# Extraction, Transmission, Host Range, Properties, and Ecology of a Virus from Gynura that Interacts with the Exocortis Pathogen

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In studies with the citrus exocortis pathogen (CEV), Weathers and Greer (1968, 1972) reported the presence of a virus causing a latent infection in gynura. This virus was mechanically transmitted from gynura to *Petunia hybrida* Vilm. in which it caused chlorotic local lesions in inoculated leaves, and to *Chrysanthemum indicum* L., causing systemic mosaic and stunting. They speculated that some of the symptoms in exocortis-infected gynura could result from an interaction of CEV and the latent virus. Semancik and Weathers (1968) found the latent virus did not interfere with development of exocortis symptoms in petunia nor with isolation of CEV from gynura. Weathers and Greer (1972) showed that the latent virus in gynura was not needed for the plant to react to CEV, but its presence seemed to increase the activity of the exocortis pathogen.

This paper reports the extraction, transmission, host range and properties of this latent virus from gynura and the interaction of this virus with its environment and the citrus exocortis pathogen. Preliminary results have been reported (Osman and Weathers, 1972; Osman *et al.*, 1972).

## MATERIALS AND METHODS

Gynura plants, both with and without CEV, were used as the donor sources of the latent virus (GLV) and petunia varieties, Burpee Blue and Radiance, as the local lesion assay hosts. All plants were grown in steam-sterilized UC soil mix in greenhouses maintained at temperatures not exceeding 24°C.

**Inoculum and Inoculation Methods.** Young leaves of source gynura were triturated in buffers of varying pH values and molarities with mortar and pestle. The juice was expressed through two layers of cheesecloth and applied to carborundum-dusted leaves with the index finger. Mechanical transmissions with gynura were done by the 'razor slash' method (Garnsey and Jones, 1967; Weathers *et al.*, 1967).

**Host Range and Physical Properties.** An experimental host range for GLV was determined by inoculating 5 to 10 plants of each species with infectious gynura tissue triturated in 0.1 orthoborate buffer, pH 9.0. Production of local lesions following return inoculations from test plants to petunia was the criterion used to determine if a plant was infected with GLV.

Rainbow chrysanthemum was the source of infectious sap for physical property determinations. To determine the thermal inactivation point, 1.0 ml samples of crude extract were heated in 10.0 ml glass tubes submerged in a water bath for 10 minutes. Other samples were allowed to age *in vitro* at room temperature, or diluted in 0.1 M orthoborate buffer, pH 9.0, to determine the dilution endpoint. Petunia served as the assay host.

**Insect transmission.** Virus-free colonies of *Myzus persicae* Sulz. were maintained on radish plants. Varying numbers of

starved and nonstarved insects were fed on infected chrysanthemum plants for varying time periods and immediately transferred by brush to healthy chrysanthemum plants and allowed to feed for varying periods of time.

## EXPERIMENTS AND RESULTS

Buffers of varying pH were evaluated for efficiency in extraction and relative infectivity of GLV from gynura. The results of these tests are reported in table 1. Increased relative infectivity was obtained with buffers in the neutral or basic range.

Basic buffers which showed increased relative infectivity were then tested for their efficiency at different molarities. Results of these tests are shown in table 2. With the exception of Tris buffer, increased relative infectivity was obtained with low ionic strength buffers. Higher numbers of local lesions were obtained over a wider range of molarities using orthoborate buffer.

**Host range**. Of all the plants tested (36 species in 10 families), only two species of chrysanthemum reacted to GLV. Korean chrysanthèmum, *C. coreanum*, developed typical mosaic symptoms. In addition to mosaic, infected leaves became wrinkled and tended to curl downward. Internodes were shortened and flowering was delayed. Rainbow chrysanthemum, *C. coccinium*, developed shortened internodes and a 'rosette' appearance. Stems often became fasciated and sometimes developed longitudinal necrotic areas.

TABLE 1
EFFECT OF DIFFERENT BUFFERS AND PH LEVELS ON THE RELATIVE INFECTIVITY OF GLV FROM GYNURA

| Buffer (0.1M) | pH | Relative infectivity* |
|---|---|---|
| Phosphate | 6.0 | 5.6 |
| | 7.0 | 15.3 |
| | 8.0 | 17.5 |
| | 9.0 | 5.2 |
| Tris (hydroxymethyl aminomethane) | 6.0 | 9.4 |
| | 7.0 | 17.6 |
| | 8.0 | 19.3 |
| | 9.0 | 13.4 |
| | 10.0 | 12.2 |
| Orthoborate | 6.0 | 6.2 |
| | 7.0 | 12.7 |
| | 8.0 | 17.3 |
| | 9.0 | 31.2 |
| | 10.0 | 14.6 |

*Average number of local lesions per half-leaf of petunia leaves. Thirty-two replicates.

TABLE 2
EFFECT OF DIFFERENT MOLARITIES OF BASIC BUFFERS ON THE RELATIVE INFECTIVITY OF GLV FROM GYNURA

| | | Relative infectivity* | | | | |
|---|---|---|---|---|---|---|
| | | Molarity | | | | |
| Buffer | pH | 1.0 | 0.5 | 0.25 | 0.1 | 0.05 |
| Phosphate | 7.0 | 3.2 | 5.1 | 6.2 | 15.3 | 18.3 |
| | 8.0 | 3.4 | 4.3 | 9.0 | 17.5 | 12.5 |
| | 9.0 | 3.5 | 3.0 | 6.0 | 5.2 | 4.0 |
| Tris | 7.0 | 3.0 | 8.1 | 14.2 | 17.6 | 20.6 |
| | 8.0 | 2.0 | 12.0 | 13.6 | 19.3 | 23.4 |
| | 9.0 | 0.0 | 5.0 | 11.5 | 13.4 | 17.1 |
| | 10.0 | 0.0 | 0.0 | 5.0 | 12.2 | 6.3 |
| Orthoborate | 7.0 | 0.0 | 13.3 | 12.5 | 12.7 | 3.2 |
| | 8.0 | 0.0 | 18.5 | 15.2 | 17.3 | 5.8 |
| | 9.0 | 0.0 | 34.7 | 26.3 | 31.2 | 11.4 |
| | 10.0 | 0.0 | 8.5 | 7.9 | 4.6 | 2.3 |

*Average number of local lesions per half-leaf of petunia. Thirty-two replicates.

Studies were undertaken to determine the comparative concentration of GLV in systemically infected hosts. Healthy gynura plants were inoculated by means of grafts and Korean and Rainbow chrysanthemums were mechanically inoculated by means of sap. Another set of gynura plants was concurrently inoculated with GLV from gynura plants and CEV from citron plants. At weekly intervals, equal amounts of fresh tissue were collected from each set of infected plants, extracted in 0.1 M orthoborate buffer, pH 9.0, and rubbed on half leaves of petunia in a randomized incomplete block design. Local lesions in petunia leaves were counted after 9 to 11 days. The virus reached higher concentrations in the two species of chrysanthemum than in gynura plants infected only with GLV. Gynura plants concurrently infected with CEV supported a higher concentration of GLV than did gynura plants singly infected with GLV, indicating a synergistic interaction between CEV and GLV.

**Physical Properties.** The thermal inactivation point of GLV was between 73° and 75°C, and longevity *in vitro* between 4 and 5 days. Longevity in frozen crude spa was between 8 and 9 months.

The dilution endpoint of GLV was between $1:10^3$ and $1:10^4$ in sap from chrysanthemum sources and between $1:10^2$ and $1:10^3$ in sap from gynura. In one experiment, the dilution endpoint was determined using infective sap from different sources. The sources of GLV and data from this experiment are shown in table 3. Infectivity of GLV was retained at a dilution of $1:10^5$ in the presence of CEV in sap from gynura, indicating synergism between CEV and GLV.

TABLE 3
DILUTION ENDPOINT OF GYNURA LATENT VIRUS FROM DIFFERENT HOST PLANTS

| | Dilutions | | | | | |
|---|---|---|---|---|---|---|
| Virus source | 1:1 | 1:10 | $1:10^2$ | $1:10^3$ | $1:10^4$ | $1:10^5$ |
| Gynura singly infected with GLV | 3.0* | 2.4 | 1.2 | 0 | 0 | 0 |
| Gynura dually infected with GLV + CEV | 33.0 | 24.0 | 10.0 | 3.2 | 2.2 | 0 |
| Rainbow chrysanthemum singly infected with GLV | 40.6 | 29.0 | 12.8 | 7.0 | 0 | 0 |
| Korean chrysanthemum singly infected with GLV | 23.4 | 11.6 | 7.1 | 2.0 | 0 | 0 |

*Average number of local lesions per half-leaf of petunia for 32 replicates.

**Aphid Transmission.** In a series of experiments, aphids were prestarved from 0 to 5 hours and allowed an acquisition feeding time of 5 to 10 minutes. Ten insects were then immediately transferred to each test plant. Increased preacquisition starving increased the efficiency of the vector to transmit GLV. Prestarvation periods of 3 and 4 hours resulted in the greatest transmission. Most aphids failed to survive 5 hours of starvation. Preacquisition starvation for 3 to 4 hours was thus adopted in all transmission tests.

In another series of experiments, aphids were allowed acquisition feedings from 1 to 16 minutes. Five aphids were used per plant and allowed to remain on receptor plants at least 30 mintues before being removed. Results of these tests showed that aphids acquired the virus from donor plants after 2 minutes but that maximum infectivity of the vector was obtained with 4 and 8 minutes of acquisition feeding.

Other tests were made in which a large colony of *M. persicae* was allowed 4 to 8 minutes of acquisition feeding on donor plants. Varying numbers of these insects were than transferred to receptor test plants and left for at least 30 minutes before they were removed. In one test, transmission was accomplished by a single insect. In general, an increased number of aphids involved in a transfer was followed by an increase in the number of infected plants.

Tests were made to determine the retention of infectivity of GLV in *M. persicae*. Starved as well as nonstarved aphids were given an acquisition feeding time of 4 to 8 minutes. Five insects per plant were transferred every 2 minutes to a series of test plants. The results of these tests indicate that starved aphids remained infective up to 8 minutes.

**Effect of Temperature and Light on GLV**. Investigations of the effects of air temperature on concentration and accumulation of GLV in petunia, gynura, and chrysanthemum and the influence of light intensity and photoperiod on local lesion development in petunia are reported.

Effects of air temperature on virus concentration in gynura and chrysanthemum were studied in a series of experiments. Gynura plants were grown in growth chambers at 15°, 20°, 25°, 30°, and 35°C. Comparative samples from inoculated plants at each temperature were collected at weekly intervals and assayed to petunia plants growing at 20°C. In plants at 25°C, the virus reached the highest concentration 6 weeks after inoculation while in plants at 20° and 30°C it reached its highest concentration at 4 weeks. No virus was detected in plants grown at 15° and 35°C. Virus concentration was sufficient in all plants after 2 weeks at 20°, 25°, and 30°C to produce local lesions in petunia. Initially, virus accumulation was favored by the higher temperature. After the virus reached maximal amount in plants at 30°C, virus concentration declined sharply but levelled off in the case of plants at 25°C. Growth of healthy plants was also favored by those temperatures that favored virus accumulation. Symptoms of exocortis disease in gynura were most severe at 25°C and least at 20°C. No symptoms of exocortis were noted at 15° or 35°C.

A similar virus accumulation pattern was noted in chrysanthemum plants. Highest concentration was reached at 8 weeks in plants grown at 35°C, at 6 weeks in plants at 15° and 20°C and at 2 weeks in plants at 30°C. Symptoms of GLV in chrysanthemum plants were more severe at 20°C than at any other temperature. No symptoms developed and no virus was recovered in plants kept at 35°C. There was a direct correlation between virus activity and growth of healthy plants. That is, temperature favoring growth of healthy plants also favored increased concentration of virus.

Effects of light intensity and duration on the development of local lesions in petunia were studied by subjecting petunia plants inoculated with GLV to different light intensities. Except for plants kept totally in the dark, there was no significant difference in local lesions among plants held at different light intensities. Lesions in petunia plants grown in the dark were fewer in number and developed a greenish color rather than the typical chlorosis.

In another experiment, effects of day length on development of local lesions in petunia were studied. Inoculated plants were maintained at photoperiods of 8, 18, and 24 hours of light at ca 1500 ft-c. No difference in number or size of local lesions was observed.

In another experiment the effects of air temperature on local lesion development in petunia were studied. Results (table 4) show petunia plants kept at 20°C produced more lesions than plants

TABLE 4
EFFECT OF AIR TEMPERATURE ON LOCAL LESION DEVELOPMENT IN PETUNIA*

| Temperature (C) | No. of lesions | Size of lesion (mm) | Incubation period (days) | Longevity of lesions (days) |
|---|---|---|---|---|
| 15 | 26 | 1-2 | 25-30 | 9-14 |
| 20 | 43 | 2-3 | 9-11 | 5-7 |
| 25 | 21 | 3-5 | 7-9 | 3-5 |
| 30 | 7 | 5-10 | 6-8 | 3-5 |
| 35 | 0 | — | — | — |

*Four experiments. Average of 64 replicates.

kept at other temperatures and the relative size of lesions increased with increase in temperature. At 15° C, lesions were initially uniformly chlorotic and later became necrotic. As temperature increased, lesions became less delimited and at 30°C coalesced into large expansive lesions. No lesions developed nor was virus recovered at 35°C.

The incubation period was inversely correlated with air temperature; that is, as temperature increased, the length of the incubation peirod decreased and *vice versa.*

## DISCUSSION AND CONCLUSIONS

The virus causing a latent infection in gynura is a member of the Carlavirus group (Harrison *et al.*, 1971), and appears to be a strain of chrysanthemum virus B (CVB). The Carlavirus group includes carnation latent, potato M, potato S, lilac mottle, pea streak, red clover vein mosaic and freesia mosaic.

The physical properties are similar to those reported for chrysanthemum virus B (Hollings, 1957). Longevity of GLV in frozen sap however was different from CVB; GLV remained infective for several months while CVB lost infectivity in a matter of days. This variance may be attributed to differences in strain, virus source or extracting medium.

In our host range studies various chrysanthemum varieties showed systemic symptoms, petunia varieties developed primary lesions and tobacco was not susceptible. Chrysanthemum virus B was described (Brierley and Smith, 1953) as causing a mosaic in chrysanthemum, local lesions in petunia, but no infection in tobacco. Similarity in host range would suggest that GLV is an isolate of CVB although some discrepancies indicate them to be different strains. For instance, *Nicotiana glutinosa, N. clevelandii*, and *Vicia faba* were reported to be hosts of CVB (Hollings, 1957) but these plants failed to react to GLV in our tests.

Gynura latent virus is transmitted by sap more efficiently when extracted in low molarity buffers in the basic or neutral range than in acidic buffers of high molarity. Orthoborate buffers (pH 9.0) were found to be the most efficient extracting buffers over a wide range of molarities. Results of aphid transmission tests showed that *M. persicae* is capable of transmitting GLV from chrysanthemum to chrysanthemum. Preacquisition starvation of the aphid increased its efficiency to transmit GLV and also increased retention of infectivity. Similar results have been reported by Hollings (1957). Preacquisition fasting of *Macrosiphoniella sanborni* for 3 hours increased the number of chrysanthemum plants infected with CVB. Hollings (1957) also found that starved *M. persicae* retained infectivity up to 45 mintues. The probable minimum period for the aphid to acquire GLV is 2 minutes, increasing up to 8 mintues. In our tests, infectivity of *M. persicae* was lost after 8 minutes.

Although Hollings (1957) obtained relatively low percentages of transmission with aphids, his results led to the conclusion that CVB is transmitted in a sylet-borne manner. Our results indicate that GLV is also transmitted by *M. persicae* in a stylet-borne manner.

Optimum temperature for development of local lesions in petunia was 20°C, with an incubation period of 9 to 11 days. The incubation period was inversely correlated with temperature. That is, the incubation period decreased with increasing temperature and *vice versa.* Characteristics of local lesions varied with temperature. Large, expansive chlorotic lesions developed at higher temperatures while at lower temperatures small delimited necrotic lesions developed. Optimum temperature for virus accumulation was 25°C in gynura and 20°C in chrysanthemum. Symptoms of GLV were most severe in chrysanthemum at 20°C.

The concentration of gynura latent virus appears to be enhanced in gynura by the presence of CEV. The concentration of GLV in gynura plants doubly infected was considerably higher than in plants singly inflected with GLV. The dilution endpoint of GLV was extended when sap

was derived from gynura plants doubly infected with GLV and CEV, and higher yields of GLV particles were obtained from plants concurrently infected with CEV (Osman *et al.*, 1972).

The virus in gynura is similar in host range, symptoms, insect transmission and particle size (Osman *et al.*, 1972) to chrysanthemum virus B. The gynura latent virus reacted positively to lilac mottle virus antiserum (Gumpf, Osman, and Weathers, unpublished), a member of the Carlavirus group, as in CVB. We suggest that our virus is a strain of chrysanthemum virus B.

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