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Host Range of the Fatal Yellows Disease

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ABSTRACT. Thirty selections of citrus, citrus relatives, and their hybrids and 17 grafted combinations were tested for susceptibility to isolates of fatal yellows. The disease became systemic in self-rooted alemow, *C. excelsa*, Etrog citron, Cleopatra mandarin, and Eustis limequat plants. Symptoms in other selections varied from fairly extensive (e.g., *C. depressa* and Indian lime variant), to trees with only a few affected leaves at onset (e.g., sweet orange, mandarin, limes, lemon), to trees with no symptoms. Some trees of sweet oranges declined on alemow rootstocks, but did not decline on rough lemon, Rangpur lime and trifoliolate orange rootstocks. The disease is difficult to transmit, the incubation period is variable (18 days to 6 months), and infections are difficult to maintain.

In the orchard, fatal yellows (FY) is a disease of alemow rootstock of lemon trees (1). Several isolates of the agent have been transmitted by grafting through a series of alemow seedlings. This paper reports results of attempts to transmit three of the isolates to other citrus species, citrus relatives, and hybrids.

In developing leaves of alemow the disease induces epinasty; thin chlorotic areas in the leaf blades; failure of blades to attain normal size; midvein curvature; vein clearing; collapse of and sinking of veins into the mesophyll on the leaf's abaxial side; while on the adaxial side, veins enlarge. Elongating stems sometimes twist and some blighting of tips may occur. Sometimes as leaves reach maturity, a type of vein clearing may appear that differs from the earlier vein clearing in expanding leaves. It is fainter and the veins become cleared throughout the leaf (hereafter called vein netting). On mature leaves, the adaxial sides of veins continue to enlarge, with some veins splitting open and becoming corky. Old leaves may become mottled. Occasional maturing stems become rubbery, while bumpy areas accompanied by gumming may appear on others.

MATERIALS AND METHODS

Three strains of the agent were

used in the tests. One designated 10-37 is moderately virulent in alemow and causes only occasional blighting of tips. New growth is normal at first, but soon becomes symptomatic.

A severe strain of fatal yellows (30-23) causes an array of symptoms similar to 10-37, but the blighting of stem tips is more prevalent and death progresses down the branches and into the trunks, often killing the tree. On trunks and on branches that do not die, axillary buds may repeatedly begin to grow and then abort. When scions are made from such stems and trunks for inoculum, they usually fail to grow or the axillary buds make meager growth and then abort.

A third strain (10-33) appears to be different from the other two in that several of the prominent symptoms are not induced. Series of undersized chlorotic leaves which may have sunken marginal veins alternate with series of normal leaves. Vein netting in maturing leaves is common. Older leaves may show a chlorotic mottle and although growth of some trees is retarded, the trees mostly grow as vigorously as the controls.

The host plants tested for susceptibility were propagated in several ways. For selections that produced a high percentage of nucellar seedlings, propagations were

usually from seed, but some rooted cuttings were used. Other selections and clonal materials were grafted onto seedlings.

Unless otherwise noted, inoculum was from symptomatic portions of alemow seedlings. Inoculations were accomplished by grafting scions onto tips and sides of either the trunk or branches. Buds and leaf patches were placed into "T" cuts. Recipients usually received from two to four pieces of inoculum. The agent transmits irregularly and the incubation period varies from 18 days to several months. Trees were periodically pruned to induce symptoms. Inoculations were repeated if the recipient did not develop symptoms within a few months and if any of the following occurred: a) scions or buds grew but the growth was symptomless; b) scions or buds did not grow; or c) the inoculum died. Some recipients seemed to reject infected scions, and also the 30-23 inoculating scions often would not grow and develop symptoms. For one or another of these reasons, in experiments 372, 373, 411, 420, 421, 473, and 476, recipients were first grafted with healthy alemow scions and the growth from them was inoculated with FY-diseased tissues.

RESULTS

The citrus selections, their relatives, and hybrids that were tested for susceptibility to FY are listed in tables 1 and 2. Table 1 lists trees on their own roots (seedlings and cuttings), while table 2 lists trees on rootstocks. Infection types are divided into two categories; hosts in which symptoms became systemic (S) and hosts in which symptoms were localized and in which new growth was only affected for a short time (L).

Self-rooted trees. When inoculated with the 10-37 or the 30-23 isolate, the disease became sys-

temic in alemow, *Citrus excelsa*, Etrog citron, Cleopatra mandarin and Eustis limequat and devitalization or death ensued. Other trees became locally symptomatic. A few of them expressed symptoms that were fairly well distributed in the trees at onset but vigor of the recipient was not affected (e.g., *C. depressa* and the Indian lime variant). They tended to recover. Many hosts expressed symptoms locally in shoots near inoculating scions at onset; but, in later growth flushes, symptoms failed to appear (e.g., sweet orange, West Indian lime, and rough lemon).

Trees on rootstocks. There were several reasons for using trees on rootstocks. It was a quick method for propagating clonal material, a knowledge of the susceptibility of certain crafted combinations was desired; and it could be determined whether the agent had moved through the trunks of symptomless selections into the rootstocks by severing the trunk near the budunion and observing rootstock sprouts for symptoms. None of the selections on rootstocks developed systemic leaf symptoms; although, on their own roots, Etrog citron and Eustis limequat did. Details of transmissions to various grafted trees are listed in table 2.

Rough lemon/alemow. In experiments 315, 316, and 318 alemow seedlings were top worked with rough lemon by placing buds on opposite sides of the alemow trunks at 28 and 32 cm above the soil. After shoots had grown from the buds, round holes were punched into mature leaves and infected leaf discs from alemow were inserted into the holes and taped in place. In addition, an infected alemow scion was grafted into one of the two shoots. For experiments 315 and 316, symptoms appeared locally and erratically in red rough lemon branches with some branches

TABLE 1
SYMPTOMS IN SEEDLINGS OR CUTTINGS INOCULATED WITH 3 ISOLATES
OF FATAL YELLOWS

	Expt. no.	Inoculations*	Trees with inoculum sympto- matic	Receptor trees with leaf symptoms†
ISOLATE 10-37				
W.I. Lime	236	5S	1	0/1†
	248	1A	1	1/2 L,R
	301	4B or 4L	1	0/3
	319	3S. I	2	1/2 L,R
Sour orange	475	2S,4L. II	3	1/3 L,R
<i>C. depressa</i>	476	3S	3	3/3 L,R
<i>C. excelsa</i>	312	2S,4L. II	3	3/3 S†
	339	1B,4L	—	3/3 S†
Red rough lemon (JBC)	341	1B,4L	—	1/1 L,R†
	341	1B,4L. II	2	0/2 §
Florida rough lemon seedling	372**	1S. I	3	2/3 L,R
Florida rough lemon cutting (VE)	373**	1S. I	3	3/3 L,R
Palestine sweet lime	474	2S,4L. II	3	2/3 L,R
Eureka lemon	236	2S	2	0/2
Rangpur lime	386	3S,3L	2	2/2 L
	420**	2S,2L. II	2	2/2 L
Etrog citron	301	3L	—	1/1 S
	442	3S,1L	—	3/3 S†
	449	1S,1L	—	2/2 S†
Meyer lemon	462	2S,4L	2	0/2
Grapefruit	499	3S	2	0/3
Cleopatra mandarin	313	2S,2L	2	2/2 L
	337	1B,4L. II	1	2/2 S§
	383	2S,1B,4L. I	0	0/2††
	421	2S,1L. I	2	2/2 S
Fairchild mandarin	345	1B,4L. I	1	0/3
	351	2S. II	2	1/4 L,R
Sweet orange	224	1S	1	0/2
	236	2S. I	1	0/1
Volkamer lemon	300	2S. I	6	1/6 L,R
Trifoliolate orange	310	2S. I	1	0/5
Indian lime variant (CRC 2450) ...	280	2B. II	6	3/6 L,R
Eustis limequat	392	2S. II	4	2/4 L,R
1449 citremon	305	1S,3B,3L. I	6	1/6 L,R
Troyer citrange	352	2S. II	3	0/4
ISOLATE 30-23				
Sour orange	475	2S,4L. I	3	2/3 L,R†
<i>C. depressa</i>	413	2S (nodal). V	3	1/4 S,R
	473**	4L. I	3	3/3 L,R
	476**	3S. I	4	4/4 L,R
<i>C. excelsa</i>	340	1B,4L. II	3	3/3 S,T
Red rough lemon	342	1B,4L. III	2	2/3 L,R
Palestine sweet lime	474	2S,4L. I	3	3/3 L,R

TABLE 1. Continued.

	Expt. no.	Inoculations*	Trees with inoculum sympto- matic	Receptor trees with leaf symptoms
Rangpur lime	385	3S,1B,4L	1	2/2 L
	412	S. II	4	3/4 L,R
	420**	2S,2L. II	2	2/2 L,R
	523	5S	2	0/2
Etrog citron	423	3S,1L	1	2/3 S,T
	450	1S,2L	1?	1/2 S,T
Meyer lemon	462	2S,4L	0¢	0/2
Grapefruit	499	3S. III	0¢	0/3
Cleopatra mandarin	338	1B,4L. II	2	1/2 S,T
	384§	2S,1B,4L	0	0/2
	421**	3S,1L	2	2/2 S,T
Fairchild mandarin	346	1S,4L. I	0¢	2/3 L,R
Eustis limequat	393	3S	0¢	2/2 L,R
	411	1S	3	3/4 S,T
	465	2S,4L	0¢	3/3 S
ISOLATE 10-33				
Etrog citron	534	3S,2L	6	6/6 S

*Arabic numerals on left indicate number of inoculum pieces per tree. A = approach grafted, B = buds, S = scions, L = leaf patches or discs. Roman numerals indicate the number of times that reinoculations were made.

†No. of trees with symptoms/no. trees inoculated. Leaf symptoms in recipients: S = symptoms systemic, L = symptoms local, R = trees showed shock symptoms and recovered, T = terminal—trees died or nearly so.

‡Subinoculations from symptomatic trees were successful.

§Subinoculations failed.

††Inoculum source was Cleopatra mandarin from experiment 337.

§Inoculum was from 338 Cleopatra mandarin.

¢Scions would not grow. A characteristic of scions infected with 30-23 isolate.

**Recipients regrafted with healthy alemow which in turn was inoculated.

growing vigorously and healthy while others were stunted. After one year, the rough lemon tops were removed by severing the alemow trunk just below the lower of the two rough lemon budunions. This induced suckering from the rootstocks, and suckers on all inoculated trees were symptomatic. Thus, although symptoms did not become systemic in the red rough lemon tops the causal agent moved a few cm through them into the rootstock. For experiment 318, the Florida rough lemon buds for topworking were taken from an orchard tree showing Woody Gall (Vein enation). Symptoms were

somewhat more prevalent than they were in red rough lemon trees. Infections of rough lemon with the vein enation disease may have enhanced susceptibility.

Eureka lemon/alemow. In experiment 359, the 10-37 and 30-23 isolates caused localized symptoms in lemon shoots adjacent to symptomatic scions, but neither agent moved down through the lemon trunk into the rootstock.

Meyer lemon/alemow. The 10-37 agent caused erratic transitory symptoms in Meyer lemon. After the trunk was cut off just above the budunion, suckers from the Meyer lemon top and the alemow

TABLE 2
SYMPTOMS IN CITRUS SELECTIONS AND RELATIVES GRAFTED ON ROOT-STOCKS AND INOCULATED WITH TWO ISOLATES OF FATAL YELLOWS

	Expt. No.	Inoculations*	Trees with inoculum symptomatic	Receptor trees with leaf symptoms
ISOLATE 10-37				
Red rough lemon (W.N.)/alemow	315	1S,3L. I	4	2/5 L††
Red rough lemon (stow)/alemow	316	1S,3L. I	4	5/5 L‡
Florida rough lemon/alemow	318	1S,4L	3	5/5 L‡
Eureka lemon/alemow	359	5S,2B. I	3	3/3 L‡
Villafranca/alemow	557	3S	2	1/3 L,R
Etrog citron/rough lemon	270	2B	4	2/4 L,R
Meyer lemon/alemow	462	2S,4L	1	1/1 L‡
Clementine/sweet orange	463	2S,4L. I	3	0/3
Clementine/alemow	466	2S,4L. II	3	3/3 L,R‡
Clementine/Troyer	468	2S,4L. I	3	3/3 L,R
Sweet orange/alemow	282	2B. I	8	0/8‡
Sweet orange/rangpur lime	456	2S,4L. I	3	2/3 L,R
Sweet orange/rough lemon	457	2S, 4L	3	1/3 L,R‡
Sweet orange/trifoliolate orange	461	2S,4L. II	2	0/3
Marumi kumquat/Orlando tangelo	505	1/3 L,R	3	3S
Nagumi kumquat/alemow	500	4L	2	2/2 L§
Eustis limequat/alemow	317	1S,3L. I	4	5/5 L§
ISOLATE 30-23				
Eureka lemon/alemow	359	3S,2B. II	2	1/2 L,R
Villafranca lemon/alemow	557	3S	1	1/3 L,R
Meyer lemon/alemow	462	2S,4L	1	0/1††
Clementine mandarin/sweet orange	463	2S,4L	0§	1/3
Clementine/alemow	466	2S,4L. II	3	1/3¢
Clementine/Troyer	468	2S,4L. I	1§	2/3 L,R
Sweet orange/rangpur lime	456	2S,4L. II	0§	1/3
Sweet orange/rough lemon	457	2S,4L. II	0§	2/4 L,R**
Sweet orange/trifoliolate orange	461	2S, 4L	0§	3/4 L,R
Marumi kumquat/orlando tangelo	505	3S	1§	2/3 L,R
Nagumi kumquat/alemow	500	4S	0§	2/2 L¢
Kulu lemon (Gombru)/alemow	559	3S	1§	0/4

*Arabic numerals on left indicate number of pieces of inoculum per tree. A = approach grafted. B = buds. S = scions. L = leaf patches or discs. Roman numerals indicate the number of times that inoculations were repeated.

†No. of trees with symptoms/no. trees inoculated. Leaf symptoms in recipients: S = symptoms systemic, L = symptoms local, R = trees showed shock symptoms and recovered, T = terminal—trees died or nearly so.

‡See text.

§Some rootstocks suckers formed after severing the trunk below the union showed symptoms.

††Suckers from rootstocks did not show symptoms after severing the trunk near bud-union.

§Scions infected with 30-23 often did not grow.

¢Alemow suckers on rootstock showed symptoms. Tree declined.

**Alemow scions grafted onto rootstock suckers did not show symptoms.

rootstock showed symptoms indicating that the agent had moved through the trunk into the alemow rootstock. A second growth flush of the Meyer suckers was normal. In the same experiment, the 30-23 agent did not cause symptoms in Meyer lemon or in the alemow rootstock suckers.

Clementine/alemow. In experiment 466, two Clementine mandarins on alemow rootstocks were inoculated with the 10-37 isolate. They showed leaf symptoms in growth that immediately followed onset of the disease but not in later growth. The trunks were severed 10 cm above the bud union and suckers appeared above and below the budunion. On one plant, symptoms appeared in both alemow and Clementine. Suckers on the other plant were symptomless. Similar results were obtained with the 30-23 agent with one of three trees showing symptoms above and below the union.

Sweet orange/alemow. In experiment 282, eight trees were inoculated but none showed leaf symptoms. However, by the end of the experiment, trees 1a, 3a and 7b were in severe decline. Sections were made of 1a and 3a and FY-pathology was present in them below the budunions. Rootstocks of all trees were infected as indicated by symptomatic suckers from the rootstock. This experiment progressed as follows. In November 1974, Madam Vinous sweet orange buds were placed in trunks of alemow seedling at 30 and 35 cm above the soil. By January 1975, the upper sweet orange buds had grown out and formed trees up to 2 m tall. In July 1975, inoculating alemow buds were placed in the sweet orange trunk at 30 and 35 cm above the budunion. They grew out symptomless; so, in April 1976 these alemow shoots were reinoculated with scions. Strong symptoms appeared within four months and in Febru-

ary 1977, the symptomatic alemow was cut off. In April, suckers from the alemow rootstocks of two trees were symptomatic, and some trees were very stunted and showing symptoms of decline (i.e., root failure). In May 1977, the trees were moved to the lathhouse. In May 1978 the trunks were severed at the budunions and the trees were returned to the greenhouse. Unknown to us, the lower of the two original sweet orange buds for top working had remained latent on a few trees. The buds were a few cm below the budunion and hence below the point where the trunk was severed. These sweet orange buds grew out and shoots on three trees showed severe FY symptoms, but as shoot growth continued it became normal. The affected shoots were propagated on rough lemon seedlings by budding but growth from the buds was normal. It may be concluded that the FY agent moved down through the sweet orange trunk and that sweet orange growth coming out from the infected alemow was affected by the disease.

Sweet orange/Florida rough lemon. In experiment 457 inoculating scions with the 10-37 agent showed symptoms on 3 trees but the recipients did not develop symptoms. Inoculating scions with the 30-23 agent did not grow, but on two trees a few leaves of sweet orange showed symptoms at onset of the disease. Suckers from the rough lemon rootstocks of trees infected with the 10-37 and 30-23 agents were grafted to alemow but none of the alemow scions showed symptoms. Apparently the agent did not move through rough lemon in this case.

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