Identification of Variants of Vein-Enation (Woody-Gall) Virus Through Synergism

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

INVESTIGATIONS of the interaction of viruses show that infections of citrus by two or more unrelated viruses sometimes induce different and more severe effects than do single infections (4, 5, 6). In previous studies, two strains of yellow-vein virus (YVV) of citrus were discovered which differ primarily in their synergistic reaction with vein-enation virus (VEV) (6). Evidence of the existence of variants of VEV, based on studies of the synergistic interaction between vein-enation and yellow-vein viruses is reported here.

Citrus plants infected with VEV show the following symptoms: slight vein swellings, formation of small enations from the veins on the under surface of the leaf, and knob-like galls on the stems, trunks, and roots (3). Plants vary in their reaction to the virus, and different isolates of the virus vary in their ability to produce symptoms. Leaf enations may be few to many, the projections may vary in size and length, and galls may be absent to very abundant on stems and roots, depending on the plant infected and the isolate of the virus. This variation in the symptoms of VEV, when it occurs alone, makes identification of variants unreliable when based strictly on symptoms. As a result, differentiation of variants of VEV was attempted by combining it with YVV and examining the symptoms produced through synergistic response.

Materials and Methods

Virus-free Rough lemon (*Citrus jambhiri* Lush.), Mexican lime [*C. aurantifolia* (Christm.) Swing.], and *C. volkameriana* Pasq. were used as indicator plants. Grafting techniques previously described (4, 5) were used. Ten different isolates of VEV collected from various citrus species were inoculated into and maintained in Mexican lime and Rough lemon plants in the greenhouse. These isolates are listed in Table 1. Except for number 7, none of the isolates caused different leaf symptoms. Most of the isolates produced abundant enations of varied sizes on most leaves. In Rough lemon stock plants, woody galls were produced by some isolates, whereas other plants were free of galls. Each of the 10 isolates of VEV was inoculated concurrently with the severe Tulare strain of YVV into indicator plants.

Results

Indicator plants infected with both viruses exhibited two levels of vein yellowing, which varied with the isolate of VEV interacting with YVV (Table 1 and Fig. 1). One level consisted of severe yellowing of the veins of every leaf on the plant and severe dwarfing, which sometimes killed the plants. The other reaction consisted of mild vein yellowing which affected only part of the leaves and caused only moderate dwarfing. Isolates of VEV from plants having both galls and abundant leaf enations always produced the severe yellow-vein reaction. Isolates from

TABLE 1. COMPARISON OF SEVERITY OF VEIN YELLOWING IN INDICATOR PLANTS INFECTED WITH TULARE STRAIN OF YELLOW-VEIN VIRUS AND DIFFERENT ISOLATES OF VEIN-ENATION VIRUS

	Symptoms of vein-enation virus isolates in singly infected plants			Vein yellowing in doubly infected plants
Vein-enation isolate	Leaves with enations, per cent[a]	Amount and size of enations	Presence of (+) or absence of (–) woody galls[b]	
1	94	Abundant and of varying sizes	–	severe
2	92	"	+	severe
3	90	"	+	severe
4	94	"	–	mild
5	95	"	+	severe
6	89	"	–	severe
7	12	Sparse and small	–	mild
8	92	Abundant and of varying sizes	+	severe
9	90	"	+	severe
10	91	"	+	severe

a. Based on 500 leaves from 5 Mexican lime plants.
b. + = galls associated with virus in stock plants.
– = galls not present in stock plants.

plants without galls, but with abundant leaf enations, caused varied responses. Some isolates produced the severe vein-yellowing reaction; others produced a mild reaction. The synergistic response to isolate number 7 was only slightly more intense than infections of YVV alone.

These mild and severe reactions with double infections remained constant. In every instance, bud inoculations from the mild and severe types to healthy indicator plants produced the mild and severe symptoms, respectively. In no case did mild isolates induce severe symptoms or vice versa.

A series of cross-protection experiments similar to those described with

YVV (6) was performed. Tissue from plants with severe symptoms was grafted into plants with mild symptoms to determine if the mild type would prevent or interfere with infection by the severe type. Plants with mild symptoms developed severe symptoms when challenged, and transfers of tissue from the challenged plants to healthy plants induced severe symptoms of YVV. These results suggest that cross-protection is not effective, or that cross-protection between the variants of VEV does not function when it is interacting synergistically with yellow-vein virus.

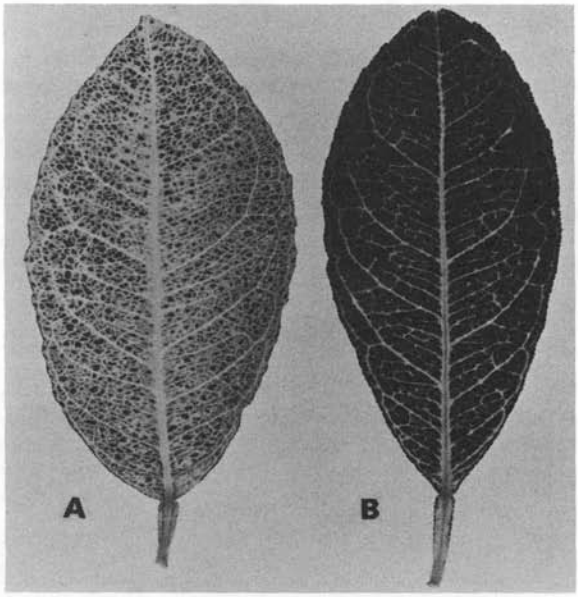

FIGURE 1. *Leaves of Rough lemon plants. A. Inoculated with YVV and a severe variant of VEV. B. Inoculated with YVV and a mild variant of VEV.*

Discussion and Conclusions

The results with double inoculations of indicator plants indicate that variants of the VEV exist, and that two distinct types of variants are recognizable: mild variants, which in combination with YVV induce mild vein-yellowing symptoms; and severe variants, that cause severe symptoms and pronounced synergistic responses with YVV.

Whether or not the two levels of severity in symptoms are due to strain differences in vein-enation virus cannot be ascertained from these results. Positive cross-protection is good evidence for virus relationship, but failure to achieve it is not always evidence of unrelatedness (1, 2). Cross-

protection is rarely absolute even under the most favorable conditions, and graft inoculation is the least desirable method to employ. Moreover, in these cross-protection tests there was the added complication of having the variants of VEV synergizing the unrelated YVV.

The behavior of VEV isolates in single infections was not correlated completely with their behavior in double infections. Isolates from galled plants always induced a severe synergistic reaction when combined with YVV, but some isolates from plants without galls likewise produced a severe synergistic response. Conversely, other isolates from plants without galls induced a mild synergistic reaction as did isolate number 7 which produced no galls and few enations in single infections. However, the behavior of isolate 7 in single infections is sufficiently different from that of the other isolates to indicate that it is an identifiable mild strain of vein-enation virus.

Literature Cited

1. BENNETT, C. W. 1953. Interactions between viruses and virus strains. Adv. Virus Res. 1: 39-67.
2. KASSANIS, B. 1963. Interactions of viruses in plants. Adv. Virus Res. 10: 219-255.
3. WALLACE, J. M. 1960. Woody galls on citrus associated with vein enation virus infection. Plant Disease Reptr. 44: 580-584.
4. WEATHERS, L. G. 1960. Yellow vein disease of citrus and studies of interactions between yellow vein and other viruses of citrus. Virology 11: 753-764.
5. WEATHERS, L. G. 1961. Responses of citrus to concurrent infection with two or more unrelated viruses, p. 187-196. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
6. WEATHERS, L. G. 1963. Use of synergy in the identification of strains of citrus yellow vein virus. Nature 200: 812-813.