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Stunting and Chlorosis Induced in Young-Line Citrus Plants by Inoculations from Navel Orange Trees Having Symptoms of Stubborn Disease

THE TERM "stubborn" was applied, about 1921, to nonproductive navel orange [*Citrus sinensis* (L.) Osbeck] trees which had responded poorly to top-working (6). Much later, Fawcett, Perry, and Johnston, after 2-years' observation of experimentally top-worked young navel orange trees, described stubborn disease in 1944, and indicated its "probable virus nature." The acorn symptom of navel orange fruit was ascribed to stubborn disease at that time and possible relationships of blue albedo, crazy top, and acorn symptoms of grapefruit (*C. paradisi* Macf.) to stubborn disease were suggested (3, 6, 8). Haas (7) later found no relationship of acorn-shaped grapefruits to crazy top, blue albedo, and low yield, and Carpenter (1) recently considered blue albedo to be a valuable but nonspecific indication of stubborn disease.

Chlorosis and stunting—symptoms frequently associated with stubborn disease (6)—were observed by us in 2 lots of citrus seedlings in 1958 during indexing operations. In the first lot, these symptoms were noted on 6 seedlings within 2 months after inoculation with buds from tree 29-36, a twiggy, stunted, nonproductive 15-year-old navel orange on a sweet orange rootstock inoculated by Fawcett *et al.* (6) in the first experiment with stubborn disease. The plants affected were Eureka lemon [*C. limon* (L.) Burm.], West Indian lime [*C. aurantifolia* (Christm.) Swingle], and calamondin (*C. mitis* Blanco). They developed no leaf or stem symptoms specific for psorosis, tristeza, vein enation, or yellow vein, and all had sound roots. About half the seedlings used for indexing tree 29-36 remained normal, and affected seedlings made partial recovery.

In the second lot, stunting and chlorosis were observed in a seedling of Tien Chieh mandarin (*C. reticulata* Blanco) inoculated with buds from shoots of Frost Washington Navel orange top-worked in 1956 on an old, medium-sized, nonproductive navel orange, tree C-189, having symptoms of stubborn disease.

This paper reports some preliminary results of studies made from 1957 to 1960 with the agent or agents present in the 2 source trees, and discusses their possible significance in relation to stubborn disease.

Experiments and Results

Forty seedlings of Koethen sweet orange in 1-gal. cans were inoculated as follows: (a) 10 received 1 bud and 1 side graft, on February 16, 1960, from tree 16A-1, a severely stunted, 2½-year-old Frost Washington Navel orange tree with symptoms of stubborn; this tree had been inoculated in the nursery with two buds from tree 29-36; (b) 10 received 1 bud, on April 14, from a West Indian lime plant with symptoms of tristeza; (c) 10 received 1 bud from tree 16A-1, on February 16, and 1 bud from tristeza-infected lime, on April 14; (d) 10 received 1 bud, on April 14, from Meyer lemon 1-54, which carries seedling yellows virus (9). Ten plants were retained as noninoculated controls. Each of the 50 plants was then budded, on April 14, with a single bud from a 2-year-old field-grown Eureka lemon seedling, the lemon buds being forced to grow after the sweet orange seedling tops were cut back on May 19. All groups were kept in a screened greenhouse.

Some of the trees inoculated from 16A-1 developed, within 10 days after they began to grow in May, 1960, weak lemon scions with small chlorotic or mottled leaves which tended to remain stiffly erect or more nearly at right angles to the upright stem than the larger and heavier leaves on control plants. Some leaves became mottled, with pale chlorotic areas extending across secondary veins. Other leaves on the same plants developed a pale creamy yellow color, except for a wedge-shaped area of green along the midvein and occasional specks of green in the chlorotic portion. Many chlorotic leaves gradually became less chlorotic after they ceased expanding. Some of the stunted plants inoculated with buds from 16A-1 made partial recovery, and others which had started to grow normally became stunted later. Reactions of the plants inoculated from 16A-1 and those inoculated from both 16A-1 and tristeza-affected lime appeared to be almost identical.

Some plants inoculated only with tristeza virus soon developed mild

chlorosis in the young lemon leaves, but 5 months after inoculation these plants appeared normal.

Plants inoculated with seedling yellows virus from Meyer lemon 1-54 forced short chlorotic lemon shoots which soon developed severe shock reaction, abscised their leaves, and within a few weeks dropped part or all their stems. Six of these plants later improved and some produced lemon shoots equal to those on some of the plants inoculated with buds from 16A-1, or superior to them. All control plants made normal growth. Plants representing each treatment are shown in Figure 1; specific measurements are summarized in Table 1.

TABLE 1. MEASUREMENTS OF DISEASED AND APPARENTLY NORMAL PLANTS OF SEEDLING-LINE EUREKA LEMON/KOETHEN SWEET ORANGE

Inoculation group and apparent condition of plants	Number of plants	Leaf blade dimensions in mm[a]		Shoot[b] length in mm	Size[c] of stock in mm² May 19	Growth[c] of stock in mm² May 19 to September 28
		Length	Width			
Seedling yellows 1-54:						
Diseased	10	75[d]	34[d]	33[d]	52	3
Normal	0	–	–	–	–	–
Navel 16A-1:						
Diseased	4	79	32	71	88	9
Normal	6	140	61	138	85	31
16A-1 + tristeza:						
Diseased	5	97	43	79	76	18
Normal	5	122	60	131	81	40
Tristeza:						
Diseased	0	–	–	–	–	–
Normal	10	134	62	133	50	34
None: (controls)						
Diseased	0	–	–	–	–	–
Normal	10	131	58	143	60	35

[a]Average of 2 leaves per plant on September 28. Measured leaves were the first 2 below a point 6 inches from the tip.

[b]Average length of lemon shoots on September 28.

[c]Averages calculated from diameters at pot level.

[d]Based on the 6 seedling yellows plants which retained lemon shoots.

Field experiments were started in 1957, in which buds from trees 29-36 and C-189 were placed in seedlings of several varieties and in the seedling rootstocks of several stionic combinations. The buds were grown into tops on some of the seedlings; on other seedlings and on the budlings they were used only for inoculation. All the inoculated seedlings sub-

FIGURE 1. *Sister plants of seedling-line Eureka lemon/Koethen sweet orange. Inoculations from left to right as follows: none (control); tristeza alone; tristeza +navel orange 16A-1; 16A-1 alone; seedling yellows from Meyer lemon 1-54.*

sequently were converted into budlings by postinoculative propagation from young-line trees presumably free of viruses. Comparable noninoculated trees were grown as controls.

Preliminary results of these field experiments agree in general with results obtained from lemons in the greenhouse. Some plants inoculated with buds from stubborn-affected trees 29-36 and C-189 were dwarfed in the nursery. Other plants, similarly inoculated, were apparently normal in the nursery, but failed to grow well after being transplanted to the field (Fig. 2) or began to grow poorly within 2 years after transplanting. The fibrous roots of stunted trees appeared to be in sound condition when examined. Eleven of the 18 young-line trees inoculated in 1957 and 10 of 16 trees propagated in the nursery in 1957-58 with buds from trees 29-36 and C-189 were visibly stunted or dwarfed by May, 1960. Inoculated trees that became stunted were: grapefruit and Satsuma on Rangpur lime (*C. limonia* Osbeck); Navel orange on Sunshine tangelo (*C. reticulata* x *C. paradisi*), on trifoliate orange (*Poncirus trifoliata* (L.) Raf.), and on Troyer citrange (*C. sinensis* x *P. trifoliata*); Shamouti orange on Palestine sweet lime (*C. limettioides*

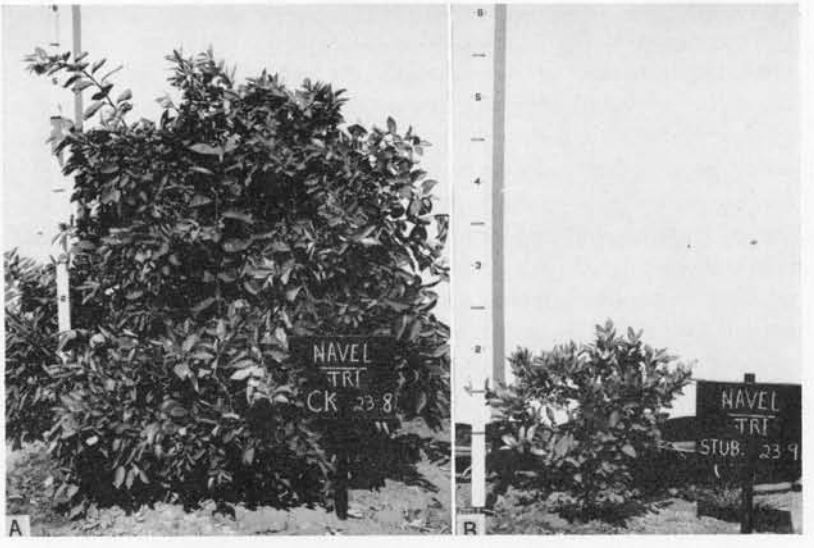

FIGURE 2. *A. Noninoculated control tree of Frost Washington Navel orange/Pomeroy trifoliate propagated in 1957 and planted in 1958. B. Sister tree in juxtaposition in same row. The rootstock of this tree was inoculated from stubborn navel orange tree 29-36 in the nursery in 1957. Pole height, 6 ft., 3 in. Photographed September 22, 1960.*

Tanaka); and Valencia orange on Troyer citrange. Propagations of 29-36 grew poorly on Orlando tangelo and rough lemon (*C. jambhiri* Lush.); those of C-189 grew poorly on Cunningham citrange, trifoliate orange, Rangpur lime, Orlando tangelo, and Palestine sweet lime stocks.

Most leaves of the dwarfed trees are abnormally small and stiff, and some are distorted. Considerable shoot growth has occurred and some nearly normal shoots have been produced, but most shoots have many multiple buds. Foliage is crowded or clustered and leaves usually drop prematurely. Although tree 29-36 and the young dwarfed trees are non-productive, they have produced many off-season blooms, which soon dropped. All noninoculated controls have made normal growth.

The trees used as sources of buds for inoculations in these experiments were indexed for known citrus viruses, with the following results: (a) lemon seedling, apparently virus free; (b) trees 29-36, 16A-1, and C-189, stubborn; trees 29-36 and C-189 are negative for exocortis and cachexia 3½ years after indexing; (c) West Indian lime, tristeza; (d) tree 1-54, seedling yellows and vein enation.

Discussion

The original stubborn experiments (3, 4, 5, 6), by themselves, neither proved transmission nor demonstrated the nature of any causal agent of the disease. Instead, they confirmed observations that healthy buds on stubborn interstocks grow poorly (6) and indicated that stubborn disease is bud-perpetuated and may be transmissible. In those experiments, tree 29-36 represented a method which, with adequate controls, might have proved transmissibility of a causal agent of stubborn disease; tree 29-36, like most inoculated trees in our experiments, consisted of an inoculated seedling rootstock on which a stunted top was grown from a bud of a normal tree (6). The close resemblance of symptoms developing in our young trees, inoculated by buds from trees 29-36 or C-189, to those described and illustrated for young trees in early reports of stubborn disease (4, 5, 6) and the relationship of tree 29-36 to the original experiments with stubborn disease have led us to conclude that the causal factor or factors of one kind of stubborn disease studied by Fawcett *et al.* (6) have been transmitted to several stionic combinations of citrus.

The results from 3½ years of indexing indicate that the graft-transmissible agent or agents responsible for chlorotic, small-leaved growth and general stunting of inoculated trees are different from the viruses of psorosis, vein enation, yellow vein, and tristeza; probably different from

those causing exocortis, cachexia, and xyloporosis. The possibility that combinations or variant forms of these viruses may have been present has not been eliminated.

Inoculations from 29-36 and from C-189 have induced dwarfing in some stionic combinations that are considered tolerant of tristeza and seedling-yellows viruses, in some tolerant of exocortis virus, and in some tolerant of cachexia and xyloporosis viruses. However, the effect of 16A-1 inoculations in some plants of Eureka lemon on sweet orange resembled that of seedling-yellows, except that the initial severe shock following seedling-yellows inoculations was absent in these plants. Reduction in leaf size, similar to that caused by 16A-1 inoculations, has been reported for orange trees affected by stubborn disease (2). The transmissible agent or agents from dwarf Navel tree 16A-1 differ from tristeza virus, and there has been little or no synergistic effect between tristeza virus and the dwarfing agent from 16A-1 in budlings of Eureka lemon on Koethen sweet orange.

The occurrence of apparently normal individuals among bud progeny of diseased sources, as well as mild or seemingly negative reactions in nearly half the inoculated young-line trees, might be due to (a) slow movement or irregular distribution of the causal agent in the host, (b) dominance of a mild form of the agent in some buds, (c) host tolerance due to physiological or genetic factors.

Considerable variation in symptoms occurs among trees assumed to be affected by stubborn disease (1, 6), and it has been questioned whether a single disease is responsible for all the symptoms ascribed to stubborn disease (1, 2). Environmental factors can cause dwarfing and poor tree condition (1), and large Navel orange trees having stubborn disease may be confused with nonproductive strains of this variety (6). The most constant symptoms attributed to stubborn disease (6) are not sufficiently specific to be diagnostic. In our experience, many trees tentatively diagnosed as having stubborn disease on the basis of gross symptomatology have proved to be adversely affected principally by factors such as root rot, cachexia, exocortis, inferior rootstocks, stionic incompatibility, and heredity.

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