better vigor and a fair to good proportion of marketable fruit. The best trees of this group are of average size, bear well, and usually have only one to a few conspicuous twiggy branches that bear stubborn fruits, have off-bloom, and undergo autumnal defoliation. Occasional large trees of this category may produce large numbers of small stubborn fruits annually.

The third group includes many trees ranging in size from average to large. These may have some twiggy branches in the top and may produce a few blued, malformed, or acorn fruits. One or more of these fruit symptoms were found in every carefully examined mature grove of Marsh, Ruby Red, Redblush, and Thompson (Pink Marsh) grapefruit. Frequently, 60 to 70 per cent of the trees in an orchard had one or more blued or acorn fruits.

At Indio, blue albedo has been found in grapefruit by late October, and the number of blued fruits seems to increase after several weeks of cool weather. Fortunately, few fruits per tree are usually affected by stubborn, although 10 to 25 or even more per tree have been found. Blued grapefruit have an unpleasant flavor that becomes distinctly bitter as an aftertaste. Some small, rough, dense fruits have a pungent odor approaching that of trifoliate-orange fruits. During the windstorms that commonly occur early in the harvest season in the desert areas of California and Arizona, some blued fruits are shed. Apparent regreening of large blued grapefruit has been noted in late spring at Indio. Observations in Arizona during 1954 and 1955 indicated that stubborn fruits are more susceptible than normal ones to frost damage; large numbers of frost-damaged fruits either fell or mummified.

Stubborn symptoms were not detectable in budlings of nucellar, good old-line and stubborn old-line Marsh grapefruit during the first season in the nursery. Blued grapefruits have been found in three-year-old nursery trees. In some four- to six-year-old plantings of Redblush and Ruby Red grapefruit at Indio, 5 to 16 per cent of the trees have had both acorn and blued fruits as well as precocious bloom and a tendency to twigginess. Stubborn symptoms have been seen in Marsh trees more than forty years

old and one large tree of Webber Java Pink grapefruit has yielded both normal and stubborn fruits for over twenty-five years.

Navel Oranges. Observations were made on both Washington Navel and Robertson Navel orange trees in California and Arizona. The range of stubborn symptoms in Washington Navel parallels that found in grapefruit. Some differences are noted here. In Arizona more than 10 per cent of the Washington Navel trees may be of the dwarf type and comparable to those described for the first group in grapefruit. Distinct acorn fruits are rarely found on them, but a few blued fruits occur. Similar fruits have not been found on vigorous old Washington Navel trees in Arizona, but nursery stock propagated from them continues to produce many dwarf trees.

The navel trees in the second group are perhaps more conspicuous than grapefruit trees in the same group, being twiggier and with a more flattened top; they bear variable crops including many poor, coarse, insipid fruits, some of which are acorn-shaped or blued.

Trees of normal appearance make up the third group. On close inspection these may yield acorn-shaped or malformed fruits. Such trees would escape notice ordinarily, except at harvest time.

Tentative diagnosis of stubborn disease in navel trees by growth habit alone is especially hazardous in some desert areas having highly variable soils and shallow hardpans, for trees grown on such unfavorable sites can simulate the dwarfness associated with stubborn. Cold, heat, or drought injury may aggravate their poor appearance.

Blue albedo is much less common in fruits of Washington Navel orange than in grapefruit. Fruits with only the blue-albedo symptom are usually less than 2 inches in diameter and rough. The albedo tends to be dense and fibrous. Such fruits are borne usually on ordinary branches and often on an inflorescence with a normal fruit. Small, hard, blued fruits may occur on Washington Navel trees; they are more numerous on those sprayed with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a fact which parallels the results obtained in spraying grapefruit with 2,4,5-T (*1*).

In the Salt River Valley of Arizona, where the winters are relatively cold for citrus, navel trees with severe stubborn are more prone to cold injury than vigorous trees are. The resulting dieback weakens an already ailing tree, and the following spring it produces many weak shoots susceptible to further damage by extremes of cold or heat. Such trees finally become useless.

In the desert area of California and Arizona many of the Robertson Navel orange trees have the dwarf growth habit of stubborn-diseased trees and bear poor crops, including numerous stubborn fruits.

Valencia Oranges. Valencia oranges appear to be either generally free of stubborn disease or little affected by it. In Arizona several hundred Valencia trees and large quantities of fruit in packing houses have been examined at harvest time without more evidence of stubborn infection than an occasional small, hard, blued fruit and a few weak trees. No symptoms were evident on a large Valencia tree that had been formed by top-working on Marsh grapefruit, even though a single branch of the Marsh produced several large acorn fruits in 1956 and 1957. The other Valencia trees of the same composition in this block were also vigorous.

Other Sweet Oranges. Many sweet orange varieties have been inspected for symptoms of stubborn disease in Arizona and California. Acorn fruits have been found in Rico Number 1, Koethen, Trovita, Mediterranean Sweet, Hamlin, and Butler oranges, and some fruits with blue albedo have been found. Some trees of these varieties have a dwarf, stubborn growth habit. Stubborn is suspected in some Shamouti orange trees because of their fruit shape and growth habit.

Tangelos. Apparently, all Thornton tangelo trees in the Indio area are affected by stubborn disease. In a single orchard of this variety, two severely diseased trees about

15 feet tall bear large numbers of stubborn fruits annually, often with strong external bluing of the thinner peel. No other citrus variety has displayed such a close association of blue albedo with acorn shape. The other trees are 20 to 25 feet tall and each has one or more large twiggy branches that bear stubborn fruits.

Stubborn disease may be present in three other varieties of tangelo in Arizona and California. Blue albedo occurs in fruits of Minneola tangelo at Indio, in fruits of Sampson tangelo in Arizona and California, and in fruits of an unidentified tangelo tree at Riverside. This last tree has many small fruits less than 1.5 inches in diameter; normal fruits often exceed 3 inches. The small fruits have thick, firm, irregular peel, but no obvious internal breakdown.

Shaddocks. Both blue-albedo and acorn fruits have been seen in the Hawaiian and Sunshine varieties of shaddock at Riverside, and blue albedo has been found in fruits of Kao Panne. Affected fruits of shaddock are mostly much smaller than normal fruits. They have a dense, fibrous albedo, aborted seeds, and roughened peel and are usually lopsided.

EXPERIMENTS

Search for an Indicator Plant. A great obstacle to research on stubborn disease is the lack of a suitable indicator plant that will give a consistent diagnostic reaction, expressed preferably much sooner than the onset of adequate fruiting. Even fruit symptoms may not be entirely satisfactory, as acorn fruits occur erratically and the specificity of blue albedo has not been determined. Twenty-five species and varieties of *Citrus* were inoculated in 1955 with buds from a Marsh grapefruit tree of the dwarf stubborn type. After two years these showed no evidence of stubborn disease in the leaves, bark, peeled stems, or roots. In 1957, thirty-five others in two series were inoculated with buds from this same Marsh tree and with those from a second stubborn Marsh tree. No foliage symptoms had developed on these after 8 months. All sixty of the species and varieties will be observed for several years, and others will be added.

Indexing of Stubborn-affected Trees for Known Viruses. Indexing of trees believed to have stubborn disease was begun in 1955. Each is being indexed on sweet orange for psorosis, on Mexican lime for tristeza and vein enation, on Palestine sweet lime for xyloporosis, on Orlando or Wekiwa tangelo for cachexia, on Rangpur lime for Rangpur lime disease, and on trifoliate orange or Morton citrange for exocortis. The goals are (*a*) to learn whether any of these viruses occur in each tree, (*b*) to detect any consistent association of known viruses with stubborn disease, and (*c*) to obtain stubborn sources free of other viruses. To achieve this last goal it is now believed that pure sources of stubborn virus, if such exist, should be sought in vigorous old trees having acorn-shaped fruits and perhaps blue albedo, but free of other suspected symptoms of stubborn disease and symptoms of other known virus diseases. At present, 15 grapefruit, 18 Washington Navel orange, 3 Valencia orange, and 3 tangelo sources are under test.

Comparative Study of Stubborn Disease in Several Hosts. In 1955 several kinds of citrus were inoculated with buds from stubborn-affected trees to study their reaction to this disease. Meanwhile, the inoculum sources are being indexed to determine what other viruses may be present in them. The scion varieties budded on Rough lemon rootstock were Frost's nucellar lines of Marsh grapefruit, Washington Navel orange, Valencia orange, and Lisbon lemon, and Furr's[3] nucellar lines of Dancy tangerine and Orlando tangelo. Two trees of each were inoculated in the rootstock with buds from a stubborn tree of each of the following varieties: Marsh grapefruit; Washington Navel and Robertson Navel oranges; Valencia, Mediterranean Sweet, Hamlin,

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and Shamouti oranges; and Minneola tangelo. Duplicate control trees of each scion and stubborn tree were produced also.

Typical symptoms of cachexia were obtained on Orlando tangelo inoculated with buds from the stubborn-diseased Washington Navel orange tree. Frost Lisbon lemon trees inoculated with buds from the same navel tree were only 2 feet high at two years of age and suffered severe frost damage during the winter of 1956–57. Control trees of this lemon were 5 feet tall, vigorous, and undamaged by frost.

Studies on the Origin of the Dwarf Stubborn Tree Type. Dwarf stubborn-affected trees of both grapefruit and navel orange apparently originate from buds on vigorous trees whose progenies are mostly vigorous trees of the variety concerned. Exploratory studies suggested that two years after inoculation the growth of Frost Marsh grapefruit trees of nursery size was distinctly retarded by inoculation with buds adjoining the fruit stems of blued fruit, but not by inoculation with similar buds from twigs bearing atypical fruit. Both kinds of inoculum were taken from the same tree.

Paired budlings have therefore been produced from eight vigorous 30-year-old Marsh grapefruit trees, each pair comprised of a budling derived from the buds adjoining the fruit stem of, respectively, an acorn or blued fruit and a typical fruit on the same tree. These will be studied to learn whether trees derived from these two kinds of budwood have equal or dissimilar growth and fruiting characteristics.

Comparative Study of Marsh Grapefruit Lines. The progenies of several Marsh grapefruit lines are being studied comparatively in relation to (*a*) growth and general behavior, (*b*) total yield and fruit-size distribution, (*c*) fruit quality, (*d*) age at which stubborn becomes apparent and its progressive development, and (*e*) the diagnostic value of the field symptoms of stubborn disease. The lines to be compared are two recent seedlings of Frost Marsh, Frost nucellar Marsh, two good old-line Marsh, and six distinct stubborn-affected trees of Marsh. These are being propagated on Rough lemon rootstock and will be used for field plantings, where they will be replicated in randomized single-tree plots.

DISCUSSION

The current and earlier investigations on stubborn disease of citrus pose many questions. If it is assumed that stubborn disease is a virus disorder, has the causal virus been correctly designated (*5*) or is stubborn caused by an unidentified virus entity or complex? Are the diagnostic symptoms currently in use reliable or misleading? Is there natural transmission of stubborn by insects, in seeds, by root grafts, and in other ways? Is stubborn disease as widespread, both geographically and within kinds and varieties of citrus, as the data indicate? Is this disease an important factor in declining yields, small-fruit-size problems, and untimely senescence in some varieties? The answer to these questions await two mutually dependent events: the discovery of a suitable indicator plant and procurement of uncontaminated sources of the stubborn virus entity or complex.

Natural transmission of the causal agent of stubborn disease is an almost unexplored aspect of this disease problem that needs thorough study, for some competent observers feel that it is spreading in certain citrus groves. Others believe that the apparent spread is only delayed expression of the disease. Studies are required on the specificity of the blue-albedo symptom and on the biochemical and physiological problems associated with blue albedo and poor fruit quality.

Stubborn disease has been reported from countries in the Mediterranean area (*2, 11*); it must therefore be ascertained whether the disorders there and in the United States are identical. Such diseases as little leaf in Israel (*3, 12*), the Safargali disorder of oranges in Egypt (*4*), greening of grapefruit in South Africa (*9*), stunt bush and

stem pitting of grapefruit in New South Wales (*13*), and the dwarf disease of Satsuma orange in Japan (*15*) may possibly be allied to stubborn.

Nucellar budwood sources are more likely to be free of stubborn than old lines and when horticulturally acceptable they should be recommended to nurserymen and growers as a means of minimizing the continued perpetuation of stubborn disease in varieties suspected of carrying it. Where nucellar lines are not available, steps should be taken to procure or produce them.

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