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Mechanical Transmission of Infectious Variegation Virus in Citrus and Noncitrus Hosts

GRANT AND CORBETT (3) reported mechanical transmission of the virus of infectious variegation in juice from Eureka lemon to Eureka lemon and sour orange seedlings and from the thus-infected Eureka lemon to healthy Eureka lemon by the mechanical and leaf piece-graft methods of inoculation.

This paper describes results of tests to determine the importance of sucrose solutions, activated charcoal, air temperature, and maturity of tissues for mechanical transmission of the virus. It also gives results of inoculation of citrus and noncitrus species and varieties with juice from plants exhibiting symptoms of infectious variegation.

Materials and Methods

Unless otherwise stated, all inoculations were made by the Carborundum-gauze pad method. Leaves to be inoculated were dusted with 500-mesh Carborundum, inoculated, and rinsed with tap water.

Experimental Results

EFFECTS OF SUCROSE, ACTIVATED CHARCOAL, AND DILUTION.—A 20 per cent sucrose solution was used at a rate of 1 ml/g of leaf material; activated charcoal (Merck 18351) was used at a rate of 0.05 g/g of leaf material. Where water was used, it was at the same rate as sucrose solution. Inoculum was prepared from Eureka lemon leaves of varying degrees of maturity, all of which exhibited symptoms characteristic of variegation. The leaves were mixed, weighed into 7 samples of 10 g each, and triturated in a mortar with a pestle. Crude juice was obtained by

TABLE 1. MECHANICAL TRANSMISSION OF INFECTIOUS VARIEGATION VIRUS FROM EUREKA LEMON TO SEVILLE SOUR ORANGE AND DUNCAN GRAPEFRUIT SEEDLINGS AFTER VARIOUS TREATMENTS OF THE INOCULUM

Treatment of Inoculum	No. of test plants with symptoms of				No symptoms		
	variegation		psorosis				
	sour orange	Duncan grapefruit	sour orange	Duncan grapefruit	sour orange	Duncan grapefruit	percentage infection
None	3	1	0	4	7	5	40
Frozen	1	2	1	2	8	6	30
Frozen + activated charcoal	0	0	1	5	9	5	30
Frozen + water	4	2	0	1	6	7	35
Frozen + water + activated charcoal	2	4	2	6	6	0	70
Frozen + sucrose	3	3	0	6	7	1	60
Frozen + sucrose + activated charcoal	6	2	1	4	3	4	65
Control	0	0	0	0	10	10	0
Total	19	14	5	28	56	38	

squeezing the pulp through cheesecloth at room temperature and was treated further as indicated in Table 1 for inoculation of 10 seedlings (5- to 7-leaf stage) each of sour orange and Duncan grapefruit. Of the 140 plants inoculated, 66 developed symptoms of psorosis or infectious variegation (Table 1). The variegation symptoms, which were persistent, included chlorotic blotches, curling, and twisting of the leaves. The psorosis symptoms, which were not persistent, included veinlet banding, vein accentuation, and some chlorotic blotches.

It is evident from Table 1 that dilution with water or sucrose solution and addition of activated charcoal increased the infectivity of the inoculum. In subsequent experiments, 20 per cent sucrose and activated charcoal were therefore added to the inoculum.

EFFECT OF TEMPERATURE.—The original studies on transmission by mechanical means were conducted during January through March (3). Subsequently, it was found that during May and June the percentage transmission by mechanical means was much less than during January through March, even though the inoculation methods were identical.

To test the effect of temperature upon mechanical transmission, plants of Eureka lemon and *Crotalaria spectabilis* Roth were inoculated and maintained at 2 different temperatures. One group was maintained for 3 weeks in a cool chamber at 20°-21°C and the other in a greenhouse where temperatures fluctuated from 20°-35°C.

Seventy-six per cent of the Eureka lemon plants at 20°-21°C became infected compared with only 8 per cent at 20°-35°C. The symptoms were all of the variegation type. Eighty per cent of the *C. spectabilis* plants exhibited necrotic rings at 20°-21°C whereas none exhibited symptoms at 20°-35°C (Table 2).

TABLE 2. NUMBERS OF EUREKA LEMON AND CROTALARIA SPECTABILIS PLANTS INFECTED, DIVIDED BY THE TOTAL NUMBERS MECHANICALLY INOCULATED WITH INFECTIOUS VARIEGATION VIRUS AT HIGH AND LOW TEMPERATURES[a]

Test no.	Test plant	Cool chamber 20-21°C	Greenhouse 20-35°C
1	Eureka lemon	5/6	0/9
2	Eureka lemon	2/5	2/5
3	Eureka lemon	5/5	0/5
4	*C. spectabilis*	4/5	0/5

[a]Inoculum was juice of young leaves of infected plants showing optimum symptoms of variegation in the greenhouse. Inoculum for Test 1 was held at −10°C for 4 hours prior to inoculation; inocula for Tests 2, 3, and 4 were held at −10°C for 9 days prior to inoculation.

Inoculum held at -10°C for 9 days prior to inoculation was just as infectious as that held for only 4 hours.

EFFECT OF AGE OF LEAF USED AS SOURCE OF INOCULUM.—We conducted 6 tests in which we compared young leaves with mature leaves as sources of inoculum (Table 3). From the data it is concluded that mature leaves are a poor source of inoculum.

TABLE 3. NUMBERS OF EUREKA LEMON PLANTS INFECTED, DIVIDED BY THE NUMBERS MECHANICALLY INOCULATED WITH INFECTIOUS VARIEGATION VIRUS OBTAINED FROM LEAVES OF DIFFERENT AGES

Test no.	*Young leaves*	*Mature leaves*[a]
1	3/5	4/5
2	5/5	0/5
3	1/5	0/5
4	4/5	0/5
5	4/5	0/5
6	3/4	0/4
Total	20/29	4/29

[a]The mature Eureka lemon leaves used as a source of inoculum in Test 1 had been inoculated 16 days previously and exhibited chlorotic lesions. In all other tests, the leaves had been systemically infected.

HOST RANGE AS DETERMINED BY MECHANICAL INOCULATION.—In attempts to find other citrus species that show more distinctive symptoms of infectious variegation, the following citrus species were inoculated: *Citrus limon* (L.) Burm. F. 'Eureka, Walden cascade' and 'Meyer'; *C. aurantium* L. 'Seville sour'; *C. paradisi* MacFad. 'Duncan'; *C. grandis* (L.) Osbeck 'Pink Shaddock; *C. aurantifolia* (Christm.) Swing. 'Key lime'. Inoculated plants were kept in the greenhouse for 4-8 months and the symptoms were recorded and compared with those induced in Walden cascade lemon.

The noncitrus plants that follow were inoculated with infectious variegation virus from Eureka lemon: *Crotalaria spectabilis* Roth; *C. juncea* L.; *C. capensis* Jacq.; *C. mucronata* Desv. (*C. striata* DC.); *Vigna sinensis* (Torner) Savi 'Lady Finger', 'Lady Finger Round', 'Blackeye Ramshorn'; *Cucumis sativus* L. 'Marketer'; *Cucurbita moschata* Duchesne 'yellow crookneck squash'; *Phaseolus vulgaris* L. 'Tendergreen'; *Pisum sativum* L. 'Little Marvel'; *Zinnia elegans* Jacq.; *Capsicum frutescens* L., *Lycopersicon esculentum* Mill. 'Marglobe', *Solanum tuberosum* L. 'U.S. D. A. seedling 41956'; *Gomphrena globosa*

L.; *Datura stramonium* L.; *Cassia occidentalis* L.; *Nicotiana tabacum* L. 'Turkish', and *N. glutinosa* L. Inoculum consisted of juice from young leaves triturated in the presence of sucrose and activated charcoal.

All citrus species mechanically inoculated with infectious variegation virus became infected and developed symptoms of variegation. Eureka lemon (Walden cascade) exhibited the most striking symptoms and was the most satisfactory test plant.

Only plants of 2 species of 2 genera in the family Leguminosae developed symptoms. Plants of *Crotalaria spectabilis* developed brownish necrotic ringspots on the inoculated leaves 13-30 days after inoculation (Fig. 1). The ringspots enlarged slightly and vein necrosis occurred when they came in contact with a vein. The inoculated leaves abscised and systemic infection did not occur. The virus was recovered from the inoculated leaves of *C. spectabilis* by inoculation to plants of Eureka lemon, which developed only a few chlorotic to necrotic rings on the inoculated leaves. Symptoms of variegation did not occur.

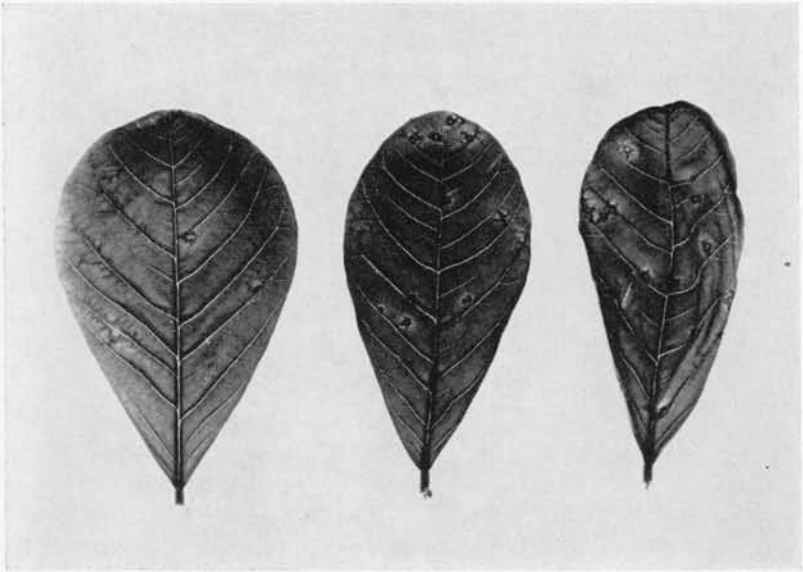

FIGURE 1. *Leaves of Crotalaria spectabilis 10 days after mechanical inoculation with infectious variegation virus from Eureka lemon. (Photograph by Harriett Long.)*

Inoculated plants of *Vigna sinensis* 'Lady Finger', 'Lady Finger Round', and 'Ramshorn Blackeye' developed no immediate symptoms on the primary (inoculated) leaves. In 10-30 days, however, the trifoliate leaves developed chlorotic vein clearing, leaf twisting, and curling (Fig. 2). The inoculated leaves turned yellow and abscised. The

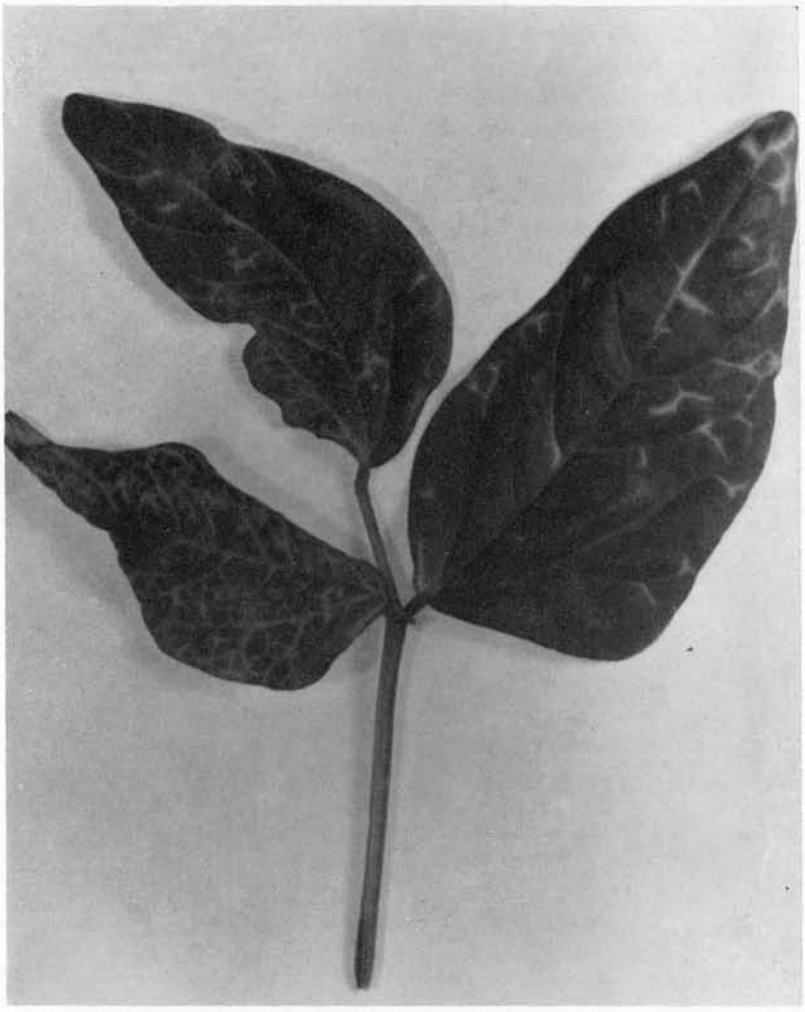

FIGURE 2. *Leaves of Vigna sinensis 'Ramshorn Blackeye' 30 days after mechanical inoculation with infectious variegation virus from Eureka lemon.*

infected plants of Lady Finger were stunted; stem necrosis developed and the plants eventually died. The virus was transferred from infected cowpea plants to plants of Eureka lemon, which developed symptoms of variegation.

No symptoms developed on other noncitrus species and no virus was recovered.

It appears that *C. spectabilis* may act as a filter plant for part of the virus complex associated with infectious variegation, for upon transmission from *C. spectabilis* to Eureka lemon, symptoms of variegation were not obtained; only a few chlorotic to necrotic rings developed on the inoculated leaves. Plants of *Vigna sinensis* (cowpea) apparently support multiplication of both viruses or strains of the infectious variegation complex.

Discussion

There has been uncertainty whether infectious variegation of citrus should be considered a separate disease from the crinkly leaf disease of lemons (1, 4). Wallace (5) concluded from cross-protection studies that infectious variegation is caused by a strain of psorosis virus. Grant and Smith (2) transmitted a variegation disease of grapefruit occurring in Florida to several citrus species and considered the symptoms to be characteristic of infectious variegation.

From the studies reported here, it is apparent that more than one virus or virus strain is involved in infectious variegation. When inoculated at greenhouse temperatures (20-35°C) with inoculum from Eureka lemon, plants of sour orange and Duncan grapefruit sometimes exhibited symptoms of variegation and sometimes those of psorosis. When inoculated and maintained at lower temperatures (20-21°C), such plants developed symptoms of variegation only. This may be explained on the basis of a differential rate of virus or virus strain multiplication at different temperatures or a masking of the psorosis symptoms at lower temperatures.

The enhancement of infectivity induced by adding sucrose and activated charcoal to the inoculum may be explained by the ability of activated charcoal to adsorb inhibitors present in the sap or by the ability of sucrose to prevent the disruption of mitochondria and consequent release of inhibitors. Intact preparations of mitochondria are usually made in isotonic sucrose. Mitochondria contain many enzymes, some of which may be deleterious to infection. The sucrose solution also had the effect

of diluting the sap, which may be why infectivity was increased, since infectivity was increased merely by addition of water. The leaves were frozen because freezing made it easier to grind the tissue completely.

The reason why plants kept at low temperatures (20-21°C) became more susceptible (as judged by symptoms) to infection by mechanical inoculation has not been established. It may be that the virus multiplies more readily at low temperatures or it may be that higher temperatures affect the physiological state of the plant so that it is rendered resistant to infection. Either a differential rate of virus multiplication at relatively high temperatures or an increased state of resistance of test plants at such temperatures may explain why some plants developed symptoms of variegation while others developed symptoms of psorosis. Symptom development may also be related to virus concentration because the percentage transmissions obtained from young tissue exhibiting maximum symptoms was greater than that obtained from older tissues. However, older leaves may contain considerable virus but because of maturity or nature of the tissue the virus is not released upon trituration. That the virus is present in older leaves is evident by the success of transmission in leaf tissue grafts.

Results of the present investigation make it possible to study the etiology of infectious variegation from the standpoint of possible separation of the viruses by differential hosts, physical and chemical properties of purified preparations, morphology of the particles associated with the disease, and their serological relationships.

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