Abscission of Citrus Fruit and Ethylene Production by Clementine Tangerine Fruit Grafted onto Seedlings Infected with Stubborn Virus

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ABSTRACT

We grafted twigs bearing immature, healthy fruit of six *Citrus* cultivars onto virus-infected seedlings with mottled-leaf symptoms of stubborn disease. Many fruit of Clementine tangerine, Pineapple orange, and Redblush grapefruit abscised within 5 months when grafted onto infected seedlings, but few abscised when grafted onto normal seedlings. Fruit of Eureka lemon, Rough lemon, and Rangpur lime did not abscise in a similar period even when grafted onto infected seedlings. Fruit of Clementine tangerines which abscised from infected seedlings averaged 1,100 ppb ethylene in their internal atmosphere; that of normal fruit averaged 100 ppb ethylene. More ethylene was produced by partly colored, abscised Clementine fruit from stubborn-affected seedlings than by freshly-picked green fruits from normal plants. Significant differences in ethylene production by foliage of normal and infected plants were not detected. The evidence, some circumstantial, supports the hypothesis that virus infection caused necrosis, which increased ethylene production, which caused the premature abscission of fruit of susceptible cultivars. Phytopathology 60: 155-157.

Infection by the virus that causes stubborn disease induces premature abscission of fruit of some varieties of citrus (2). For example, Clementine tangerine (*Citrus reticulata* Blanco) fruit were retained when grafted onto healthy plants, but they abscised before maturity when grafted onto plants affected by stubborn disease (8). Recent studies indicate that abscission of citrus fruit or foliage caused by chilling, chloride excess, or chemical sprays, is associated with increased ethylene in the internal atmosphere (4, 5, 10, 11). The objective of the present investigation was to determine whether ethylene production was correlated with fruit abscission subsequent to infection by the virus that causes stubborn disease.

Materials and Methods.—Twigs bearing healthy, immature fruit of Clementine tangerine were grafted 17-19 July 1968 onto Duncan grapefruit (*C. paradisi* Macf.) seedlings in 15-cm pots in the greenhouse. Twigs bearing healthy, immature fruit of Pineapple orange (*C. sinensis* [L.] Osb.), Redblush grapefruit, Rough lemon (*C. jambhiri* Lush.), and Eureka lemon (*C. limon* [L.] Burm. f.) were grafted onto sweet orange seedlings in June 1968. On 4 June 1968, twigs bearing healthy, immature fruit of Rangpur lime (*C. limonia* Osb.) were grafted onto plants with Rangpur lime tops. The grafting technique has been described (8). Some seedlings were normal and free of stubborn virus; others, graft-inoculated, showed the mottled-leaf symptom of stubborn virus infection (8). Grafts were considered successful if fruit remained attached to the stem for 30 days. A fruit that subsequently dropped from the grafted stem prior to normal harvest was usually considered as abscised, unless the fruit was split or showed stylar-end breakdown.

Ethylene production by the fruit was measured at Pasadena, Calif., and Orlando, Fla., by using flame-ionization gas chromatography.

Results.—*Abscission of grafted fruit*.—Clementine fruit grafted onto virus-infected seedlings abscised at a rapid rate; few fruit abscised from comparable noninfected seedlings (Table 1). Immediately prior to abscission, yellow-orange color developed within a few mm of the button of the fruit. The abscised fruit from infected seedlings showed varying amounts of yellow-orange color at the stem end; the stylar end of the fruit was green. Fruit on normal seedlings remained entirely green until the onset of cooler temperatures in the fall, when yellow-orange color developed first at the stylar end of the fruit. Fruit of Pineapple orange and Redblush grapefruit also abscised from stubborn-affected seedlings, and similarly showed yellow-orange discoloration at the stem end. Few Eureka lemon, Rangpur lime, and Rough lemon fruit abscised from affected seedlings (Table 1).

Ethylene in fruit samples.—Five Clementine tangerine fruit that had abscised from stubborn-affected seedlings and five similar fruit taken from a normal tree were collected on 30 August 1968 at Indio, California, and sent in polyethylene bags to Orlando, Florida, for analysis. On 3 September, air samples in the internal atmosphere of the fruit were taken as described by Burg & Burg (1), by submerging the fruit in water and inserting a hypodermic syringe underneath the rind or inside the center of the fruit and withdrawing 2 ml of air. The air was analyzed by flame-ionization gas chromatography to determine the ethylene content. Fruits abscised from stubborn-affected seedlings averaged 1,100 ppb ethylene in the internal atmosphere of a single fruit; that of a normal fruit averaged 10 ppb ethylene. The atmosphere in the polyethylene bags enclosing five control fruit contained 100 ppb ethylene; that enclosing five comparable affected fruit contained 5,700 ppb ethylene.

More ethylene was produced by partly colored Clementine fruit that had abscised from stubborn-affected seedlings than by freshly-picked green fruit taken from normal plants (Table 2).

Ethylene in leaf samples.—Normal grapefruit leaves and comparable leaves showing the mottled-leaf symptom of stubborn disease were collected and sent to Orlando in polyethylene bags. Samples collected on 23 October 1968 were analyzed on 26 October. A change in the rate of ethylene production caused by infection with stubborn virus was not detected; virus-

TABLE 1. Effect of stubborn virus infection of citrus seedlings on abscission of healthy, immature fruit grafted onto them

Seedlings disease status when fruit was grafted onto them	Kind and no. of seedlings grafted	Fruit remaining on grafted stems, no. months after grafting				
		1	2	3	4	5
Var.						
Clementine tangerine	Grapefruit					
Infected	59	136	43	14	5	4
Normal	61	162	136	129	115	107
Pineapple orange	Sweet orange					
Infected	21	48	41	30	9	5
Normal	51	107	101	90	89	85
Redblush grapefruit	Sweet orange					
Infected	24	46	43	40	32	18
Normal	35	68	67	66	63	56
Eureka lemon	Sweet orange					
Infected	6	24	24	24	24	24
Normal	4	16	16	16	15	15
Rangpur lime[a]	Rangpur lime tops on various rootstocks					
Infected	25	72	72	72	71	71
Normal	25	87	87	87	87	86
Rough lemon	Sweet orange					
Infected	12	52	52	52	52	51
Normal	4	35	34	34	34	32

[a] Ten fruit with stylar-end breakdown and 3 with splits also abscised from infected seedlings; 2 fruit with stylar-end breakdown and 1 split fruit abscised from normal seedlings.

affected and normal leaves in comparable bags developed 2 ppb ethylene/g fresh wt per 48 hr.

In a second trial, mottled leaves from affected plants and normal leaves from control plants were collected at the Pasadena laboratory. Discs of equal size were cut from normal and affected leaves and incubated for 24 hr at 27°C in light (16 hr) and dark. Ethylene production was below 50 ppb/g fresh wt for both mottled leaves of affected plants and normal leaves of control plants, and no significant differences in ethylene production in foliage related to infection by stubborn virus were detected. Leaf abscission did not occur in either control or affected plants.

DISCUSSION.—When healthy, immature fruit of Clementine tangerine, Pineapple orange, and Redblush grapefruit were grafted onto seedlings affected with stubborn disease, most of the fruit on affected plants abscised, while few asbscised from normal plants. The evidence presented herein, some circumstantial, suggests the following sequence of action: virus infection in phloem tissue caused necrosis, which increased ethylene production, which in turn caused fruit abscission of susceptible varieties. However, premature fruit abscission did not occur when lemons and Rangpur lime fruit were grafted for the same number of months onto seedlings affected with stubborn disease.

Stubborn and greening are considered related diseases, with identical or a related causal virus (2). Schneider (13) noted that greening virus kills mature phloem in the leaves; a similar effect might be expected in other organs. Fruit of some varieties (including sweet orange, grapefruit, and some tangerines) harvested from branches with stubborn disease are often malformed and contain excessive numbers of aborted seeds (2, 8). Ethylene emanation is a plant response to injury, and the amount of ethylene produced by infection with several viruses in several herbaceous host plants was proportional to the degree of necrosis (14).

Ethylene, whether produced endogenously in response to injury or by chemicals which release or cause the release of ethylene, is associated with fruit abscission (4).

In the present trials, abscised fruit had more ethylene in their internal atmosphere than did normal fruit. The area of the peel at the stem end that changed from

TABLE 2. Association of fruit abscission, extent of yellow-orange color near button end, and stubborn-virus infection with increased ethylene production by Clementine tangerine fruit[a]

Disease status of fruiting plants	Fruit sample				
	No.	Weight, g	% Abscised	Yellow-orange color near button	ppb C_2H_4/g fresh wt/24 hr[b]
Normal	5	190	0	0	5
Infected	7	210	57[c]	trace	20
Infected	6	175	100[d]	25 to 50%	55

[a] Fruit collected 5 September 1968 was placed into 500-ml closed flasks 6 September, and enclosed air was analyzed 24 hr later at Pasadena, Calif.

[b] Parts per billion ethylene per g of fresh wt in 24-hr period.

[c] Three of 7 fruits showed trace of yellow-orange near button and were pulled from tree before abscission was completed.

[d] Fruit abscised during a 3-day period preceding sampling date, 5 September.

green to yellow-orange increased as the concentration of ethylene in the fruit increased (Table 2). Ethylene is generally recognized as the constituent that degreens citrus fruit (6). Normal fruit degreens first at the stylar end.

It is now known that degreening of citrus fruit is delayed by external applications of gibberellic acid (3); and naturally occurring auxins, cytokinins, and gibberellins delay abscission of plant parts (9). Ethylene opposes the retarding effects of these naturally occurring growth regulators (12). Therefore, we speculate that knowledge of the endogenous levels of cytokinins, auxins, and gibberellins as well as ethylene in normal and stubborn-affected leaves and fruits of the various citrus varieties may aid to clarify why the stubborn-affected lime and lemon fruits did not abscise, and why the stylar end of the stubborn-affected Clementine fruit did not degreen. It is known that abscission of leaves does not generally occur until ethylene levels reach about 1 ppm (7). These levels were not reached in the stubborn-affected Clementine leaves.

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