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### *Seedling Yellows in California*

**S**EEDLING YELLOWS DISEASE of citrus was first described in Australia in 1952 by Fraser (4), who demonstrated that the causal virus is transmitted by *Toxoptera citricidus* (Kirk.), the most efficient vector of tristeza virus known. Fraser determined experimentally that West Indian lime seedlings infected with seedling yellows develop vein clearing and stem pitting, the symptoms generally accepted as those of tristeza (1, 8, 11, 14). Fraser was able to separate from seedling yellows infections a virus that caused this "tristeza-like symptom" on lime but not seedling yellows on lemon. These first studies led to the assumption that the seedling yellows reaction of Eureka lemon is caused by a virus distinct from but always accompanied by tristeza virus. Furthermore, it was demonstrated that in naturally-infected field trees of lemon, grapefruit, and sour orange in Australia, "tristeza-stem pitting virus" is present but not the "seedling yellows virus." Seedling yellows virus was obtained frequently from naturally-infected trees of sweet orange and mandarin, but it was always accompanied by tristeza virus, that is, a virus that caused vein clearing and stem pitting on lime seedlings.

In 1955, McClean and van der Plank (13), reporting on studies of seedling yellows and stem pitting in tristeza of citrus in South Africa, agreed in general with Fraser regarding symptomatology and host range, and also that plants infected with seedling yellows always carried the "stem pitting component." They concluded that tristeza is caused by a virus complex and that seedling yellows virus is a part of that complex.

In 1957, Wallace (15) reported that the seedling yellows reaction had been obtained by inoculating with virus from numerous trees of mis-

cellaneous varieties maintained in the orchards of the University of California but not with virus from trees of sweet orange in commercial plantings that had been naturally infected with tristeza virus. This means that the naturally occurring tristeza (quick decline) in sweet orange in California is not associated with the virus, virus strain, or virus complex that causes seedling yellows. From his preliminary studies, Wallace favored the opinion of McClean and van der Plank that the virus that causes seedling yellows is a part of the tristeza virus complex and not a distinct virus.

In 1959, Fraser (5) presented an interpretation of the nature of seedling yellows differing from her earlier one. Briefly, her conclusions were:

1. The virus that causes yellows symptoms on small seedlings of lemon, grapefruit, and sour orange also causes the symptoms originally described as tristeza, that is, decline and death of budded trees of certain scion-rootstock combinations.
2. Seedling yellows virus is always accompanied by, or in mixture with, a distinct, unrelated virus.
3. The accompanying virus, which also occurs in nature separate from seedling yellows virus, does not cause decline of budded trees but is the cause of the stem-pitting disease of grapefruit.

McClean (12) in 1960 emphasized the variable nature of the so-called "tristeza virus complex," and, to avoid confusion in describing symptoms or host reactions, made a distinction between tristeza, seedling yellows, and lime reaction. *Tristeza* was used in its original conception as a disease of citrus trees on sour orange rootstocks; *seedling yellows* referred to the dwarfing and yellowing of small seedlings of sour orange, lemon, grapefruit, and other citrus as originally described by Fraser; and *lime reaction* was used to describe the well-known vein clearing and wood pitting of sour lime. McClean (12) stated, "The virus responsible for the disease occurs naturally in some kinds of citrus trees but not in others, and is always accompanied by virus that induces symptoms of vein clearings and vascular pitting in limes, and tristeza disease in composite trees of sweet orange on sour orange roots."

### *Symptoms of Seedling Yellows*

The name "seedling yellows" was used first by Fraser (4) in Australia to describe the reaction of seedling plants of Eureka lemon, Marsh

grapefruit, and Seville (sour) orange inoculated by means of tissue grafts when in the 4 to 8 leaf stage. The symptoms were reported to be "very severe stunting which resulted in the cessation of growth after three to six leaves had been formed by axillary shoots. The leaves were reduced in size, yellow, at first marginally and finally the whole blade. There was no vein clearing or flecking, or mosaic pattern." It was stated that the affected seedlings remained in that phase for 15 months without signs of recovery and that the presence of seedling yellows virus in West Indian lime altered and intensified the symptoms caused by tristeza virus. Lime plants infected with seedling yellows virus not only developed such previously accepted symptoms of tristeza as vein clearing and cupping of leaves, but also became extremely chlorotic and ceased growth of new shoots. Also, after about 3 months the leaves of the lime plants displayed corking and splitting of the leaf veins. In a later paper (5), Fraser suggested the existence of strains of seedling yellows virus.

McClellan in South Africa (12) has found the seedling yellows symptoms and host range to be much the same as described by Fraser (4, 5), but he indicated that not all sources of the seedling yellows virus complex caused the extremely severe symptoms first described by Fraser. McClellan also stated that lime seedlings may react severely to some sources of seedling yellows virus and mildly to others.

In California, our studies have shown a range of symptoms, from mild to extremely severe, on Eureka lemon seedlings inoculated with the seedling yellows virus complex from various sources. Also, as observed by McClellan (12), the reaction of lime seedlings did not always indicate that an inoculum source carried the seedling yellows component of the virus complex. Furthermore, our studies clearly showed that lemon and sour orange seedlings that develop severe yellows symptoms after inoculation with seedling yellows virus from some sources will later recover by developing symptomless basal shoots which grow quite normally.

### *Investigations of Seedling Yellows in California*

Because of space limitations of papers published in these Proceedings, it is not possible to report in detail the results of studies that have been made on seedling yellows in California during the past 3 years. An attempt is made to describe briefly some of the experimental results and to emphasize areas of agreement and disagreement between our results and those of other workers.

#### PROCEEDINGS of the IOCV

SOURCES OF SEEDLING YELLOWS VIRUS IN CALIFORNIA.—In addition to sources reported (15), all Meyer lemon trees which gave a positive lime reaction that have been further indexed on Eureka lemon seedlings have been found to carry seedling yellows virus.

STRAINS OF SEEDLING YELLOWS VIRUS IN MEYER LEMON.—Different Meyer lemon trees have yielded strains of virus that differ in virulence and some trees carry 2 or more strains. From 2 Mexican lime seedlings inoculated from the same Meyer lemon and grown for 4 weeks in a heat chamber at 38° to 40°C, 4 different strains of seedling yellows virus were obtained (3).

VIRUS SEPARATION STUDIES.—Virus strains or components that cause the lime reaction but not yellows are readily separated from a seedling yellows complex. As reported by McClean (12), sour orange seedlings that have shown seedling yellows symptoms in early stages of infection commonly yield, at a later time, virus that causes only the lime reaction. Also, the writers have found that the seedling yellows component commonly disappears from experimentally-infected Eureka lemon seedlings. The nonpersistence of the yellows component is associated with, and probably responsible for, "recovery" of infected sour orange and lemon seedlings, that is, renewal of normal or almost normal growth on plants that once showed good symptoms of yellows.

EXPERIMENTAL INFECTION OF 10-YEAR-OLD LEMON TREES.—Field trees of lemon (10 years old) graft-inoculated with a very severe strain of seedling yellows virus and pruned back when inoculated developed good symptoms of seedling yellows. Yellowing and stunting were still noticeable 2½ years after inoculation. From such trees seedling yellows virus was recoverable at 12 and 18 months after inoculation. Symptoms did not develop on a tree that was not pruned back, but seedling yellows virus was obtained from this tree when tested 24 months after inoculation. Similar trees inoculated with a somewhat milder strain of seedling yellows virus showed only slight indications of symptoms on the new growth that followed the pruning, but tests showed that seedling yellows virus had persisted for at least 18 months.

Regardless of the presence or absence of top symptoms, the inoculated field lemon trees produced heavier than normal crops of fruit the second season after inoculation. This suggested a tristeza-like, bud union girdling effect even though the trees were on rough lemon rootstock. On inoculated trees the bark above the union was two times as thick as below, and the inner face of the scion bark was darker than normal.

On inoculated trees there were also varying degrees of "honeycomb" pitting.

COMPARISON OF SEEDLING YELLOWS AND TRISTEZA VIRUS STRAINS ON SWEET ON SOUR BUDLING TREES.—Nine-months-old budlings of Valencia on sour orange were graft-inoculated with three "strains" of seedling yellows virus, here identified as SY 1, SY 2, and SY 3. Strain SY 1 originated from a tree of African lemon (also listed as Spanish lemon) propagated from budwood imported from South Africa in 1913. This strain is extremely severe on lemon, grapefruit, and sour orange seedlings. Strain SY 2 was obtained from a Meyer lemon tree growing at Orland, California. On the basis of its effects on indicator seedlings, it has been classified as moderately severe. Strain SY 3 was found in a tree of Kawano Wase Satsuma grown from an importation from Japan prior to 1924. Under greenhouse conditions, strain SY 1 caused severe chlorosis, defoliation, stunting, and early flowering and fruiting. Strain SY 2 and SY 3 caused similar but slightly less severe effects. Three strains of tristeza virus from naturally-infected field trees, selected on the basis of their effects on Mexican lime seedlings, caused much milder reactions on sweet on sour budlings than did SY 1, SY 2, and SY 3, except that 4 of the 8 test trees inoculated with one of the tristeza strains developed symptoms as severe as those obtained with mild strains of seedling yellows virus.

Standard nursery trees of Valencia on sour and navel on sour inoculated in the field with seedling yellows virus from different sources developed symptoms somewhat faster than similar trees inoculated with field sources of tristeza virus. However, there was no significant difference in the ultimate effects on the trees of the two groups. All infected trees eventually reached a rather advanced stage of disease, but very few of them collapsed and died.

CROSS PROTECTION STUDIES.—Eureka lemon seedlings about 15 inches high were graft-inoculated with one strain of tristeza virus. Three months later, groups of these were challenged with 5 different strains of seedling yellows virus. Healthy lemons were inoculated with each strain of seedling yellows virus at the same time to serve as controls. In general, the tristeza-infected lemons developed yellows symptoms as severe as the controls and there was no clear-cut evidence of protection.

Different results were obtained, however, when lemons that had recovered from seedling yellows were reinoculated. Recovered lemon seedling plants were increased by cuttings and by budding to sweet

orange and rough lemon seedlings. Index inoculations with buds and twig grafts from the selected recovered lemons to lime and lemon seedlings showed that the yellows component had not persisted but that the plants carried virus that caused the lime reaction. When the clonal progeny of the recovered lemon seedlings were reinoculated with the same seedling yellows virus strain originally used on them and with two other strains, there was good protection. The virus that remained in recovered lemon seedlings after the severe yellows component had disappeared sometimes caused a slight reduction in growth. Whether this virus is or is not tristeza virus has not been established. The fact that in recovered lemon plants it protects against a challenging inoculation with seedling yellows while, in our limited tests, naturally-occurring tristeza virus did not afford this protection to lemons suggests that the virus retained in lemon plants after the yellows component disappeared may differ from tristeza virus.

TRANSMISSION OF SEEDLING YELLOWS VIRUS BY *APHIS GOSSYPHII*.— Because of the inefficiency of *Aphis gossypii* as a vector of tristeza virus in California, the writers have not conducted transmission experiments with this aphid species. However, in limited tests, Dr. R. A. Flock (personal communication) succeeded in infecting lime seedlings with seedling yellows virus by means of *Aphis gossypii* which first fed on Meyer lemon. The writers were supplied with 2 strains of seedling yellows virus obtained by Dr. Flock and have used these in their experimental study.

### *Discussion and Conclusions*

In general, results of studies in California are in agreement with those reported from South Africa by McClean (12), and there are no wide differences between our interpretation and that of McClean as to the relationship of seedling yellows to tristeza. Also, our results are similar to those described by Fraser (4, 5) in Australia, although there are some differences in interpretation. In her latest paper, Fraser (5) concludes that seedling yellows virus is the cause of tristeza, that is, the decline of susceptible budded trees, and that the other virus that always accompanies seedling yellows virus and which causes the lime reaction is the stem-pitting virus. The basis for this conclusion according to Fraser is that the virus found in naturally-infected trees of lemon, sour orange, and grapefruit did not, in her experiments, cause decline of

oudded trees although it caused vein clearing and stem pitting on lime seedlings.

If Fraser's observations and interpretations are correct, there must be wide differences in the natural occurrence of the virus mixtures or complexes in Australia as compared to California. One established difference is that the seedling yellows complex is not spreading naturally in California in contrast to a seemingly general spread in Australia where seedling yellows is present in nearly all field trees of sweet orange and mandarin. The nonpersistence of the seedling yellows component in infected lemon, sour orange, and grapefruit seedlings may, as Fraser suggests, explain why seedling yellows has not been found in naturally-infected field trees of these species in Australia. According to Fraser, the virus obtained from trees of these three species causes the lime reaction but not yellows, and it does not cause tristeza on budded indicator trees.

In contrast to these findings of Fraser, McClean in South Africa reports that tristeza in sweet orange on sour orange stocks is induced "by the virus components present in many grapefruit and by ones which do not give a normal seedling-yellows reaction." In California, trees of grapefruit on sour orange stock experimentally infected by graft-inoculation with field sources of tristeza virus developed good symptoms of tristeza, but no naturally-infected grapefruit trees have been found. Likewise, limited tests have failed to disclose any naturally-infected lemon trees. The absence of an efficient insect vector in California probably explains the lack of infection of lemons and grapefruit with any of the components or mixtures of the tristeza-seedling yellows virus complex. Whatever the explanation, it can be stated without contradiction that tristeza (decline of budded sweet on sour orange stock) in California has resulted, perhaps exclusively, from virus that does not cause seedling yellows. Thus we cannot agree with Fraser that only the seedling yellows virus causes tristeza.

It is true that budded indicator trees inoculated with seedling yellows virus in California develop tristeza and that under some conditions the effects are more severe than on trees infected with field sources of tristeza virus which do not carry the yellows component. But yellows-free inoculum has been responsible for the loss of commercial trees from tristeza in California.

The work of Grant and Higgins (7) and Grant (6) suggests the probability that most field sources of tristeza virus are comprised of more than one strain. Using the reaction of indicator seedlings of lemon,



sour orange, grapefruit, and lime, the writers have demonstrated that there are numerous so-called strains of seedling yellows virus. Furthermore, Desjardins *et al.* (3) have shown that a single source of seedling yellows yielded several seedling yellows virus strains or cultures. Experimental studies indicate that all sources of seedling yellows contain virus strain mixtures or virus complexes from which a virus can be separated that will cause the lime reaction but not yellows.

In consideration of Fraser's premise that seedling yellows is caused by a virus that is distinct from and unrelated to the "lime-reaction virus" (stem-pitting virus) which is always present in a seedling yellows infection, it seems that such a claim cannot be made on the basis of present evidence. It would seem somewhat unlikely that two unrelated viruses would always occur in mixture, that is, that the seedling yellows virus would never be found in the absence of the other virus and that 2 unrelated viruses would always be simultaneously transmitted by 2 species of aphids.

On the other hand, the results of preliminary cross-protection studies raise some questions regarding the nature of the components of the seedling yellows complex. Naturally-occurring California tristeza virus experimentally introduced into lemon seedlings did not protect against seedling yellows. However, lemons that had recovered from seedling yellows and containing virus capable of inducing the lime reaction but not that capable of causing seedling yellows showed a high degree of protection when inoculated with seedling yellows virus strains that caused severe symptoms on virus-free lemons.

Thus, it seems that there are two important facts to establish. The first of these is to determine whether or not the virus that persists in lemon, sour orange, and grapefruit after the seedling yellows component disappears will cause tristeza on budded indicator trees. Experiments are now in progress for this purpose. The second is to determine whether or not the virus that persists in lemons after recovery from seedling yellows will, when inoculated into healthy lemon seedlings, protect them from a challenge inoculation with seedling yellows virus.

If such protection is not afforded and if the virus that persists in the lemons causes tristeza when transmitted to budded trees, we can conclude that the persisting virus is the same as the naturally-occurring field tristeza virus. On the other hand, if this virus provides protection in plants that develop no symptoms and thus go through no recovery reactions, we can conclude that it differs from field strains of tristeza virus.

Finally, the nonpersistence of the yellows inducing virus component in hosts that are very susceptible during early stages of infection is indeed a strange reaction if the yellows symptoms are caused by certain strains or combinations of strains of tristeza virus. An explanation of nonpersistence of the yellowing component in the mixed infections might demonstrate the nature of seedling yellows and the relationship of the virus components present in seedling yellows inoculum. It is the opinion of the writers that presently available information does not clearly define the role of the different virus components involved in the tristeza-seedling yellows-stem pitting diseases of citrus.

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