Stubborn Disease Symptoms in Grafted Fruit of Citrus and Poncirus

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THE TECHNIQUE (7) of grafting twigs bearing immature fruit into seedlings proved so useful in studying stubborn disease symptoms under controlled conditions that I pursued it further. Contrasted with the experiments in 1967–68, in which the test conditions lacked uniformity and emphasized comparisons between normal and diseased fruit within varieties, the experiments of 1968–69, which are presented here, were conducted under reasonably uniform conditions. They permitted better comparisons than the first trials. Tests were repeated with several varieties used in 1967–68, and 20 additional varieties were included.

Materials and Methods

Fruit-bearing twigs were obtained from the following: Moro, Pineapple, Valencia, and Vaniglia Sanguigno sweet orange; Redblush grapefruit; Owari satsuma; King mandarin; Changsha, Clementine, Dancy, and ponkan mandarin; Minneola, Seminole, and Thornton tangelo; Brazilian sour orange; chinotto; Rangpur lime; red shaddock; Eureka lemon; rough lemon; Mexican lime; karna; Palestine sweet lime; calamondin; lemonquat; Rubidoux and Christian trifoliate orange; Sacaton and CPB (Crops Physiology and Breeding, USDA) 4475 citrumelo. Except for the lemonquat, a hybrid of Texas origin, the species and varieties have been described (7, 8). Fruiting twigs were taken mostly from virus-free nucellar trees; the Clementine mandarin tree was infected with exocortis virus.

Madam Vinous sweet orange and Duncan grapefruit seedlings, 30–45 cm tall and growing in 15-cm pots, were grafted with single buds or side grafts derived from plants infected with stubborn virus. The usual virus source in these trials was an 11-year-old Minneola tangelo nucellar seedling tree severely affected by stubborn disease. A few other stubborn-affected nucellar trees of recent origin also provided the virus. Inoculated seedlings showing the mottled-leaf symptom of stubborn disease, a yellow and green blotching differing from minor-element deficiencies (1, 5, 6, 9), were considered to be infected.

From late April to mid-July, 1968, twigs bearing young fruit of the different varieties were collected and grafted onto infected and normal seedlings, with techniques previously described (7). Grafts were considered successful if the fruit remained attached to the grafted stem for 30 days. Fruit abscission occurring thereafter was attributed to the effect

of stubborn-virus infection unless other causes were definitely implicated. Observations and measurements include seed abortion, stylar-end greening, acorn shape, and the color of peel and albedo. Fruit of each variety were harvested when normal fruit were well colored and mature.

All plants were grown in warm glasshouses. Daily maxima of 32–40°C prevailed for several hours, and daily minima were mostly above 25°C except in the coldest months when the maxima were of shorter duration and the minima were sometimes as low as 15–20°C.

Results

Results are summarized in Table 1. Fruit grafted onto normal seedlings had typical numbers of good seed and degreened normally, starting at the stylar end of the fruit. The percentage of preharvest fruit drop of a given variety of fruit was often much higher from infected plants than from normal plants. The remaining fruit on infected plants developed stylar-end greening or failed to degreen normally, and the seeds were aborted.

Complete seed abortion was the most striking fruit symptom among varieties severely affected by stubborn disease. Rarely, a few plump but darkened seeds were found. Excessive seed abortion also occurred in some fruit varieties that did not show well-marked external stubborn symptoms. Stylar-end greening developed less frequently than seed abortion; it was not found in the absence of seed abortion. Only calamondin retained greened fruit in the absence of seed abortion until harvest time; this variety was harvested in late October and may have been immature.

Acorn-shaped fruit were commonly found among mandarins on infected plants; this symptom was unusually well developed in the King variety. Some acorn-shaped and malformed fruit were found in other severely affected varieties, and a trace of acorn shape was seen in Eureka lemon. Blue albedo was observed only in the peel of Thornton tangelo, Redblush grapefruit, and karna.

On the basis of total symptoms, fruit of orange, grapefruit, tangelo, and mandarin varieties were most severely affected by stubborn disease. The seed abortion, stylar-end greening, and acorn-shape symptoms were frequently combined in mandarin fruit.

Fruit of sour orange, shaddock, citrumelo, lemon, rough lemon, and lemonquat varieties, and of chinotto and karna, on infected plants showed slight to severe seed abortion and often remained green instead of developing normal peel color at maturity. They occasionally showed other symptoms of stubborn.

Symptoms of stubborn disease were lacking in fruit of calamondin and in the 3 lime and 2 trifoliate orange varieties, although fruit of these varieties on infected plants were smaller than those on healthy plants.

The time of grafting of fruit-bearing twigs affected the development of

TABLE 1. PREHARVEST FRUIT ABSCISSION AND SEVERITY OF STUBBORN DISEASE IN FRUIT OF CITRUS AND PONCIRUS VARIETIES GRAFTED ONTO ORANGE OR GRAPEFRUIT SEEDLINGS WITH MOTTLED-LEAF SYMPTOM OF STUBBORN DISEASE[a]

Fruit-bearing scion variety	Date of grafting	Preharvest fruit abscission		Grafted fruit harvested from infected plants			
		Normal plants (%)	Stubborn-affected plants (%)	Harvested (no.)	With aborted seed (%)	With stylar-end greening (%)	Development of typical peel color
Sweet orange							
Moro	June 10	0	89	4	100	0	delayed
Pineapple	July 3	12	88	6	100	0	delayed
Valencia	April 29	40	44	47	85	0	delayed
Vaniglia sanguigno	June 7	6	87	2	100	0	delayed
Tangelo							
Minneola	July 22	0	67	1	100	100	delayed
Seminole	July 23	25	25	5	100	0	delayed[b]
Thornton	July 22	0	75	2	50	0	delayed[b]
Mandarin							
Clementine	June 10	0	100	0[c]			
Clementine	July 10	32	97	4	100	0	delayed
Changsha	July 6	15	54	6	100	67	delayed
Dancy	July 6	25	20	8	100	75	delayed
King	July 10	16	57	6	100	100	delayed
Owari satsuma	July 10	35	31	7	71	71	delayed
Ponkan	July 8	33	33	2	100	100	delayed
Miscellaneous							
Redblush grapefruit	May 22	37	69	14	100	0	delayed[b]
CPB 4475 citrumelo	May 1	50	50	4	50	0	normal
Sacaton citrumelo	April 29	50	33	4	25	0	normal
Chinotto	July 16	0	60	6	100	0	delayed
Brazilian sour orange	July 16	25	75	6	66	0	delayed
Red shaddock	May 22	50	50	2	100	0	delayed
Calamondin	June 10	68	81	8	0	0	delayed[d]
Rough lemon	May 21	9	8	37	92	41	delayed[d]
Rough lemon	July 16	8	0	12	8	0	delayed[d]
Lemonquat	July 10	50	50	5	0	0	normal[b,d]
Karna	May 24	0	0	8	100	0	normal[b,d]
Eureka lemon	May 20	25	29	17	?	0	normal
Mexican lime	May 20	45	54	12	0	0	normal
Palestine sweet lime	July 16	8	8	10	0	0	normal
Rangpur lime	July 16	42	50	10	0	0	normal
Christian trifoliate orange	April 29	58	74	5	0	0	normal
Rubidoux trifoliate orange	May 1	45	67	4	0	0	normal

a. Fruit harvested from normal seedlings showed no symptoms of stubborn disease.
b. Blue albedo was common in peel of indicated variety.
c. There were originally 37 fruit but all abscised; of the 21 fruit in controls, none abscised.
d. Fruit resembled poorly grown fruit, not sharply distinct from normal fruit.

stubborn symptoms and seemed to influence fruit drop. All Clementine mandarin fruit dropped long before harvest from twigs grafted to infected plants on 10 June. On fruiting twigs grafted a month later, a few fruit remained until harvest on diseased plants; they developed severe symptoms of stubborn disease. Rough lemon fruit on twigs grafted to infected plants on 21 May developed a high percentage of seed abortion and stylar-end greening. Those same symptoms were almost absent from fruit on similar grafts made 2 months later.

Discussion

The principal fruit symptoms associated with stubborn disease of citrus can be induced experimentally in many varieties under controlled glasshouse conditions. Excessive seed abortion, stylar-end greening, acorn shape, delayed or erratic greening, and blue albedo are considered to be important symptoms of stubborn disease. The specificity of fruit symptoms has not been fully established, but excessive seed abortion in normally seedy varieties (2) appears to be a key symptom of stubborn disease and to have some influence on other fruit symptoms. Field observations on excessive seed abortion indicated that this condition was not associated with 6 other citrus viruses, and it occurred in the present tests in the absence of other viruses (2). In experiments reported here, stylar-end greening occurred only in the presence of excessive seed abortion. Delayed development of normal peel color at maturity, which is reported as a symptom of greening disease, occurred in conjunction with seed abortion in all varieties, with the questionable exception of calamondin. Excessive seed abortion was associated with acorn-shaped and malformed or lopsided fruit in mandarin and other varieties.

Blue albedo is probably not a specific symptom of stubborn disease (3) and is often observed in field-grown fruit in the absence of excessive seed abortion. Nevertheless, blue albedo is frequently encountered in field-grown fruit on stubborn-affected trees of grapefruit, orange, and tangelo.

The responses of 29 citrus varieties to stubborn disease agree with, and extend the results of, the earlier report on this work (7). Fruit of Dancy, Changsha, King, and ponkan mandarin developed incontestable symptoms of stubborn disease; fruit drop was not excessive on infected plants. The fruit of these varieties are of medium size and not too heavy to support themselves well on grafted twigs. They normally produce large numbers of good seeds so that excessive seed abortion is conspicuous in affected fruit. These characteristics suggest that further trials should be made to test mandarin varieties as indicators of stubborn disease; they are reported to be affected by most of the stubborn and stubbornlike diseases of citrus (4).

Time of grafting seemed to influence fruit drop and development of symptoms of stubborn in Clementine

mandarin and rough lemon fruit. The behavior of the varieties and earlier observations (7) indicate that grafting of fruit-bearing twigs should be timed to achieve good fruit retention and maximum development of symptoms. Varietal differences in fruit retention may influence the selection of experimental materials.

Grafting of fruit-bearing twigs onto other plants to obtain fruit responses to stubborn disease within a single season offers new possibilities in the rapid indexing for this virus and in other studies of stubborn disease. The technique may enable further comparisons of the apparently related stubborn, greening, little leaf, leaf mottling, and likubin (4). In all these, the fruit develop one or more of the symptoms associated with stubborn disease. This technique may be valuable in studies on impietratura (10), in which damage to fruit is a response to virus infection.

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