CHAPTER 6

Other Virus Diseases of Citrus

Citrange Stunt and Ringspot, Two Previously Undescribed Virus Diseases of Citrus

J. M. WALLACE and R. J. DRAKE

THE PURPOSE of this paper is to report and describe experiments with two previously unreported virus diseases of citrus. The first was found associated with citrus tatter-leaf infections (5) and has been named citrange stunt. Its causal virus is referred to as citrange stunt virus (CSV). The second was first found associated with psorosis and was thought to be caused by a strain of psorosis virus. The name citrus ringspot is suggested for this disease, and the causal virus is identified as citrus ringspot virus (CRV).

Citrange Stunt

Seedlings of *Citrus excelsa* West. (= *C. aurantifolia*), a kind of lime, inoculated directly from Meyer lemon [*C. limon* (L.) Burm. f.] show symptoms of tatter leaf at first, but eventually new growth is free of symptoms. Tissue inoculations from the symptomless parts to healthy *C. excelsa* give no symptoms, but symptomless portions reinoculated with tatter-leaf virus (TLV) develop typical symptoms of TLV. This indicates that the symptomless portions are free of TLV. However, inoculations from these same symptomless portions of *C. excelsa* to citremon [*C. limon* (L.) Burm. f. x *Poncirus trifoliata* (L.) Raf.] and Troyer citrange

[*C. sinensis* (L.) Osb. x *P. trifoliata*] cause the same symptoms described by the authors (6) on citremon (Fig. 1) and Troyer infected directly from Meyer lemon. These are: occasional, slightly depressed, blotchy areas on green twigs which develop into superficial lesions that cause the twigs to bend or curve; cessation of growth on the lesion side, causing the twig to bend sharply from its normal direction and resulting in a zigzag pattern of growth; deep pits that develop in the wood as the twig becomes older and often occur as a series of vertically aligned pits; and with continued growth, the merging of these pits into longitudinal

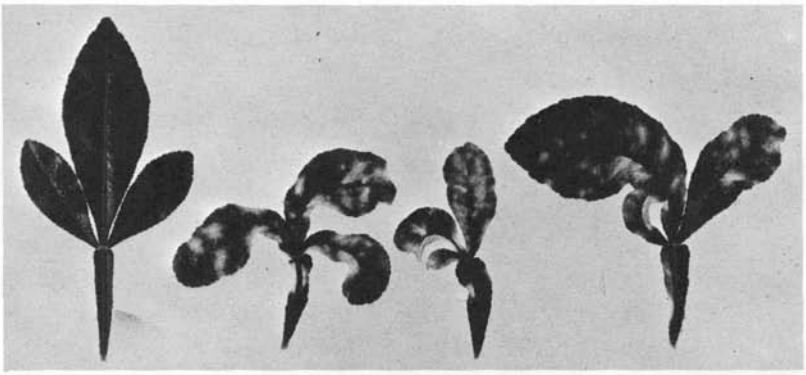

FIGURE 1. *Healthy leaf of citremon and leaves from a plant infected with citrange stunt virus.*

grooves to give a fluted appearance to the trunk (Fig. 2). The overall effects usually result in severe stunting.

TRANSMISSION EXPERIMENTS.—Semancik and Weathers (4) showed that a component of the tatter-leaf virus complex can be transmitted by juice inoculations to citrus and to a number of herbaceous plants. Apparently, these authors transmitted the virus that causes the symptoms previously described on Troyer citrange (6) and not the virus that causes the tatter-leaf symptoms on *C. excelsa* (5). Several attempts to transmit a virus from this complex with aphids failed. Furthermore, failure to find this virus in any citrus other than Meyer lemon (6) suggests that it has not been spread naturally. As previously indicated, CSV has been transmitted by tissue grafts from *C. excelsa* to citremon, Troyer, and other citranges.

HOST RELATIONS.—Trifoliate orange, one parent of citrange, appears highly resistant to or immune to this virus, but sweet orange, the other parent, is a symptomless host with the exception of some slight spotting

of mature leaves. However, it appears that only hybrids between these two species are severely affected by CSV. Morton, Etonia, Carrizo, and Rusk citranges develop strong symptoms, as do certain other trifoliate hybrids such as citrumelo, citremon, and citrangequat. In limited tests, *C. taiwanica, C. amblicarpa, C. volkameriana, C. paradisi, C. reticulata, C. sinensis, C. aurantium, C. jambhiri* (Rough lemon), *C. limon*, and Meyer lemon developed none of the symptoms shown by the trifoliate orange hybrids. However, many of these are symptomless hosts of CSV.

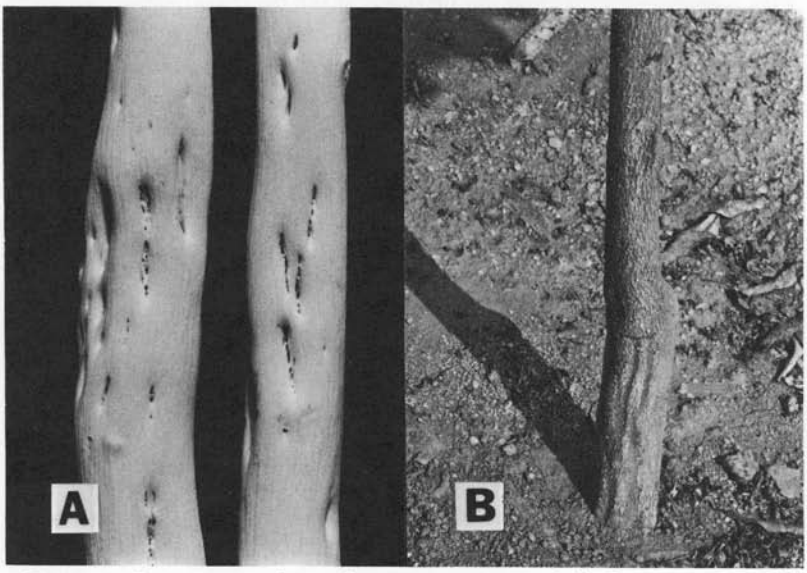

FIGURE 2. *Stem-pitting effects of citrange stunt virus. A. Citremon stems with bark removed. B. Grooving of Troyer citrange rootstock.*

Some of them occasionally display a few small, irregular, brownish or gum-colored spots on mature leaves which have a slight resemblance to the mature-leaf spotting of psorosis A (2).

We have established that the virus causing the symptoms we have described on citranges is not the same as that causing the symptoms originally described as tatter leaf on *C. excelsa*. Consequently, some other name for the second virus of this complex is required. Because this virus appears to affect principally the citranges or related hybrids, it is proposed that the disease be called citrange stunt and the virus be known as citrange stunt virus.

IMPORTANCE AND CONTROL.—Because inoculated seedlings of Troyer citrange were often seriously affected by CSV and because sweet orange

on Troyer citrange rootstock is now the most widely used combination in California, experiments were started to determine its effect on such trees. Three years after inoculation, young sweet orange trees on Troyer rootstock were chlorotic, unthrifty, and about one-half the size of healthy controls. The Troyer rootstock portions showed deep fluting or grooving. In 1963, Calavan *et al.* (1) reported severe decline of Satsuma orange on Troyer citrange rootstock inoculated from a Meyer lemon for the purpose of studying the effects of seedling-yellows virus. The injury unquestionably resulted from the presence of the tatter-leaf-associated virus.

This virus has such a severe effect on Troyer citrange rootstock that it could cause great loss, particularly in California, should a vector spread it to commercial plantings. At present, only two control measures can be suggested: to eradicate all infected Meyer lemons and establish virus-free clones from seedlings of this citrus variety or from sources free of the virus, and to include Troyer citrange or other suitable indicators for CSV (6) in all indexing programs. The writers have confirmed the report by Garnsey (3) that Rusk citrange is an excellent indicator of the virus associated with tatter-leaf virus.

That this virus can exist separately from the tatter-leaf virus has been demonstrated experimentally. If, by chance, it exists somewhere in citrus by itself, not mixed with tatter-leaf virus, indexing only on *C. excelsa* would not have disclosed its presence. Fortunately, the Citrus Variety Improvement Program in California provided other safeguards. All foreign citrus importations that were maintained after completion of the short-term seedling indexing for virus infection were indexed under quarantine on budded trees of navel orange on Troyer rootstock for periods of three to five years. Had this virus been present, it would have been detected by its effect on the Troyer rootstocks. Also, prior to release to nurserymen, most of the virus-free domestic selections had been propagated on Troyer citrange rootstock and observed for several years.

Citrus Ringspot

In 1944, some sweet orange seedlings inoculated from a field lemon tree in California displayed flecking, typical of psorosis A, on young leaves, but also showed some yellowish spots that later developed into small rings which persisted. The old lemon tree in the field had a small lesion on one limb that resembled a psorosis bark lesion. From this source of inoculum, ringspot symptoms were produced on several kinds of citrus. This was originally believed to be an unusual strain of psorosis virus that produced ringspot leaf symptoms and small bark lesions on

lemon. Neither of these symptoms is caused by ordinary strains of psorosis A. Ringspot symptoms were subsequently encountered in other inoculations from field trees, sometimes with leaf symptoms of psorosis, but in two instances without these symptoms. One of the latter two sources was tested against psorosis, and it was found that it did not protect sweet orange against psorosis-A lesion inoculum. Efforts were then made to identify the virus that caused the ringspot symptoms. It now seems advisable to describe these symptoms for the benefit of investigators of citrus virus diseases.

SYMPTOMATOLOGY AND HOST RANGE.—After inoculation with tissue-grafts, the tender new leaves of test plants show faint chlorotic spots. Small portions of the veins may also be cleared or chlorotic. The spots and chlorotic parts of the veins on the upper surface of the leaves later become yellowish, and green islands appear in many of the spots so that small rings are formed. Occasionally, small necrotic areas develop in the spots and rings. As the leaves mature, the yellowish spots and rings become very conspicuous, and many of them coalesce to make larger blotches. At times, the early vein symptoms resemble the vein-clearing of tristeza, but the symptoms tend to spread into adjacent tissues giving a feathery, banded appearance. Sweet orange leaves occasionally display large patterns resembling mature-leaf symptoms (2) of psorosis A. The spots, rings, leaf blotching, and vein symptoms also appear on the under surface of leaves, but are without much yellow color. The leaf symptoms described are illustrated in Figure 3.

As symptoms begin to appear on inoculated seedlings of certain citrus varieties, a shock effect may occur that results in leaf drop and necrosis of soft stems, or a lesion may appear farther down the stem. Occasionally, the lesion encircles the stem, causing death of the parts above.

Extensive studies of the host range of CRV have not yet been made, but very strong symptoms have been observed on Eureka lemon, Rough lemon, Szinkom mandarin, sour orange, sweet orange, Mexican lime, citremon, and *C. excelsa*. Symptoms have developed also on *Severinia buxifolia* and *P. trifoliata*.

RELATION OF CRV TO OTHER CITRUS VIRUSES.—Citrus ringspot virus has been found in trees infected with psorosis-A virus, but it has also been obtained free of psorosis A. Sweet orange plants experimentally infected with ringspot virus were not protected when later inoculated with psorosis-A lesion-bark inoculum. This suggests that the two viruses are not closely related. On the other hand, sweet orange seedlings previously infected with psorosis A from non-lesion inoculum or with blind-

pocket virus developed no ringspot symptoms when later inoculated with ringspot virus. In the same experiment, sweet orange seedlings previously infected with one isolate of concave-gum virus developed severe ringspot when inoculated with ringspot virus. These plants were indistinguishable from healthy control plants inoculated with CRV. Further studies are needed before conclusions can be reached regarding the relation of CRV to these other viruses.

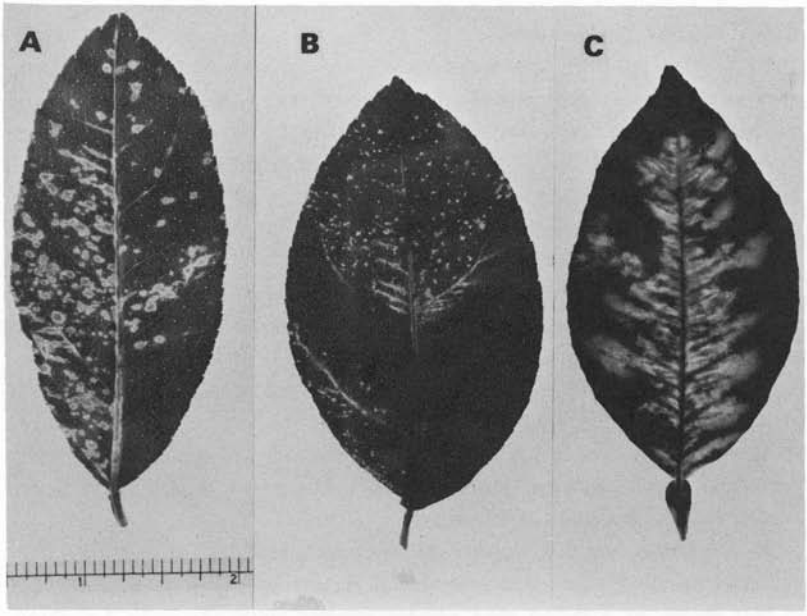

FIGURE 3. *Leaf symptoms of citrus ringspot. A. Bright spots, rings, and vein symptoms on Eureka lemon. B. Spotting and feathered veins on lemon. C. Symptoms on sweet orange similar to mature leaf symptoms of psorosis A.*

TRANSMISSION.—To date, CRV has been transmitted only by tissue grafts. No studies have been made on possible insect vectors. Considerable effort was made to transmit CRV mechanically to citrus and numerous herbaceous plant species, but without success. When symptoms appear in test plants graft-inoculated from orchard trees, they are usually mild and may not appear on all plants inoculated from a given source. However, by serial transfer, CRV can be established in a quite virulent form and is thereafter easily maintained in glasshouse trees. No field studies have been made.

IMPORTANCE.—At the present time, citrus ringspot seems to be of no

economic importance, at least it has not been associated with any recognized disorders of citrus, and recognizable symptoms have never been observed on orchard trees in California. Little is known of its distribution, but the writers have observed symptoms on citrus leaves collected in Central America and Florida. In 1966, strong CRV symptoms were seen on leaves from an orchard tree in Sicily, and mild symptoms were seen on two trees in Spain.

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