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Experiments on Mechanical Transmission of Citrus Viruses

MECHANICAL TRANSMISSION of citrus variegation virus, first reported by Grant and Corbett in 1960, has undoubtedly opened a new era for research on citrus viruses (2). Not only has it provided an indispensable tool for a basic study of citrus variegation virus, but it has also stimulated attempts to transmit other citrus viruses.

The virus that causes citrus variegation is supposed to be a severe strain of crinkly leaf virus. Both viruses have been described as strains of psorosis virus (4). The relation of crinkly leaf virus to psorosis virus has, however, recently been challenged (1).

This paper describes the mechanical transmission of crinkly leaf virus to various species of citrus and to herbaceous plants with unpurified leaf sap and also with a partially purified preparation.

Strain of Crinkly Leaf Virus Used

The crinkly leaf virus used in this work is the California strain 81-A-65 obtained from Dr. J. M. Wallace.

In an independent experiment, this strain has been shown to induce, by bark grafts, psorosis young leaf symptoms as well as crinkly leaf symptoms on seedlings of the following species: Eureka and Lisbon lemon, Hamlin and Trovita orange, Marsh grapefruit, Willow-leaf mandarin, and sweet lime. Never have variegation symptoms been obtained. The infectious variegation strain obtained from Dr. T. J. Grant, on the contrary, induces a pronounced variegation pattern, together with crinkly leaf symptoms, under the same conditions.

Mechanical Transmission of Crinkly Leaf Virus by Unpurified Leaf Sap

METHOD.—Young crinkled leaves from graft-infected lemon seedlings were treated according to the sucrose-charcoal method of Grant and Corbett (3). The leaves were cut in small pieces into a mortar and frozen either by placing them in a deep freeze for several hours or immediately by pouring liquid nitrogen onto them. The frozen leaf fragments were ground with a pestle in the presence of 0.05g of activated charcoal and 1 ml of a 20 per cent sucrose solution per gram of leaves. In later experiments, 0.1 M sodium phosphate pH 7.0 was used instead of water in preparing the sucrose solution. Also, charcoal did not seem to improve the transmission greatly. In any case, the cold homogenate was squeezed through several layers of cheese cloth, and the juice obtained was used to inoculate the test plants by mechanical rubbing in the presence of Carborundum (500-mesh). After inoculation, the plants were kept at 20°-25°C in a refrigerated greenhouse.

MECHANICAL TRANSMISSION TO SPECIES OF CITRUS.—All the inoculated Mexican lime, sweet lemon, and acid lemon seedlings showed clear-cut crinkly leaf symptoms after one month. Eighty per cent of the inoculated Hamlin orange and sour orange seedlings showed symptoms after two months. On sour orange leaves, the symptoms appeared first on the wings.

MECHANICAL TRANSMISSION TO VIGNA SINENSIS.—Twenty to 25 per cent of the inoculated *Vigna sinensis* seedlings (Black-eye cowpea) showed the systemic symptoms described by Grant and Corbett after three weeks, but in addition to these symptoms, yellow chlorotic local lesions were generally obtained on the inoculated leaves (Fig. 1).

Infected *Vigna sinensis* leaves were used as a source of virus with which to inoculate *Vigna sinensis,* Mexican lime, sour orange, and Eureka lemon seedlings. Sixty per cent of the inoculated *Vigna sinensis* plants showed the chlorotic local lesions on the two primary inoculated leaves and the systemic symptoms on the uninoculated leaves after 15 days. The citrus species, however, showed symptoms only after 10 weeks.

Mechanical Transmission of Crinkly Leaf by a Partially Purified Preparation

Vigna sinensis plants inoculated with crude sap from infected lemon leaves or from infected *Vigna sinensis* plants were harvested together,

FIGURE 1. *Local lesions on a Vigna sinensis leaf (Black-eye cowpea) mechanically inoculated with sap from citrus leaves affected by crinkly leaf virus. The same lesions are also obtained when the inoculation is done with the partially purified preparation of crinkly leaf virus.*

respectively, three and two months after inoculation; their leaves showed typical vein clearing symptoms.

Twenty-seven grams of such leaves were homogenized in a Virtis 45 homogenizer at full speed with 50 ml of ice-cold 0.02 M sodium phosphate buffer at pH 8.0. The homogenate was centrifuged at 0°C at 2,000 G for 25 minutes; the dark green supernatant fluid was recentrifuged at 100,000 G for 2½ hours in a Spinco ultracentrifuge. The clear brown supernatant fluid was discarded. The green pellet was resuspended in cold water and the suspension was submitted to two more cycles of low and high speed centrifugation. The final 100,000 G pellet was reddish, translucent, and contaminated in the center by a small amount of green material. After resuspension of the pellet in water, a last centrifugation at 2,000 G for 15 minutes eliminated the green material and yielded 6.5 ml of an amber-colored solution, the absorption spectrum of which showed a maximum at 259 mμ and a minimum at 240. This solution was diluted 10 times with 0.1 M sodium phosphate buffer at pH 7.0 and the diluted solution was used to inoculate *Vigna sinensis*, sour orange, and Eureka lemon seedlings.

The inoculated plants showed severe symptoms in less time than when infected with crude sap. As with crude sap, the inoculated cowpea leaves showed the yellow chlorotic local lesions. The partially purified prepa-

ration has been examined in the electron microscope. No particles could be seen.

Comparison Between Crinkly Leaf Virus and Citrus Variegation Virus by Mechanical Transmission

Vigna sinensis plants inoculated with crude sap from leaves of lemon seedlings graft-infected with citrus variegation virus (Florida strain of Dr. Grant) have shown chlorotic local lesions and vein-clearing symptoms much earlier than those inoculated under the same conditions with crinkly leaf virus. In certain experiments, the symptoms appeared as early as eight days after inoculation.

Conclusion

The mechanical transmission of citrus variegation virus achieved by Grant and Corbett was duplicated with crinkly leaf virus. The symptoms obtained with the latter virus appear generally later than those given by the variegation virus. These facts strengthen the hypothesis that crinkly leaf virus and citrus variegation virus are closely related and that the variegation virus is a severe strain of crinkly leaf virus.

Finally, a partially purified infectious preparation of crinkly leaf virus was obtained by differential centrifugation.

Literature Cited

1. Fraser, L. R. 1961. Lemon crinkly leaf virus, p. 205-210. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
2. Grant, T. J., and Corbett, M. K. 1960. Mechanical transmission of the infectious variegation virus of citrus. Nature 188: 519-520.
3. Grant, T. J., and Corbett, M. K. 1961. Mechanical transmission of infectious variegation virus in citrus and non-citrus hosts, p. 197-204. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
4. Wallace, J. M. 1959. A half century of research on psorosis, p. 5-21. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.