Relationship of Symptoms to the Presence of Tatter-Leaf Virus in Several Citrus Hosts

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TATTER-LEAF VIRUS of citrus (TLV) was described by Wallace and Drake (7) in 1962. This disease is one of the few citrus viruses transmitted to herbaceous plants by sap (mechanical) inoculation (2, 4, 6). Included in its extensive range of herbaceous hosts are local lesion hosts which can be used for rapid, quantitative assays (4, 6).

Tatter-leaf virus is known to occur naturally only in Meyer lemon [*Citrus limon* (L.) Burm. f. hyb.], a symptomless carrier, but it has been transmitted experimentally to a number of other citrus varieties (8). Wallace and Drake (8) reported that plants of Citremon [*Poncirus trifoliata* (L.) Raf. x *C. limon* (L.) Burm. f.] and Troyer citrange [*P. trifoliata* (L.) Raf. x *C. sinensis* (L.) Osb.] showed striking symptoms when inoculated with TLV, whereas seedlings of trifoliate orange (*P. trifoliata*) were less affected. They also reported that inoculated seedlings of sweet orange (*C. sinensis*) became systemically infected with TLV, but remained symptomless.

This paper reports experiments on the susceptibility of trifoliate orange, sweet orange, and certain citranges to TLV infection, and the relationship of virus distribution in these hosts to symptoms.

Materials and Methods

Experiments were conducted in a screened and partially shaded greenhouse cooled by evaporative coolers. Temperatures usually ranged between 21 and 35°C, and light intensity ranged from 1,200 to 2,000 ft-c near bench level at midday. The plants were grown in steam-sterilized potting soil and were fertilized regularly. All plants were inspected frequently and sprayed to control mite and insect infestations.

Several isolates of TLV from Meyer lemon were used in this study. Some isolates carried tristeza virus, but all were free of psorosis and exocortis viruses.

Most graft inoculations were made by chip grafting two pieces of stem tissue, without buds, of the inoculum source to the desired indicator plant. Grafts were wrapped with 6 mil. plastic tape and plants were topped to force new growth. In several experiments a small rectangular piece of leaf tissue from the inoculum source was placed under a flap of

bark of similar size in the indicator plant. One edge of the leaf piece was allowed to protrude above the edge of the bark flap so that the longevity of the inoculum could be observed. The leaf-piece grafts were wrapped with plastic tape and the indicator plants treated as above.

Inoculum for sap inoculations was prepared by macerating host tissue in cold, neutral potassium phosphate buffer (.05 M) (4). Unless stated otherwise, dilution of the inoculum was standardized at a ratio of five 6.4 mm discs of leaf tissue per ml of buffer. The macerate was quickly applied to the primary leaves of cowpea [*Vigna sinensis* (Torner) Savi var. Early Ramshorn] previously dusted with 500-mesh Carborundum. Test plants were inoculated when the primary leaves had expanded, but before the trifoliate leaves appeared. Inoculated leaves were rinsed with cool tap water and the plants were incubated under moderate light at 21 to 24°C (4). Lesions were counted 5 to 7 days after inoculation.

Results

SWEET ORANGE.—In the past three years we graft-inoculated more than 20 sweet orange seedlings with tissue from TLV-infected Meyer lemon trees. All inoculated plants remained symptomless, but TLV was consistently transmitted from them by grafting and by sap inoculation. Tissue assays indicated that, as in Meyer lemon, TLV was distributed throughout the stems and leaves of infected sweet orange plants. Furthermore, TLV was transmitted from tissue of the first new growth that appeared following inoculation. This indicates rapid multiplication of the virus and invasion of host tissues.

CITRANGES.—Rusk citrange was used in most tests because it developed better symptoms than Troyer or Carrizo (3). Tatter-leaf virus symptoms in leaves and stems of Troyer and Rusk have been illustrated and described previously (3, 8). However, the range of symptoms of TLV within individual plants has not been fully described. Rusk citrange plants infected with TLV usually show strong symptoms, which are not uniformly distributed throughout the plant. On certain shoots, every leaf may be severely affected, and marked distortion of the stem is associated with irregular areas of chlorosis. Corky areas often develop on the stem. Only a few leaves on other shoots of the same plant may show symptoms, and stem symptoms will be mild (Fig. 1).

The intensity of symptoms in individual leaves often varies greatly. Some leaves become almost entirely chlorotic and severely distorted (Fig. 1,A,C). Only one or two leaflets may be affected on other leaves and

only a single small chlorotic spot (Fig. 1,B,D) may appear on some leaves. Symptoms of TLV develop when the leaf is quite young, but usually become more distinct as the leaf matures. New growth arising from a mature portion of a plant with strong symptoms often shows no symptoms on the first 4 to 8 leaves.

The intensity of TLV symptoms in Morton citrange was similar to that observed in Rusk, but the chlorotic areas were often more diffuse. Symptoms in Troyer were similar to those in Rusk, but milder. Carrizo seldom showed more than an occasional blotching and leaf distortion with little or no stunting or stem distortion.

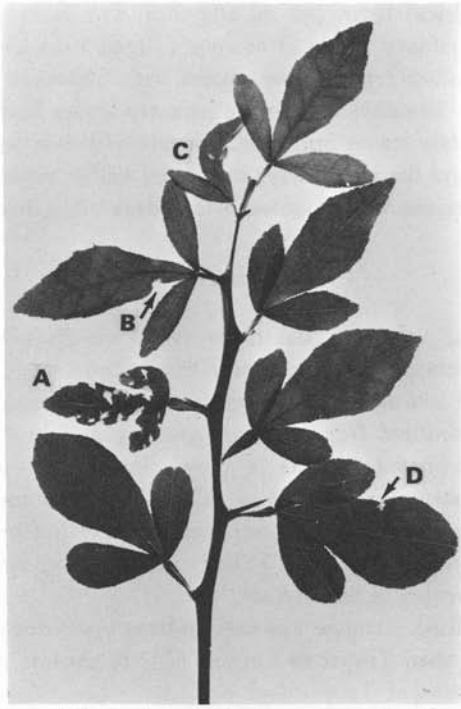

FIGURE 1. *A shoot of Rusk citrange showing uneven distribution of symptoms of tatter-leaf virus. Leaves A. and C. have severe symptoms; B. and D. exhibit single chlorotic spots. The remaining leaves are symptomless.*

Our first attempt to transmit TLV by grafts from infected Rusk, Troyer, and Carrizo seedlings to healthy Rusk plants was not especially successful. None of the 10 Rusk plants inoculated, 5 with Troyer and 5 with Carrizo tissue, became infected, and only 2 of 5 inoculated from Rusk became infected. The symptomless plants were cut back repeatedly for

over 1 year, but failed to show symptoms. However, 5 Rusk plants inoculated from the original Meyer lemon source, at the same time, all developed strong symptoms within several months. Sporadic transmission also occurred in subsequent experiments where citrange was the inoculum source.

Experiments showed that TLV could be easily sap-transmitted from citrange to cowpea if tissue with visible symptoms was used as inoculum, but transmission was rare from tissue which showed no symptoms (4). To ascertain whether absence of symptoms means absence of the virus, samples were taken from areas with and without symptoms (Fig. 2). Fourteen discs from 1 Rusk citrange leaf showing strong TLV symptoms were assayed, each on 4 primary leaves of cowpea. The total lesion count resulting from the the assay of each disc is shown in Figure 2. Transmission experiments with leaves from TLV-infected Troyer and Carrizo plants produced similar results. These sap-inoculation studies indicate that the concentration of TLV was very much higher in tissue with symptoms than in symptomless tissue.

Tests were also made to determine whether failure to transmit the

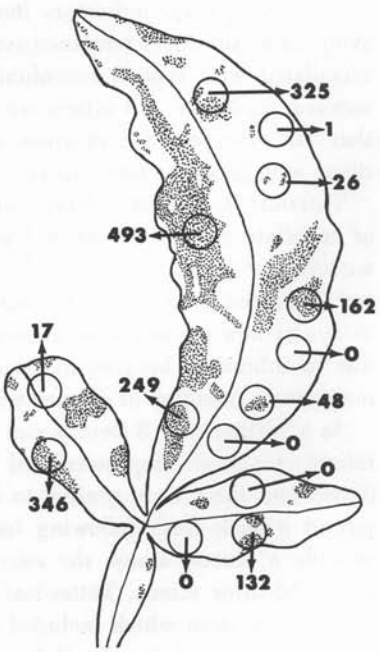

FIGURE 2. *Differential retrieving of tatterleaf virus by inoculation with sap from an infected leaf of Rusk citrange. Stippled areas correspond to chlorotic areas on the original leaf. Each disc was assayed separately by sap inoculation on four cowpea leaves. The number alongside the disc is the total number of local lesions for each assay.*

virus from symptomless leaf tissue was due to absence of the virus or to a titer below the level detectable by sap inoculation. Graft inoculations were made with leaf pieces cut from diseased and symptomless areas of citrange leaves. Because Rusk was believed to react inconsistently to inoculum containing very low quantities of TLV, sweet orange seedlings also were used as indicators. The sweet orange seedlings were assayed by sap inoculation to cowpea or by grafting on Rusk. In general, these preliminary graft transmission tests confirmed the sap transmission results and indicated that TLV was absent from symptomless leaf tissue. The exceptions noted occurred when leaf pieces were cut to include veinlets leading to an area showing symptoms.

In our last experiment, the following types of inocula from Rusk leaves were used: 1) areas containing visible TLV symptoms, 2) symptomless areas on TLV-affected leaves, 3) symptomless leaves from an infected shoot, and 4) symptomless leaves on a symptomless shoot from an infected plant. Each leaf piece was divided and the 2 portions grafted, one to Rusk citrange and the other to a sweet orange seedling. The Rusk indicators were examined for visible symptoms, whereas infection in the sweet orange indicators was determined by assay on cowpea.

Four of 5 Rusk indicators inoculated with type 1 inoculum showed symptoms; all others remained symptomless. All 5 sweet orange seedlings inoculated with type 1 inoculum and 1 of 10 inoculated with type 2 assayed positively. All others were negative. These results also indicate that TLV was confined to areas with visible symptoms and to vascular tissue with access to these areas.

TRIFOLIATE ORANGE.—Symptoms of TLV failed to appear in seedlings of trifoliate orange during a 1-year period following graft inoculation with Meyer lemon tissue. Seven months after inoculation, the seedlings were indexed to determine whether infection had occurred. Three sap assays of new growth were all negative. These negative results were not due to inhibitors because macerates of trifoliate orange leaf tissue did not prevent infection of cowpea with other sources of TLV.

As a further test, 8 stem pieces and 10 leaf pieces were cut from a trifoliate orange seedling inoculated 7 months previously with Meyer lemon tissue, and these were grafted to individual sweet orange seedlings. The second growth flush following inoculation was assayed on cowpea. To provide a second assay, the sweet orange seedlings were grafted with Rusk indicator scions. Tatter-leaf virus was transmitted only from that piece of the stem which included a portion of the original Meyer lemon inoculum. Stem pieces of trifoliate orange cut 1 cm above and below the

Meyer lemon inoculum piece failed to transmit TLV. These results indicated that trifoliate orange is either highly resistant to or immune to TLV infection.

Discussion

Results suggesting that certain citrus viruses are incompletely distributed within a particular host have been reported occasionally. These reports result from failure to obtain transmission from a systemically infected host or from inconsistent or negative results in cross-protection experiments. However, most systemically distributed citrus viruses can be transmitted consistently by grafting, and transmission is not usually correlated with symptoms in the inoculum piece. For example, psorosis virus can be transmitted with equal ease by leaf pieces from symptom-bearing or symptomless leaves. In contrast, TLV in citranges is limited primarily to areas showing visible symptoms.

Systemic viruses are not always completely distributed throughout the host tissues. Reid and Matthews (5) report islands of virus-free tissue in leaves of Chinese cabbage (*Brassica pekinensis* Rupr.) systemically infected with turnip yellow mosaic virus. The unequal distribution of TLV in citranges merits special attention, however, because it apparently results from interaction of the factors controlling TLV susceptibility in the two parents, sweet orange and trifoliate orange. Crosses between an immune plant (trifoliate orange) and a susceptible but latent host (sweet orange) produce progeny (citranges) with tissues that react in an intermediate and mixed fashion towards the virus. This results in unequal distribution of the virus in the citrange host. Thus, symptoms in citranges are closely correlated with presence of the virus and apparently result from this interaction.

The TLV symptoms we observed in various hosts were comparable to those reported (8) previously, except in trifoliate orange. Wallace and Drake (8) indicated that they observed some symptoms of TLV infection in trifoliate orange, although these were slow to appear. They did not report attempts to recover TLV from inoculated trifoliate orange plants. All selections or strains of trifoliate orange may not be immune to TLV. There is also the possibility that susceptible "strains" of trifoliate orange may, in fact, be hybrids, and inoculations of TLV may prove to be useful for distinguishing between trifoliate orange and hybrids that closely resemble it. The presence of tristeza in some of our isolates of TLV had no observable effect on TLV symptoms.

The severe effects TLV produces when infected commercial varieties

are grown on Troyer rootstocks (1, 3, 8) pose a potential threat to citrus production. These results also suggest that trifoliate orange may perform more favorably than citranges.

The factors involved in the non-uniform distribution of TLV in citranges do not necessarily account for the unequal distribution of other citrus viruses. Nevertheless, these results clearly show that a citrus host can be non-uniformly infected by a virus and that citrus virus-host relationships should be considered individually.

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