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Author

Fraser, Lilian

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THE RELATION OF SEEDLING YELLOWINGS TO TRISTEZA

Lilian R. Fraser¹

New South Wales Department of Agriculture,
Sydney, New South Wales

INTRODUCTION

In 1952 I described a virus reaction on seedling citrus varieties as "yellows," attributing it to an undescribed virus (3). This was based on two assumptions. In the first place I accepted the hypothesis then current that tristeza disease and the stem-pitting disease of grapefruit were different effects of the one virus and that the symptoms of vein flecking or clearing and xylem pitting of inoculated West Indian lime seedlings were seedling reactions to tristeza infection. The second assumption was that a type of decline which affects grapefruit on sour orange stock was tristeza disease, differing only in symptom intensity from the disease affecting oranges on this stock. In surveys of citrus in New South Wales I had been unable to obtain yellows from grapefruit trees, however severely they may have been affected with stem pitting. Moreover, on a basis of symptom type, yellows appeared quite different from the vein flecking. It was therefore concluded that seedling yellows could not be a tristeza symptom. As a result of further work, it is now considered that the original view was incorrect and that both basic assumptions are untenable.

Yellowing of seedlings of various citrus varieties in Brazil was attributed by Costa, Grant, and Moreira (2) to the tristeza virus. McClean and van der Plank (6), also, have shown that yellows is a symptom produced by the tristeza virus. The last two authors concluded that tristeza is caused by a virus complex consisting of a stem-pitting component, which alone is capable of causing stem pitting in grapefruit, and a yellows component which is never found separately but always in mixture with the stem-pitting component. In this connection they stated (6): "Nothing seems to have been published to allow it to be decided whether the two components are strains of a single virus or whether they are distinct viruses or even distinct virus complexes."

Knorr and Price (4) have advanced the view that stem pitting and tristeza are strains of a single virus and that sweet orange sorts out one component and grapefruit the other. Other investigators, in accepting the vein-flecking and pitting reaction of West Indian lime seedlings as a symptom of tristeza, are committed to the view that stem pitting and tristeza are caused by the one virus. Among these, Wallace (8) favors the view that the virus which causes seedling yellows is part of the tristeza complex and not a distinct virus.

Both tristeza disease and stem-pitting disease occur in New South Wales. Tristeza is not economically important, because the susceptible sour orange stock has been used in only a limited area in the southwestern part of the state, and from the beginning has been a complete failure. Records of nurserymen since 1870 and the experience of the New South Wales Department of Agriculture over the past thirty-five years have been to the effect that orange trees worked on this stock soon die. From the descriptions of

¹ Plant Pathologist, Biological Branch, New South Wales Department of Agriculture, Sydney, N.S.W., Australia.

tree appearance and behavior it seems clear that the reason for this must have been the presence of the tristeza virus. Its most efficient vector, the tropical citrus aphid, *Toxoptera citricidus* (Kirk.), is also present. The virus and its vector were probably introduced from South Africa in the early days of the New South Wales colony. Grapefruit stem-pitting disease was first observed in the latter country in 1937 (7).

In attempting to assess the relationship of yellows to tristeza it is necessary, first, to define the limits of the field diseases of tristeza and stem pitting, and secondly to try to relate, as accurately as information permits, the seedling symptom to the various phases of field disease. To do this, one must rely on symptom development in citrus seedlings, and symptoms are likely to vary with the stage of growth and the vigor of the indicator used, the season, temperature, light intensity and duration. Any of these can delay the onset or modify the type of reaction. Added to this is the disadvantage of having to use seedlings, which must be chosen for uniformity on a visual basis. There is the risk in this sort of analysis of equating a field disease with the wrong seedling reaction.

Although tristeza is of no practical importance in New South Wales because the stocks in general use are not affected by it, stem-pitting disease in grapefruit constitutes a considerable threat. Surveys have therefore been made to determine the extent to which this disease is present in commercial varieties in all citrus areas in the state.

In survey and experimental work, speed and uniformity of reaction are important. For this reason I work with the youngest seedlings it is possible to handle (3- to 4-leaf stage), of optimum vigor and rigorously culled for uniformity. Inoculations are made in the late spring or early summer, because in New South Wales later inoculation leads to slower and somewhat variable reaction and modification of symptoms in some cases. In spite of very careful selection for uniformity, some variation in reaction has been traced to nonuniformity of seedlings. West Indian limes and some varieties of sour orange seedlings have been relatively uniform, but nonuniformity is a feature of lemon, citron, and grapefruit seedlings, and these had to be abandoned for survey purposes. All work has been carried out in insect-proof greenhouses, because the prevalence of *Toxoptera citricidus* makes outdoor work unreliable.

The results of this field survey and experimental work can, in my opinion, be most logically explained by the hypothesis that stem-pitting disease and tristeza disease are caused by unrelated viruses. The evidence for this hypothesis, as presented in the following sections, is (a) that the West Indian or Key lime seedling reaction of vein flecks and pitted xylem is an indicator symptom of the virus causing stem-pitting of grapefruit; (b) that the symptom described under the name of yellows is the reaction by seedlings of hypersensitive species to infection by the virus causing tristeza disease; and (c) that the rapid deterioration of sweet orange on sour orange rootstock, which was originally described as the tristeza disease, cannot be brought about by infection with the virus of stem pitting alone.

WEST INDIAN LIME SEEDLING REACTION AS AN INDICATOR OF STEM-PITTING DISEASE

Stem-pitting disease of grapefruit, in its most severe form, causes the development of deep furrows and contortions in trunks and main branches, longitudinal pits or furrows in the xylem surface of year-old branches, small and distorted fruit, poor bushy growth, and decreased vigor. Histologically, there is a failure in the area of the pit for xylem to form at the normal rate, some gum production, and some failure of xylem to lignify, with consequent crushing and distortion of the cambium surface. The phloem elements are also somewhat contorted, forming wedges above the abnormal xylem, with occasional lignified elements.

West Indian lime indicator seedlings budded from such grapefruit show, in growth made subsequent to inoculation, vein flecking, vein clearing, and sometimes leaf cup-

ping. Usually, after a season's growth, pits or furrows can be seen in the xylem surface. Histologically, these pits are similar to those of twigs of diseased grapefruit, but the effect is somewhat more severe.

In New South Wales, stem-pitting disease appears to exist in a multiplicity of strains ranging in degree of pitting, growth retardation, and fruit distortion from very severe to extremely mild. At the extreme end of the range are vigorous trees without even the mildest pitting or fruit effect. When inoculated to West Indian lime seedlings, all symptom types produce vein flecking, vein clearing, and xylem pitting in various degrees. Inoculations from symptomless grapefruit quite consistently produce the mildest and most transitory symptoms on indicators—a few vein flecks in the leaves formed immediately after inoculation, with few or none on subsequent leaves and with only an occasional mild pit after one or more season's growth. Isolates from various sources show an almost continuous gradation in symptom severity from this, to that produced by the severest strain. The most severe strains cause more or less total vein clearing, leaf cupping, severe overlapping or grouped pits, and, ultimately, cracking of the bark of the stem, corking, and splitting of veins in the indicator seedlings. The effect on vigor of growth of the indicator seedling varies in the same way. The mildest strains restrict growth not at all; the most severe strains restrict it seriously.

Like seedlings of West Indian lime, those of sweet lime, citron, Yuzu, and *Citrus acida* show vein flecking and clearing and xylem-furrowing symptoms following inoculation. Seedlings of Marsh grapefruit are moderately sensitive, and some slight vein flecking and xylem furrowing are usually produced in these and also in sour orange seedlings, the intensity of the symptoms depending on the virus strain used for inoculation. Seedlings of most sweet orange and mandarin varieties, Rough lemon, and Rangpur lime are insensitive and rarely show symptoms.

Histologically, the symptoms are similar in all citrus varieties, differing in degree according to the severity of the strain of virus and the susceptibility of the citrus seedling species.

It is considered that these symptoms on seedling indicators are evidence of the presence of the virus which causes stem pitting of grapefruit. On the basis of these symptoms, surveys of citrus have shown that the virus is present in all cultivated citrus trees of every variety in coastal New South Wales, with the exception of *Poncirus trifoliata* and some of its hybrids. Some virus-free trees have been located in western New South Wales. On field-grown trees, however, symptoms of stem pitting are shown only by some grapefruit varieties and occasionally by citron and smooth Seville orange when very severe strains are present.

It is considered that the differences in severity of stem-pitting disease in the field, and of symptom production in indicator seedlings, are due to the existence of numbers of strains. This conclusion is based largely on the histological similarity of the symptoms, but some work has also been done which indicates that blocking or cross protection occurs between strains. Details of this work will be published elsewhere.

SEEDLING YELLOWS, A REACTION TO THE TRISTEZA VIRUS

When the sources of inoculum are Eureka and Lisbon lemon, sour orange varieties, grapefruit, citron, shaddock, and pomelo, only the symptoms of vein flecking and xylem pitting are produced in indicator seedlings. When, however, inoculum is taken from sweet orange varieties, mandarin varieties, Rough lemon, or Rangpur lime, the yellows symptoms are shown by certain seedling indicator varieties. The leaves of growth made subsequent to inoculation are small and yellowed, the yellowing generally being marginal at first and then involving the whole leaf. Growth ceases after the first few leaves are formed, though a certain amount of restricted growth may occur at a later stage.

The citrus species and varieties which show the yellows effect when inoculated as seedlings are sour orange, lemon, grapefruit, citron, shaddock, and pomelo. It will be noted that these are the species or varieties which, in the field, have been found infected only with the virus which causes vein flecking and clearing. They are also the varieties which, when used as stocks for sweet orange, are susceptible to the tristeza disease.

The yellows reaction is also shown by West Indian lime seedlings in combination with the symptoms of stem pitting. In other yellows-susceptible seedling varieties, especially grapefruit and sour orange, vein flecking and xylem pitting may occur in conjunction with yellowing, the intensity varying from one isolate to another, presumably with the strain of stem pitting present. Those species and varieties which carry this yellows virus in the field, i.e., varieties of orange and mandarin, Rough lemon, and Rangpur lime, show no reaction as seedlings when inoculated with yellows.

The yellows virus is readily transmitted by the tropical citrus aphid and appears to be very invasive. In New South Wales, except for a relatively few trees in the arid western parts, all mandarin, orange, and Rough lemon trees carry this yellows virus as well as the stem-pitting virus. So far it has not been found unaccompanied by stem-pitting virus.

Some variation in yellows severity on indicator seedlings may indicate the presence of strains. There is, however, no intergradation between the vein flecking—xylem furrowing symptoms of stem pitting and the yellows symptoms; inoculations from lemon, grapefruit, and sour orange produce the vein flecking—stem pitting; buds from orange, mandarin, Rough lemon, and Rangpur lime produce both.

PRODUCTION OF TRISTEZA SYMPTOMS IN INDICATOR SEEDLINGS

When very young indicators composed of seedling sweet orange or mandarin scions on sour orange stock are inoculated with buds from varieties containing the yellows virus plus stem-pitting virus, tristeza symptoms develop in six to eight weeks. When similar indicators are inoculated with buds containing the stem-pitting virus alone, i.e., buds from lemon, grapefruit, or sour orange, tristeza symptoms are not produced. If the stem-pitting strain used is the mildest type, little or no reduction of growth occurs. If a severe strain is used, the inoculated tree is reduced in vigor, but symptoms of yellowing and cessation of growth are absent. If these trees are then reinoculated from a source carrying the yellows virus, tristeza symptoms appear. No evidence of protection by stem pitting against yellows, or blocking of the one by the other, has been obtained.

This type of experiment has been repeated many times over the past six years. In no case has it proved possible to bring about tristeza where the yellows virus was absent. In one series, commenced four years ago, 33 selections of stem pitting varying from very mild to very severe were used as inoculants. Half of the trees worked with each selection were inoculated with yellows virus from the one source four months after the inoculation with stem pitting. These trees developed tristeza symptoms in the following spring, and declined severely. The trees inoculated with stem pitting, only, are still growing with moderate to good vigor, their condition depending on the strain of stem pitting used.

The yellows virus is able to invade trees of sour orange, grapefruit, lemon, citron, shaddock, or pomelo only when these are very young. It is impossible to infect mature trees in the field. A particular example of this behavior of mature trees has been examined on a number of occasions. In a fairly large number of orchards in coastal New South Wales, Marsh grapefruit trees on Rough lemon stock have been top-worked with mandarin or orange varieties. These have grown extremely well. Fourteen trees on which one or more unworked grapefruit limbs still remained have been sampled

for virus content. The grapefruit limbs contained only stem-pitting virus; the orange or mandarin limbs contained both stem pitting and yellows. This is in line also with Marloth's (5) observations that the sour orange suckers which sometimes develop after the death of the top of the tree from tristeza, are healthy and vigorous in appearance. One such case has come to my notice. Buds from a tree of Valencia orange on sour orange rootstock in a western New South Wales orchard, newly affected with tristeza, were collected in November, 1953, and yielded yellows and stem-pitting virus. The tree was not removed, and suckers developed from the rootstock after the death of the scion. These suckers, sampled a year later, yielded stem-pitting virus only.

CONCLUSIONS

The name tristeza was first given to the condition of collapse or more or less rapid decline of trees of sweet orange on sour orange rootstock. It has since been given a much wider meaning, however. It has been applied to the slow deterioration of grapefruit on sour orange and other stocks, and to a condition where no evidence of decline of citrus on sour orange stock is present (1), the criterion being the production of the vein-flecking reaction on West Indian lime seedlings.

The range of symptoms seen is easily understood if it is accepted that there are two viruses, tristeza and stem-pitting, and that the West Indian lime reaction is produced not by the tristeza but by the stem-pitting virus.

The tristeza virus is capable of infecting some species and varieties without causing symptoms. The tolerant types are orange, mandarin, Rough lemon, and Rangpur lime. Other varieties are hypersensitive and can be infected only as very young seedlings. As mature trees they show field immunity. Tolerant scions on tolerant stocks are not injured by the presence of the virus. Tolerant scions on hypersensitive stocks decline swiftly because of the effect of the virus transmitted from the scion to the stock. Hypersensitive scions on either tolerant or hypersensitive stocks are not affected, because no infection can occur.

The stem-pitting virus appears to be capable of infecting almost all citrus varieties, but most varieties behave as symptomless carriers. A few such as grapefruit, West Indian lime, and sweet lime are rather severely injured. Many strains occur. No hypersensitive varieties have been found.

The evidence in support of the thesis that stem pitting and tristeza are caused by distinct viruses is as follows:

1. Two distinct types of symptoms occur on inoculated seedling indicators, namely, yellows and vein flecking—xylem pitting.
2. There is a discontinuity of symptom type between the two diseases in the field.
3. Stem-pitting isolates unaccompanied by yellows (i.e., obtained from lemon, sour orange, grapefruit) do not cause the tristeza type of decline when inoculated to seedling combinations of sweet orange on sour orange roots. A slow deterioration of a different type is caused by the most severe strains of stem pitting in sensitive varieties.
4. The varieties and species which show extreme sensitivity to yellows as seedlings are those which are susceptible to tristeza when used as stocks for sweet orange or mandarin in the field.
5. The varieties and species which show strongest reactions of vein flecking and xylem pitting as seedlings show symptoms of stem-pitting disease in the field.
6. The viruses occur together in mandarins and oranges, without mutual interference or blocking.
7. The presence of stem pitting, mild or severe, is no protection against infection by tristeza, but a mild strain of stem pitting can exert a degree of blocking against a severe strain of stem pitting.

The question remains whether tristeza is caused by yellows virus only, or is a

synergistic effect of yellows and stem pitting together. The answer must wait until the separation of yellows from admixture with stem pitting has been effected. There are at present no reasonable grounds for assumption either way.

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