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Progress in Citrus Virology: Mechanical Transmission

MECHANICAL TRANSMISSION of citrus viruses is a key that can unlock the door to a wide new field of knowledge and basic information on the virus diseases of citrus. Variegation virus was the first citrus virus to be mechanically transmitted by means of juice extracts (3). It has been transmitted not only from citrus to citrus but also to some herbaceous plants (4). Mechanical transmission has made possible studies on the properties of the virus as determined by thermal inactivation, tolerance to dilution, and aging *in vitro*. It has also made possible detection of inhibitors in the source plants and has permitted the determination of the time required for maximum concentration of virus in the source plant.

Similar studies with tobacco mosaic virus resulted in much basic information of value to the understanding of other plant viruses. Even today, studies on the basic structure of tobacco mosaic virus are contributing to our understanding of viruses in general. Studies on citrus variegation virus will help make possible development of techniques needed for further understanding of viruses of citrus and other woody plants.

Although the results of the studies on citrus variegation virus have been published (3,4,5), it was felt that a discussion of some of the recent findings and problems encountered would be of special value to members of the International Organization of Citrus Virologists who are particularly concerned with citrus diseases.

BIOASSAY.—A local lesion host is the cornerstone for progress. Any host that will produce countable lesions as a result of mechanical inoculations will expedite studies on the virus.

The first local lesions observed as a result of mechanical transmission with citrus variegation virus (CVV) were on young leaves of Eureka lemon [*Citrus limon* (L.) Burm. f.]. Unfortunately, they were not satisfactory for bioassay because they coalesced and systemic infection followed (3). The next local lesion host discovered for CVV was *Crotalaria spectabilis* Roth. The reaction was satisfactory, but considerable time was required to grow usable plants and the leaves did not offer identical test surfaces (4). Chlorotic local lesions were produced on the cotyledons of the cucumber, variety "Chicago Pickling" by Desjardins and Wallace (1) with their strain of infectious variegation which they consider to be a strain of citrus psorosis virus. Such chlorotic lesions are not desirable for bioassay work because they are not easily counted. The most satisfactory local lesion host for CVV thus far discovered is Black cowpea [*Vigna sinensis* (Torner) Savi] (5). Variation occurred, however, in color of the lesions produced; some were red and others light green to yellow. These differences in lesion color were found to be related to periods of light and dark after inoculation. Small red lesions were produced if the plants were kept in the dark for one or two days after inoculation and then exposed to ordinary day and night conditions. Chlorotic lesions were produced under normal day and night conditions (5).

The number of lesions varied on primary cowpea leaves inoculated with the same inoculum. Thus it was necessary to inoculate at least ten plants (20 primary leaves) per test and to include three replications of any given test.

The presence or absence of Carborundum on the primary leaves to be inoculated was the most important single factor in the production of local lesions. Leaves without Carborundum produced none to few lesions, whereas leaves dusted with Carborundum produced 50-150 lesions. The size or mesh of the Carborundum particles had some effect; best results were obtained with 500 mesh.

The size, age, and physiological condition of the Black cowpea plants to be inoculated caused some variation in the results. In general, eight or nine cowpea seeds were planted to a uniform depth of one-half inch in quart cans of composted soil. The five most uniform plants were selected and inoculated when they were five to seven days old. The variation in plant size depended on light conditions. Cloudy days and those of short day length retarded development and expansion of the primary leaves. All plants were inoculated before the development of trifoliate leaves. Leaves on slightly wilted plants produced fewer lesions and were

subject to mechanical injury. Relatively no difference in lesion number occurred when the plants were inoculated with a gauze pad or a pipe cleaner. In all cases, pressure that caused visible injury of the primary leaves tended to reduce lesion number.

Washing of leaves with water after inoculation reduced the number of local lesions.

VARIATIONS IN VIRUS CONCENTRATION.—Systemically infected Lady Finger Round cowpea plants varied in their concentration of virus. During November through January maximum virus concentration occurred 12-16 days after inoculation. In the spring, summer, and fall it was desirable to dilute the inoculum so that the symptoms were less severe and the trifoliate leaves would not drastically curl and drop off. Once the cowpea trifoliate leaves had curled and the plants were approximately 30 days old the virus concentration was low.

In all the tests, inoculated control plants were used to determine the virus concentration of the inoculum for comparison with the treatment under test. Again, three replications of a given test were needed to establish a reasonable basis for measuring the results.

USE OF BUFFERS.—The use of buffers was found desirable in routine virus transfers from cowpea to cowpea. The most desirable pH of these buffers was pH 7 for 0.1 M phosphate and pH 8 for 0.1 M borate buffer. A range of pH and different buffers should be tried on other citrus viruses.

TOLERANCE TO DILUTION.—When crude juice from systemically infected Lady Finger Round cowpea plants was diluted with 0.1 M phosphate buffer pH 7.0, the number of local lesions increased at a ½ dilution, and then decreased with lesion number approximately inversely proportional to dilution. No infection occurred at a dilution of 1/128.

AGING IN VITRO.—In crude juice the virus survived for four hours but not for eight. When inoculum was diluted ½ with 0.1 M phosphate buffer, the virus survived for 8 hours but not for 24.

THERMAL INACTIVATION.—The virus was still infectious after a 10-min exposure at 55°C but not at 60°C.

While recognizing that the physical properties of any virus depend greatly upon the virus concentration in the host, it may be concluded from the studies on such physical properties of CVV that it is a relatively unstable virus.

PRESENCE OF INHIBITORS.—When crude plant juice from Lady Finger Round cowpea was used as inoculum, an increase in the number of lesions occurred on Black cowpea leaves with a vol./vol. dilution with 0.1 M phosphate buffer pH 7. No increase or decrease in lesion number occurred when similar inoculum was diluted vol./vol. with healthy cowpea leaf juice, whereas when inoculum was diluted vol./vol. with crude leaf extract of sweet orange (*C. sinensis* Osbeck), grapefruit (*C. paradisi* Macf.), or Eureka lemon, the number of local lesions produced on Black cowpea test plants was drastically reduced. These results indicated that citrus juice contained a virus inhibitor. Inhibitory action also occurred when vol./vol. dilutions were made with juice from cucumber (*Cucumis sativus* L.) cotyledons or leaves.

The inhibitor appeared to be relatively thermostable, for healthy citrus leaf and cucumber cotyledon juice still retained some of the inhibitory effect after being heated in boiling water for 10-15 min. Most of the inhibitory effect, however, was lost after 30 min in boiling water.

When the virus concentration was high in the crude juice inoculum, little advantage was obtained by the use of additional buffers and stabilizing chemicals. On the other hand, when the virus concentration was not high, the addition of buffers and chemicals increased the number of lesions. Lesion number was also found to increase in relation to time. For example, the use of vol./vol. dilution of 0.1 M borate buffer increased lesion number from 22 lesions per primary leaf at 0 hour's aging to 123 after 6 hours of aging (5). The time lapse for best results was not necessarily constant; in some tests a sample aged for 4 hours was more infectious than one at 0 or 6 hours. When crude juice inoculum was diluted ½ with distilled water, the average number of local lesions decreased or remained the same after 10 min of aging, then increased slightly after 20, 40, and 80 min, but was definitely reduced after 160 min of aging.

The influence of dilution, buffers, ionic concentration, and aging on the number of infections produced may apparently result from an effect on inhibitors in the sap or from an action on aggregates of virus particles.

Limited tests indicated that activated charcoal, sucrose, and glycine brought about an immediate response (inoculation immediately after mixing) in the number of lesions, whereas other treatments increased lesion numbers only after aging.

EFFECT OF RIBONUCLEASE.—All plant viruses thus far studied are nucleoprotein, composed of ribonucleic acid and protein. The ribo-

nucleic acid is very sensitive to the action of the enzyme ribonuclease (RNase). The enzyme RNase has little or no effect on most viruses until the protein coat or cover is removed by phenol or some other chemical (2). This, however, was not the case with CVV. When 1 μg/ml of RNase was added vol./vol. to crude juice from systemically infected Lady Finger Round cowpea, infectivity was almost completely removed. Likewise, when 1μg/ml of RNase was wiped or sprayed on the surface of Black cowpea primary leaves before inoculation with CVV, none or very few lesions were obtained. The RNase on primary surfaces of Black cowpea was effective in preventing infection for more than 24 hours but lost its protective effect if left on the leaf surface for more than 3 days. Washing the RNase-sprayed leaves with water reduced the protective effect of RNase.

These results suggested that RNase naturally occurring in citrus leaves (6) may be important in limiting the success of mechanical transmission of many of the citrus viruses.

THE CHALLENGE.—The International Organization of Citrus Virologists is an informal organization whose principal value is one of exchanging ideas and information that will advance man's understanding of citrus virus diseases. In the past, much time and effort has been expended in describing disease symptoms and in obtaining transmission by means of tissue grafts or in a few cases by insect vectors. Strains of some citrus viruses have been recognized largely on the basis of symptoms and many citrus viruses are suspected of having strains not yet adequately demonstrated. Also, some suspected citrus virus diseases have not been proved to be transmissible.

The relatively recent advances in studies of CVV were made possible because of mechanical transmission not only from citrus to citrus, but also to some herbaceous hosts. It has been possible to determine properties of the causal virus and to initiate studies on its structure, physical characteristics, and its possible relationship to other viruses.

The future for more detailed studies on citrus viruses looks very attractive and the possibilities for a better understanding increase daily. Tanaka (personal correspondence) has obtained mechanical transmission of the virus responsible for Satsuma dwarf disease from citrus to some herbaceous hosts. The inhibitors that appear to restrict mechanical transmission of some other citrus viruses are currently being studied.

The members of the International Organization of Citrus Virologists

have a real challenge and have the enthusiasm and energy needed to meet and solve the problems that restrict our knowledge of citrus virus diseases.

Literature Cited

1. DESJARDINS, P. R., and WALLACE, J. M. 1962. Cucumber, an additional herbaceous host of the infectious variegation strain of citrus psorosis virus. Plant Disease Reptr. 46: 414-416.
2. GIERER, A., and SCHRAMM, G. 1956. Infectivity of ribonucleic acid from tobacco mosaic virus. Nature 177: 702-703.
3. GRANT, T. J., and CORBETT, M. K. 1960. Mechanical transmission of the infectious variegation virus of citrus. Nature 188: 519-520.
4. 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.
5. GRANT, T. J., and CORBETT, M. K. 1964. Properties of citrus variegation virus. Phytopathology 54: 946-948.
6. KESSLER, B., and MONSELISE, S. P. 1961. Ribonucleic acid content and ribonuclease activity of developing apple and orange leaves, as influenced by leaf and tree age. Amer. Soc. Hort. Sci. 78: 53-58.

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