4

Exocortis

Gynura as a Host for Exocortis Virus of Citrus

L. G. WEATHERS and F. C. GREER, JR.

EXOCORTIS VIRUS has been transmitted from citrus to petunia by dodder, by grafting, and by various mechanical methods (3, 4, 6, 7), and from petunia to petunia, and back to citron, by the same methods. It was graft transmitted from infected petunia to 12 species of plants in the family Solanaceae (7).

Subsequent attempts were made to transmit the virus to herbaceous plants in other families. The purpose of the study was to find suitable hosts for virus maintenance and virus extraction. We report here the transmission of exocortis virus to 2 species of plants in the family Compositae. A brief statement of these studies has already appeared (5).

Experiments and Results

Experiments were conducted in a partially shaded greenhouse cooled by evaporative coolers. Air temperatures were maintained at approximately 22°C. Plants were grown in pots of steam-sterilized UC Soil Mix.

Petunia plants infected with exocortis virus served as the inoculum source. The isolate used had been transmitted by dodder (*Cuscuta subinclusa* Dur. and Hilg.) from West Indian lime to petunia (4) and is the same as the isolate used in studies that demonstrated association of infectivity with nucleic acid preparations (2). Inoculations were made by inserting wedge-shaped pieces of stem into slits in the sides of the stems of the test plants and covering with plastic tape (7), or by making several incisions or punctures in the stems of healthy plants with a razor blade that previously had been drawn several times through the stem of an infected petunia (1). Reproduction of exocortis symptoms following back-inoculation from test plants to petunia was the criterion used to determine that a plant was infected.

Two species of plants, *Gynura aurantiaca* DC. and *G. sarmentosa* DC., showed symptoms after inoculation with exocortis virus from petunia. Exocortis virus could be retrieved in petunia and citron indicator plants

from these infected gynura plants.

Symptoms in plants of *G. aurantiaca* were similar to those in citron and solanaceous hosts but developed more quickly, usually within 14 days of inoculation. Symptoms consisted of epinasty of young developing leaves (Fig. 1) and discoloration and necrosis on the undersides of midveins and main lateral veins. The necrosis often extended into the petioles and stems, but the plant did not die. In later stages, the leaves curled downward into a "roll" (Fig. 1); some leaves became very malformed and developed irregular green blotches intermixed with the normal purple color. The plants became stunted and developed a "bushiness" from the production of an abnormal number of secondary shoots. Stems, petioles, and veins became turgid, brittle, and would break with slight pressure. Other symptoms were clearing of principal veins and malformation of flowers.

Symptoms in *G. sarmentosa* developed in 6 weeks to 3 months, and consisted of slight epinasty of the leaves and necrosis of the midvein. In later stages the necrosis advanced into the petioles and stems, killing the plant. In view of the lengthy incubation period and the severe reaction of *G. sarmentosa* to the virus, no further attention was given to its possibilities as a suitable host of exocortis virus for experimental purposes.

Attempts were made to transmit exocortis virus from infected *G. aurantiaca* to citron and petunia by a combination of razor slashes or needle punctures and foliar rubbing. The virus was successfully transmitted to citron and petunia and induced typical symptoms of exocortis.

In addition to showing exocortis

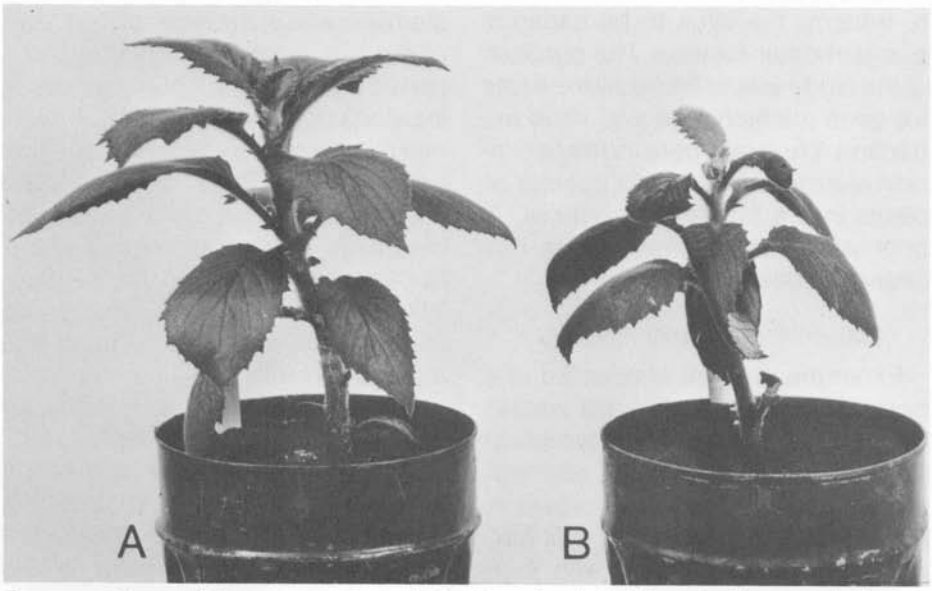

FIGURE 1. *Plants of* Gynura aurantiaca. *A. Noninoculated. B. Infected with exocortis virus.*

symptoms in the developing shoots, all petunia plants inoculated by rubbing with extracts from exocortis-virus-infected *G. aurantiaca* developed chlorotic local lesions in the inoculated leaves. Such lesions never appeared in inoculated leaves of petunia when rubbed with infectious extracts from citron and petunia. When petunia plants were inoculated with extracts from exocortis-free *G. aurantiaca* plants, chlorotic local lesions appeared in all inoculated leaves, indicating that *G. aurantiaca* carried a latent virus.

Tests were made to determine whether the latent virus interacted with exocortis virus when combined with it in *G. aurantiaca* plants. Clones of *G. aurantiaca* free of latent virus were obtained by meristem tip culture. Plants of virus-free clones were compared with plants carrying the latent virus. No differences in growth and behavior between the 2 kinds of plants were detected, indicating that the virus in *G. aurantiaca* was truly latent.

Plants of virus-free clones were inoculated with exocortis virus and compared with plants dually infected with the latent virus and exocortis virus. When exocortis virus was alone in *G. aurantiaca* plants, symptoms were similar in appearance to those described above but were noticeably less severe. Stunting and leaf malformation in doubly infected plants were more conspicuous and more severe than in plants singly infected with exocortis virus. These results indicate that there is a synergistic interaction between exocortis virus and the latent virus of *G. aurantiaca*.

When petunia plants were inoculated with the latent virus and exocortis virus, each virus seemed to invade the plants independently of the other and produce its symptoms regardless of which was introduced first or whether they were introduced concurrently. There was no antagonistic or synergistic interaction of the 2 viruses in petunia; the latent virus produced only local lesions in inoculated leaves and exocortis virus invaded systemically and caused symptoms in developing leaves.

The latent virus was not carried by plants of *G. sarmentosa*, and the addition of the latent virus to exocortis-infected *G. sarmentosa* plants did not alter its reaction to exocortis virus.

Limited attempts were made to identify the latent virus. The virus infected chrysanthemum and produced mosaic and stunting. It did not infect citrus plants. No evidence of the latent virus was observed in high speed supernatants or phenol extracts from *G. aurantiaca* that contained the exocortis virus (2).

Discussion and Conclusions

The requirements for a succulent, durable, compact host that will react reasonably quickly to the exocortis virus and will maintain the virus in high titer for long periods of time seem to be satisfied with *G. aurantiaca* plants. Exocortis-infected *G. aurantiaca* plants survived for well over a year without any evidence that the titer of the virus was diminished. This plant produces only sterile seed, but it is easily and readily propagated by cuttings. It is relatively pest free in the

greenhouse and has no special light and temperature requirements for growth. Although a systemic indicator host is less useful than a local lesion host, the short incubation period of the virus in this plant also makes it acceptable as an assay host for exocortis virus.

The presence of the latent virus is of particular interest. While not needed for the plant to react to exocortis virus, preliminary studies show that its presence increases the titer of exocortis virus in the plant. Furthermore, the latent virus reacts only locally to petunia and is readily removed in procedures used to purify exocortis virus.

The discovery of *G. aurantiaca* as a donor and indicator plant for exocortis virus facilitated studies that have resulted in the purification and characterization of exocortis virus (2).

ACKNOWLEDGMENTS.—Grateful acknowledgment is made to D. H. Hall, Extension Plant Pathologist, University of California, Davis, for providing virus-free meristem tip cultures of *Gynura aurantiaca.*

Literature Cited

1. GARNSEY, S. M., and JONES, J. W. 1967. Mechanical transmission of exocortis virus with contaminated budding tools. Plant Disease Reptr. 51: 410–13.
2. SEMANCIK, J. S., and WEATHERS, L. G. 1968. Exocortis virus of citrus: association of infectivity with nucleic acid preparations. Virology 36: 326–28.
3. WEATHERS, L. G. 1965. Petunia, an herbaceous host of exocortis virus of citrus. Phytopathology 55: 1081.
4. WEATHERS, L. G. 1965. Transmission of exocortis virus of citrus by Cuscuta subinclusa. Plant Disease Reptr. 49: 189–90.
5. WEATHERS, L. G., and GREER, F. C., JR. 1968. Additional herbaceous hosts of the exocortis virus of citrus. Phytopathology 58: 1071.
6. WEATHERS, L. G., and HARJUNG, M. K. 1964. Transmission of citrus viruses by dodder, Cuscuta subinclusa. Plant Disease Reptr. 48: 102–3.
7. WEATHERS, L. G., GREER, F. C., JR., and HARJUNG, M. K. 1967. Transmission of exocortis virus of citrus to herbaceous plants. Plant Disease Reptr. 51: 868–71.