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Responses of Citrus to Concurrent Infection with Two or More Unrelated Viruses

INVESTIGATIONS on the interactions of viruses begun in 1958 by the writer (9, 10, 11) have shown that infections of citrus by two or more viruses, presumably unrelated, sometimes induce different and more severe effects than single infections. This paper reviews the previous studies on interactions of viruses in citrus and reports on some additional investigations of this subject.

Materials and Methods

Experiments were conducted in a greenhouse maintained at approximately 75°F. Plants were grown in pots of steam sterilized U.C. Soil Mix C. (Fertilizer I) (2) and were regularly irrigated with supplemental nitrogen.

A single strain or source of each virus was used. Yellow vein came from one of the original diseased field trees of Eustis limequat [*C. aurantifolia* (Christm.) Swing. x *Fortunella japonica* (Thunb.) Swing.] (11), and so far as could be determined was not contaminated with other viruses. The strain of psorosis virus used came from a limequat field tree that was showing psorosis leaf symptoms but no evidence of other viruses. A strain of vein enation virus was supplied by Dr. J. M. Wallace from a greenhouse-grown sweet orange plant that had been infected experimentally by *Myzus persicae* (Sulz.).

Plants were inoculated by inserting buds from the diseased sources into T-slits in the bark of the test plants.

Results

PSOROSIS AND YELLOW VEIN.—When yellow vein and psorosis viruses were introduced concurrently into plants of West Indian lime [*Citrus aurantifolia* (Christm.) Swing.] characteristic leaf symptoms of psorosis dominated very quickly and almost completely; yellow vein virus occasionally induced a few vein yellowing symptoms in a young leaf, but typical symptoms of yellow vein failed to appear.

When lime seedlings systemically infected with yellow vein virus were inoculated with psorosis virus, yellow vein symptoms already established were not noticeably altered; symptoms of psorosis developed systemically in the plants and were dominant in subsequent growth. When buds were taken from these plants in the area where yellow vein symptoms were not altered and were introduced into healthy lime plants, the latter developed symptoms of psorosis and not those of yellow vein.

When systemic infection by psorosis virus preceded infection by yellow vein virus, the result was much the same as with plants inoculated simultaneously. Whenever psorosis virus was combined with yellow vein virus, leaf symptoms of psorosis were more severe and the plants more stunted than in plants infected with psorosis virus alone.

PSOROSIS AND VEIN ENATION.—When vein enation virus was combined with psorosis virus in lime plants, each appeared to invade the plant independently of the other and produce its own characteristic symptoms regardless of the sequence of introduction of the components. However, in lime plants systemically invaded with psorosis virus prior to infection with vein enation virus, the onset of vein enation was noticeably delayed as compared to plants infected with vein enation virus alone. The delay in onset usually varied from 4 to 6 days, but was as much as 10 days in some plants. The development of psorosis symptoms was not affected by prior infection with vein enation virus.

Symptoms of psorosis did not appear to be changed in any way by the presence of vein enation virus, but the severity of vein enation symptoms seemed to be affected by the presence of psorosis virus. Results of one test, selected as representative, are shown in Table 1. The effect of psorosis virus on the development and severity of woody galls, recently shown by Wallace (8) to be associated with vein enation, was not determined.

YELLOW VEIN AND VEIN ENATION.—When lime, rough lemon (*C. jambhiri* Lushington), lemon [*C. limon* (L.) Burm.], and sweet orange

TABLE 1. COMPARISON OF SEVERITY OF VEIN ENATION SYMPTOMS IN WEST INDIAN LIME PLANTS INOCULATED WITH VEIN ENATION VIRUS ALONE, VEIN ENATION AND PSOROSIS VIRUSES CONCURRENTLY, VEIN ENATION VIRUS PRIOR TO PSOROSIS VIRUS, AND PSOROSIS VIRUS PRIOR TO VEIN ENATION VIRUS

Plant	Percentages of leaves showing diagnostic vein enations on plants infected with			
	vein enation[a] only	psorosis[b] and vein enation	vein enation[c] prior to psorosis	psorosis[b,c] prior to vein enation
1	98	76	58	76
2	97	80	66	68
3	97	62	64	64
4	96	84	64	70
Average	97	75	63	69

[a]Based on 100 leaves from each plant.
[b]Based on 50 leaves from each plant.
[c]The onset of vein enation symptoms in this group of plants was delayed 4 to 5 days as compared to plants inoculated with vein enation virus alone.

[*C. sinensis* (L.) Osbeck] plants were inoculated with the viruses of vein enation and yellow vein, 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. However, yellow vein symptoms in dual infections were more conspicuous than those of yellow vein alone (Fig. 1). Furthermore, a marked synergistic reaction in doubly-infected plants caused severe stunting (Fig. 2) never produced by single infections. In some cases of mixed infections, plants perished. The synergistic reaction was evident regardless of the sequence of the inoculations.

Sweet orange, lemon, and rough lemon inoculated with yellow vein virus alone developed vein yellowing symptoms much less severe than did West Indian lime. Symptoms were generally restricted to an occassional young leaf and were followed by recovery or lack of symptoms in subsequent growth. Plants inoculated with vein enation virus alone developed only occasional enations on main veins on the undersides of the leaves (Fig. 2). Neither virus, when present alone, stunted the growth of seedlings (Fig. 2). Plants inoculated with both viruses had severe vein yellowing symptoms throughout. The vein enations took on a brilliant yellow color and appeared to be enlarged. Subsequent growth was restricted and leaves were reduced in size. In many leaves, yellowing ex-

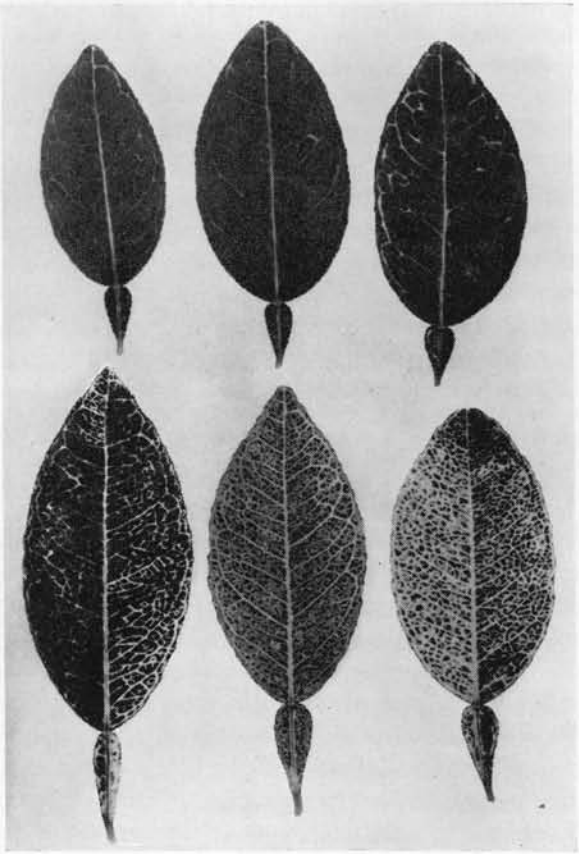

FIGURE 1. *Leaves of West Indian lime. Top, infected with yellow vein virus alone; bottom, infected with both yellow vein and vein enation viruses and showing severe symptoms of yellow vein.*

tended into all the small veinlets so that almost the entire leaf became yellow. After several months, doubly-infected plants were about 1/3 normal size but frequently were killed.

In a previous study (11), it was shown that inoculation of *P. trifoliata* plants with buds and grafts from limequat and West Indian lime plants with yellow vein symptoms produced no vein yellowing. Transfers from inoculated *P. trifoliata* plants back to healthy lime plants failed to pro-

FIGURE 2. *Response of Florida rough lemon plants to infection with vein enation and yellow vein viruses. Top, (left to right) noninoculated plant, plant infected with yellow vein virus alone, plant infected with vein enation virus alone, and plant infected with both vein enation and yellow vein viruses, showing synergistic dwarfing and severe symptoms of yellow vein; bottom, corresponding leaves from plants at the top, showing severe yellow vein symptoms in the leaf (extreme right) infected with both yellow vein and vein enation viruses.*

duce yellow vein symptoms. These results indicated that *P. trifoliata* is immune from yellow vein virus.

Tests were initiated to determine whether or not the yellow vein virus in combination with vein enation virus could be transmitted to *P. trifoliata*. Healthy *P. trifoliata* scions were grafted onto West Indian lime plants infected with both yellow vein and vein enation viruses and showing severe synergistic effects. Healthy *P. trifoliata* scions were grafted

onto lime plants infected only with yellow vein, to serve as check plants. Most of the basal shoots of these plants were pruned to encourage development of new shoots from the scions and to encourage the movement of virus into these shoots.

Yellow vein symptoms developed in all the new growth of *P. trifoliata* scions grafted on doubly-infected lime stock. Transfers of buds from these affected *P. trifoliata* tops to healthy West Indian lime plants induced yellow vein symptoms. Many of these *P. trifoliata* tops were killed. A plant of *P. trifoliata* on lime rootstock exhibiting the severe synergistic dwarfing and vein yellowing symptoms is shown in Fig. 3. None of the control plants (healthy *P. trifoliata* grafted to lime with

FIGURE 3. *Effects of yellow vein and vein enation viruses on Poncirus trifoliata on West Indian lime rootstock. Left, plant with yellow vein alone; right, plant with both yellow vein and vein enation.*

yellow vein only) developed any evidence of yellow vein. Transfers of *P. trifoliata* buds from the control plants to healthy lime plants failed to produce yellow vein symptoms, indicating failure of yellow vein virus to become established. Transfers of West Indian lime buds from the rootstocks of the control plants to healthy lime plants induced yellow vein symptoms.

As a further test, buds from vein-enation-infected West Indian lime plants were inserted into the *P. trifoliata* tops of half of the singly infected (yellow vein) control plants. The plants were pruned to force new growth from the *P. trifoliata* tops. All the plants receiving the vein enation virus developed yellow vein symptoms. The remaining singly infected control plants remained free of yellow vein symptoms. The results of these tests indicate that, in the presence of vein enation virus, yellow vein virus can invade *P. trifoliata* tissue whereas it cannot do so alone.

Other tests were made in which scions from lime plants showing severe symptoms resulting from the interaction of yellow vein and vein enation viruses were grafted to healthy *P. trifoliata* plants. Shoots were encouraged to grow from both above and below the graft union. Yellow vein symptoms appeared in new growth from both the top (lime) and stock (*P. trifoliata*) portion of the plants. Following this, the lime tops were removed and the *P. trifoliata* stocks retained for periods up to 6 months. Subsequent growth of *P. trifoliata* showed no symptoms of yellow vein. Scions were then removed from the *P. trifoliata* stock and grafted to healthy West Indian lime plants. All scions transmitted vein enation virus but none transmitted yellow vein virus, indicating that yellow vein virus was not able to maintain itself in *P. trifoliata* for any considerable period. Thus it appears that, although yellow vein virus mixed with vein enation virus can invade and produce marked yellow vein symptoms in *P. trifoliata* when the yellow vein virus is supplied from West Indian lime, yellow vein virus cannot persist in *P. trifoliata* in the absence of attached susceptible tissue.

PSOROSIS, YELLOW VEIN, AND VEIN ENATION.—Following experiments in which the viruses of psorosis, yellow vein, and vein enation were studied in paired combinations, studies were made of the interactions and effects of all 3 viruses together. In one test, West Indian lime plants were inoculated concurrently with the 3 viruses. In other tests, each of the 3 possible pairs was introduced into healthy lime plants and allowed to become established prior to inoculation with the third virus. Plants inoculated only with the pairs served as controls. In all plants containing

the 3 viruses, regardless of the inoculation sequence, severe vein yellowing and dwarfing symptoms, typical of the synergistic reaction of the mixture of yellow vein and vein enation viruses, dominated quickly and almost completely in the new growth. Symptoms in plants with the 3 viruses were almost identical to those on plants dually infected with yellow vein and vein enation viruses. Leaf symptoms of psorosis could only rarely be detected. There was no indication of an interaction of the 3 viruses. These results indicate that the synergistic behavior of yellow vein and vein enation viruses in these mixed infections overcomes the suppressive effect of psorosis virus on either yellow vein or vein enation viruses used singly.

Discussion and Conclusions

It has been established that the viruses of psorosis, vein enation, and yellow vein are not closely related (6, 7, 9). Their interactions are of particular interest in that they demonstrate experimentally that multiple infections of citrus with unrelated viruses can influence development and severity of symptoms and injury to the host plant.

Without means of obtaining quantitative data, one can only speculate about mechanisms involved in the interactions. The marked suppression of yellow vein and vein enation by psorosis virus might suggest a relationship at some particular level. Failure to obtain reciprocal and complete protection suggests, however, something quite different. The fact that yellow vein and vein enation viruses became established in plants previously infected with psorosis virus suggests that the ability of psorosis virus to suppress yellow vein and vein enation is due not to the blocking of the infection sites but to some later phase of activity. The simplest explanation is that when plants are inoculated with the virus pairs, the virus with the greater affinity, that of psorosis in this case, invades and multiplies more rapidly, possibly utilizing materials needed for the multiplication of the yellow vein or the vein enation virus.

Probably the same principle operates in the domination of psorosis virus by the combination of yellow vein and vein enation viruses. The combination of yellow vein and vein enation viruses in a mixed infection with psorosis virus may have the greater affinity and can invade and multiply more rapidly in the host, using materials that are essential for the synthesis of psorosis virus.

The increase in severity of symptoms produced by psorosis virus when accompanied by yellow vein virus, as compared to psorosis virus alone,

demonstrates that yellow vein virus affects either the activity of psorosis virus or the reaction of the host plant to that virus. This explanation has some support in the findings of Bennett (1), in his studies of dodder latent mosaic, and Rochow and Ross (3), who worked with potato viruses X and Y. These investigators showed that the presence of one virus has a marked effect on a second virus and concluded that synergistic responses are correlated with increased multiplication of one virus in the presence of another. Multiplication of the accompanying virus may be unaffected or inhibited appreciably.

Bennett (1) and Ross (4, 5) have hypothesized that marked stimulation in mixed infections of virus multiplication results from the inhibitory effects by the second virus on the mechanisms that normally limit multiplication of the first. Bennett (1) found that when tomato plants that had recovered from dodder latent mosaic were inoculated with tobacco etch virus or tobacco mosaic virus, the concentration of dodder latent mosaic virus increased and became established at new levels considerably higher than in plants infected by dodder latent mosaic virus alone. He also found that the increase in concentration of dodder latent mosaic virus resulted in the reappearance and increased severity of dodder latent mosaic symptoms. The synergistic reactions between citrus yellow vein virus and citrus vein enation virus reported herein are analogous to the interactions between dodder latent mosaic virus and the viruses of tobacco etch and tobacco mosaic. Dual infections with yellow vein and vein enation viruses always resulted in retarded growth of the host plant and an increase in severity of yellow vein symptoms. When lemon, rough lemon, and sweet orange plants that had recovered from the initial effects of yellow vein virus were infected with vein enation virus, the symptoms of yellow vein reappeared and were much more severe than those of the singly-infected plants and persisted as long as the plants were retained. It seems reasonable to assume that the mechanisms involved in the interactions of these citrus viruses are similar to the mechanisms described by Bennett (1) and Ross (4, 5).

It was shown that yellow vein virus, in combination with vein enation virus, can invade *P. trifoliata* and produce symptoms. Whether yellow vein virus multiplies in *P. trifoliata* is less certain and even rather doubtful. Yellow vein virus persisted and produced symptoms in *P. trifoliata* only in the presence of attached susceptible lime tissue. Tests by grafts showed that both viruses entered *P. trifoliata*. Upon removal of the lime tissue, yellow vein symptoms ceased to be produced on *P. trifoliata* and subsequently the yellow vein virus could not be detected by grafts from

it to healthy lime plants even though vein enation virus could be recovered from such plants. This suggests that yellow vein virus was not only failing to multiply in *P. trifoliata,* even in combination with vein enation virus, but was, in fact, becoming inactivated. It can be concluded then that the yellow vein symptoms developed in *P. trifoliata* in the presence of vein enation virus and attached susceptible tissue only because of the continued production of fresh virus, presumably by the synergistic reaction of yellow vein and vein enation viruses in the attached susceptible host.

ACKNOWLEDGMENTS.—The author gratefully acknowledges the technical assistance of M. K. Harjung, Laboratory Technician II, University of California, Riverside.

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