L. R. FRASER

Lemon Crinkly Leaf Virus

A DISEASE PRINCIPALLY OF LEMONS was described by Fawcett (1) and later with more detail by Fawcett and Bitancourt (2) under the name of crinkly leaf psorosis. The disease was transmissible by budding and bark grafting and was associated nearly always with psorosis A, although Fawcett and Bitancourt noted that not all strains of psorosis A produced crinkly leaf in lemons. Although including the disease with psorosis, they pointed out three possible explanations of the relationship of crinkly leaf to psorosis: (a) that it is a manifestation in lemon of the presence of a strain of psorosis A; (b) that it is caused by a mixture of the psorosis A virus and some other virus; and (c) that it is due to a distinct variety of psorosis virus. In view of this uncertainty, no name was applied to the causal virus. Wallace (5) referred to the disease as being caused by a strain of psorosis virus.

Presence of Crinkly Leaf in New South Wales

Symptoms of crinkly leaf were seen in New South Wales for the first time in 1954 on 16-year-old trees of Belair, Genoa, and Villa Franca lemon at the Citrus Experiment Station, Narara, and on 48-year-old Eureka lemon trees in a commercial orchard at Gosford. A single 16-year-old tree of Lisbon lemon was recorded as affected in the orchard block at the Citrus Experiment Station, Narara, though 22 trees of the same age and budline were without symptoms. Subsequently 2 blocks of Eureka lemons younger in age were located at Somersby and Hawkesbury River, in which all trees were affected, evidently as the result of the

use of infected budwood, and a few very aged affected trees of Eureka lemon were found in an orchard at West Pennant Hills.

In 1952 a number of propagations of lemon varieties had been made at Narara and these included Belair, Genoa, and Villa Franca lemons which were subsequently found to be infected, as well as other uninfected strains. It was observed in 1954 that all progeny of crinkly-leaf-affected parents showed in the nursery strong symptom development both on the lemon scion and also on the leaves of suckers of sweet orange stocks developing below some of them. It was also noticed that a yearling tree of a Lisbon selection growing in a row adjacent to the infected nursery trees showed symptoms of crinkly leaf although its parent tree and other nursery trees budded from it, showed no such development.

Since that time, surveys have been made of lemon plantings in all parts of the state. No further evidence of the disease has been found, nor any further suggestion of the spread of the disease from known infected blocks to trees in their neighbourhood.

Symptoms of Crinkly Leaf

The symptoms seen in the New South Wales material resemble the symptoms of warping and pocketing due to irregularities of growth in different parts of the leaf blade described and figured by Fawcett (1), Klotz and Fawcett (4), and Fawcett and Bitancourt (2) (Fig. 1).

As noted by Fawcett and Bitancourt (2), the intensity and distribution of symptoms were found to be variable. Some trees showed symptoms on one or two branches only, and in others most branches bore noticeably affected leaves. Seasonal variations also occurred. Fruit symptoms as described by Fawcett and Bitancourt were rather uncommon in New South Wales.

The presence of the virus seemed to have a depressing effect on vigour of old trees. Severely affected trees of Genoa lemon at Narara Citrus Experiment Station had a rather upright, restricted type of growth as compared with healthy trees. Young vigorous trees, however, grew and cropped well without noticeable reduction of vigour or yield.

Symptom Development in Seedlings

During 1956, 1957, and 1958 a series of inoculations by budding was made from selected crinkly-leaf-affected trees to seedling stocks of a number of citrus varieties growing under insect-free conditions in a glass-

FIGURE 1. *Leaves of Genoa lemon affected with lemon crinkly leaf virus.*

house. The method of inoculation used for young seedlings has been previously described (3) and proved satisfactory, 100 per cent successful takes being usual. Ten seedlings of each variety or species to be tested were inoculated in each series.

EUREKA LEMON. Small spots were produced on the immature leaves of new growth made following inoculation. The first indication was usually seen 21 days from budding on the second or third new leaf to be formed. The spots were circular clear areas 2 to 4 times larger than the oil glands and were distributed without relation to veins (Fig. 2). Their margins were usually fairly sharply defined and their numbers often so numerous that the leaves were noticeably paler in colour than unaffected leaves. The numbers of such spots were greatest on the growth made immediately following inoculation and decreased in later growth. As the spotted leaves grew and reached maturity, they developed a crinkling similar to that seen in the field.

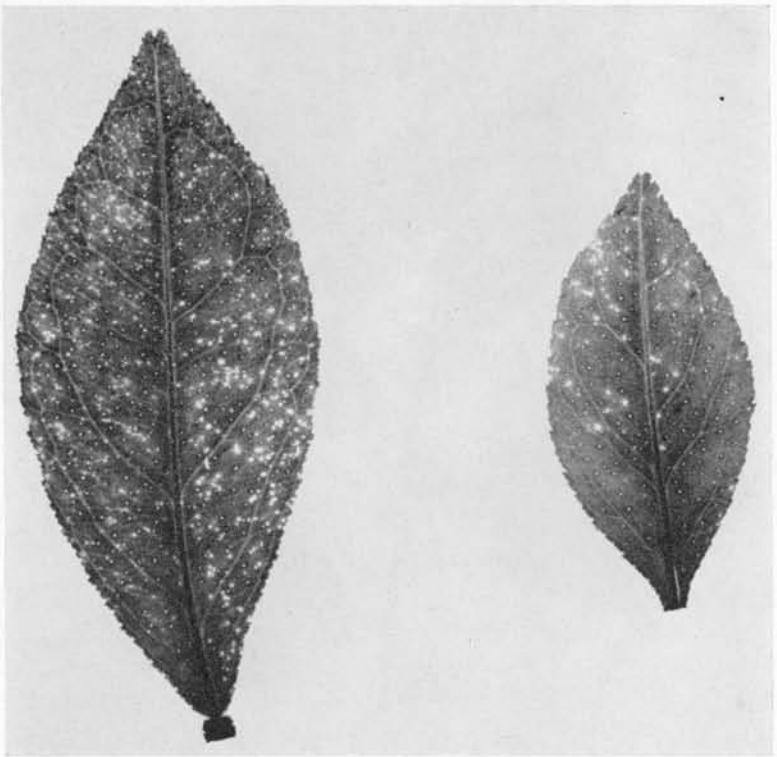

FIGURE 2. *Leaves of a seedling Eureka lemon which was inoculated with lemon crinkly leaf virus, showing development of circular clear spots.*

LISBON LEMON. The seedlings were somewhat more sensitive than those of Eureka lemon and the spots produced on early formed leaves were in some cases so numerous that they became confluent, and some distortion of the young leaf resulted.

MEYER LEMON. The spots were few or none, poorly defined, and no or very slight crinkling resulted.

PEAR-SHAPED AND KNIGHT CITRONS. The spots were few and not well defined. In a few cases very slight crinkling occurred in one or two leaves.

SWEET ORANGE. Spots were very few, but well defined, crinkling was slight.

Seedlings of sweet lime, sour orange, rough lemon, *Poncirus trifoliata,* Emperor and Cleopatra mandarin, and Orlando tangelo showed no symptoms.

No leaf pattern of the psorosis type was produced by any of the inoculated seedlings.

During November and December, 1958, buds from a crinkly-leaf-infected tree and from a psorosis-A-infected source were used in a parallel series of inoculations. In seedlings inoculated with psorosis, the characteristic oak-leaf pattern and flecking developed on new growth within 28 days. It was most marked on sweet orange and mandarin, but recognizable, though fleeting, on lemon varieties, citron, and sweet lime. In seedlings inoculated with crinkly leaf, clear circular spots and some leaf crinkling developed, as described above.

In a second series, in which crinkly leaf and psorosis were introduced simultaneously into each test plant, psorosis line pattern and crinkly leaf spotting developed concurrently in Eureka and Lisbon lemon seedlings. In sweet orange, the psorosis pattern was the dominant symptom, but a few scattered spots also occurred. In mandarin, which in earlier tests had given no reaction to crinkly leaf, the psorosis pattern was produced, but no trace of spotting or crinkling.

In a third series of inoculations, 20 seedlings each of Eureka lemon and sweet orange were infected with crinkly leaf by budding; and 6 weeks later, when spots were well developed, an inoculating bud carrying psorosis A was introduced into half the plants. At the same time, 10 seedlings each of healthy Eureka lemon and sweet orange were inoculated with psorosis A.

Psorosis leaf symptoms developed in the subsequent growth flush of all seedlings inoculated with psorosis A. No modification of symptom type or delay in development occurred where crinkly leaf had previously been introduced.

Strains of Crinkly Leaf Virus

During 1958, a series of inoculations to Eureka lemon and sweet orange seedlings was made using two sources of inoculum, one a tree of lemon (Belair) showing upright growth of poor vigour, and considerable crinkling of foliage on most branches (A), and the second a tree of lemon (Villa Franca), of moderate vigour, showing a few branches only with a low percentage of crinkled leaves (B). Symptoms produced on lemon seedlings following inoculation from budline A were more severe than

those produced by budline B, the intensity of spot development was much greater and the vigour of seedling growth was slightly depressed. Differences were also apparent, though less marked, on the sweet orange seedlings.

Discussion

No evidence can be adduced from the experiments carried out with crinkly leaf to justify its inclusion in the psorosis virus group; no evidence of interaction between the viruses of psorosis and crinkly leaf was seen. Crinkly leaf is therefore believed to be a distinct disease caused by a separate virus. It is thought that the material used by Wallace (5) carried both crinkly leaf and psorosis A and that symptoms of both viruses were present. Psorosis is very uncommon in New South Wales, and the occurrence of crinkly leaf in the absence of psorosis is therefore not surprising.

Literature Cited

1. Fawcett, H. S. 1936. Citrus diseases and their control. McGraw-Hill Book Company, Inc., New York and London.
2. Fawcett, H. S., and A. A. Bitancourt. 1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33: 837-864.
3. Fraser, L. 1952. Seedling yellows, an unreported virus disease of citrus. Agr. Gaz. N. S. Wales 63: 125-131.
4. Klotz, L. J., and H. S. Fawcett. 1941. Color handbook of citrus diseases. Univ. Calif. Press, Berkeley and Los Angeles.
5. Wallace, J. M. 1957. Virus-strain interference in relation to symptoms of psorosis disease of citrus. Hilgardia 27: 223-246.