Variation in Symptom Expression of Exocortis and Gummy Pitting in Citrus Trees on *Poncirus trifoliata* Rootstock in New South Wales

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USAGE OF TRIFOLIATE ORANGE IN NEW SOUTH WALES (N.S.W.)

Trifoliate orange, previously grown to only a minor extent in N.S.W., came into prominence during the 1940's. In the 1930's and 1940's, root rot caused by *Phytophthora* spp. resulted in heavy losses of trees on rough lemon and sweet orange stocks during a succession of seasons of higher-than-average rainfall. This was particularly so in the irrigation areas of the Murrumbidgee and Murray Rivers where the situation was aggravated by faulty irrigation practices, lack of adequate drainage, and nonuniform soil types (Fraser, 1949). In the 1950's, a succession of wet seasons and flooding of rivers in the central coast area and at Narromine also resulted in destruction of thousands of trees.

By 1942, it was evident that a suitable root-rot-resistant stock must be found to reestablish the industry in areas hard hit by Phytophthora diseases and to permit expansion. Though sour (Seville) orange had been used traditionally with great success in overseas countries for the control of these diseases, it was a failure in N.S.W., as it also has been in South Africa since the 1870's. The reason was then not known, but was later recognized as tristeza virus.

In the search for stocks alternative to rough lemon and sweet orange, all available species were screened for resistance to Phytophthora. Trifoliate orange was the only species with required root-rot resistance, but it had a worldwide reputation as a dwarfing and unreliable stock. The first mention of its use in N.S.W. as a stock, by Crichton in 1893 (quoted by Bowman, 1955), referred to its dwarfing properties.

The great demand for trees on root-rot-resistant stocks made prominent the variable performance which had prevented general use of trifoliate orange in the past.

The Trifoliate Improvement Committee (later renamed the Citrus Improvement Committee) was set up by the N.S.W. Department of Agriculture in 1942 to solve this problem. Surveys were made of all known plantings of trees on trifoliate orange in N.S.W., to clarify and define the nature and extent of the variability.

VARIATION IN OLD BLOCKS OF TREES ON TRIFOLIATE ORANGE

The most striking feature was the range of tree sizes, from large — approaching that of trees of the same age on rough lemon stock — to very small; some trees were only a few meters high at ages of up to 40 years. Tree shape also varied; some dwarfed trees were flattened and spreading, others were rounded or upright.

Phenomena shown by the stock below the bud union also varied. In the most dwarfed trees, the butt was clothed with hard, persistent scales (exocortis). Some large trees also showed scaling. Other small trees showed intermittent or partial scaling of the stock, with or without pustular corky outgrowths. Associated with this latter type was gummy pitting of the wood originating at the cambial level. In other trees, the bark of the stock was smooth without external abnormalities but often with some gummy pitting of the wood (Fraser *et al.*, 1976). All these symptoms varied greatly in intensity from tree to tree. The budunion varied from smooth and flat to abruptly benched, with indentation and some periodic gumming at the cambial level. This indentation varied from nil or mild to deep. With age, the stock greatly outgrew the scion. The stock varied from slightly enlarged in diameter compared with the scion, straight sided, or more or less strongly fluted. Some dwarf trees showed no butt symptoms at ages up to 40 years, other than a reticulum of surface cracks on the shoulder below the budunion and a few small, gummy pits.

A few blocks of Valencia and Washington navel orange and Emperor mandarin showed no dwarfing; trees were of uniform size and their stocks were considerably enlarged and fluted without any of the abnormalities associated with dwarfed trees. These were later determined to be of nucellar origin and formed the basis of a bud certification scheme developed on indexed virus-free clones.

SOURCES OF TRIFOLIATE ORANGE

As a basis for field trials, a collection was made of trifoliate orange sources. Seed was obtained from many old seedling trees in N.S.W., from other Australian states, and from the U.S.A. Suckers from the stocks of trees showing satisfactory growth were propagated directly, as cuttings, for inclusion in the collection.

A selection of 148 of these is maintained in the arboretum at Somersby Horticultural Research Station. Variation between clones of trifoliate orange is not great in the material available in N.S.W., suggesting that original imports of this stock were few and of uniform type (Fraser *et al.*, 1961).

Among selections in the Somersby collection, minor variations occur in flower type, autumn leaf color, time of blossoming and leaf fall. Some remain leafy as seedlings over the winter during their first year, becoming fully winter-dormant only in the second or third season. One, a tetraploid, is slightly larger and coarser.

HORTICULTURAL TRIALS

The possibility that strains of trifoliate orange may perform differently as stocks, and that some could produce stunted trees because of incompatibility, was investigated. Seed-source trees for trial as stocks were selected in a variety of ways and included suckers from stocks of non-dwarfed Washington navel oranges, old trifoliate orange stock trees in plantations of satisfactory growth, a range of stocks imported from other states, and trees showing minor vegetative differences. The tetraploid was included in one series of trials. A total of 33 stock selections was used in trials with scions of non-dwarfing Washington navel and Valencia orange, Marsh grapefruit, and Ellendale tangor at Griffith, Yanco, and Somersby. Although slight differences in size and performance of trees of the same age on these various stocks developed, none of the trees was stunted. In all these trials, the seedlings used for stocks were selected in the nursery for uniformity before budding, to eliminate possible hybrid types.

In other trials, seedlings from single sources were separated into categories on size, deciduous habit and type of branching. When budded with orange varieties, no significant difference was shown between them and no butt abnormalities developed. In a trial at Yanco, which included a tetraploid, no significant differences were shown between the tetraploid and other seedling stocks.

TRANSMISSION OF EXOCORTIS

Trials were set out, first on growers' properties and later at horticultural research stations at Narara and Somersby on the central coast and Griffith and Yanco in the hotter, more arid Murrumbidgee irrigation areas, to

TABLE 1
DWARFING SOURCES, AND THEIR REACTIONS ON ETROG CITRON

Source	Symptoms in source tree	Etrog citron reaction
3531	Washington navel orange, dwarfed, few old scales, pustules, gummy pitting, discontinuous indented ring	+
3532	Washington navel orange, moderately dwarfed, benched at union, few scales	+
3533	Washington navel orange, moderately dwarfed, slight scaling, severe gummy pitting, discontinuous ring, benched	+
3534	Washington navel orange, non-scaling, moderate benching	+
3535	Washington navel orange, severely dwarfed	+
3536	Washington navel orange, dwarfed, small patches of pustules and scales	+
3537	Valencia orange, dwarfed, not scaling (off-type stock)	-
3538	Marsh grapefruit, intermediate size	-
3539*	Marsh grapefruit, dwarfed	+

* 3539 produced a positive reaction for xyloporosis in Orlando tangelo.

TABLE 2
EFFECT OF INOCULATION WITH BUDS FROM DWARFED TREES ON SIZE AND CROPPING OF BELLAMY NAVEL ORANGE ON TRIFOLIATE ORANGE ROOTSTOCK

Inoculation	Cumulative yield to July 1977 No./tree	Cumulative yield to July 1977 (kg)/tree	Fruit weight (g)	Tree size (m) Height	Tree size (m) Width
Bellamy nucellar navel orange	8753	1363	156	3.9	4.1
3531	5256	843	160	2.8	3.7
3532	5465	874	160	2.7	3.5
3533	6839	1038	152	3.4	4.0
3534	7270	1098	151	3.2	3.7
3535	4532	711	157	3.6	3.0
3536	5019	841	168	2.0	3.3
3537	7559	1210	160	3.9	3.6
3538	4598	685	149	2.5	3.1
3539	4587	695	152	2.6	2.8

determine whether a transmissible agent present in the scions of dwarfed trees was responsible for dwarfing and scaling.

The dwarfing agent associated with the scaly butt condition proved to be bud-transmissible in the symptomless scion (Benton *et al.*, 1950). Trees propagated from dwarfed, exocortis-affected sources reproduced the characters of the parent tree. Size differences between affected and healthy trees, and onset of scaling, started to show up at about 4 years.

The variation shown by trees in old commercial blocks on trifoliate orange which had been propagated from budwood from trees on rough lemon stock was reflected in the transmission trials, though in lesser degree.

Highest percentage transmissions of the pathogens causing scaling were obtained from old-line lemon varieties. Over 90 per cent of these showed strong exocortis symptoms at about 4 years. Transmission of exocortis, using Washington navel and Marsh grapefruit sources for inoculation of nucellar varieties, varied considerably and was generally less than 75-80 per cent.

TRANSMISSION OF EXOCORTIS AND DWARFING

Griffith Viticultural Research Station. In a trial at the Griffith Viticultural Nursery in 1947, trees of two bud sources from an orchard at Dooralong were compared: 1) a dwarfed tree with exocortis scaling; and 2) an apparently normal tree in the same orchard.

Scaling was recorded on half the trees budded with the exocortis budline at 4 years, and in a total of 67 per cent of the trees after 13 years. During these years, a portion of the original scaling trees showed periodic remission of symptoms and finally developed flaky scales and pustules. In most remaining trees, at 32 years of age, gummy pitting of the wood has developed; however, 15 per cent of these trees developed no external butt symptoms, though minor gummy pitting occurred and all were dwarfed to varying degrees.

Of the trees inoculated with budwood from the apparently healthy tree, one is showing a small area of flaky scales and pustules in 1979. In 1959, budwood of two dwarfing selections used in other trials was used to inoculate some of these trees. By 1979, not all of the inoculated control trees had reacted to the challenge inoculation. Thirty-three per cent of trees inoculated with one dwarfing budline now known to carry xyloporosis, and 60 per cent of trees inoculated with the other budline, which is not known to carry other viruses (except tristeza), have remained free of scaling.

Inarching of dwarfed and exocortis-affected trees at Dooralong. Trees in a commercial orchard, showing a full range of dwarfing and butt characters, and including non-dwarfed trees, were inarched in 1942 and 1943 about 30 cm above the bud union with selected seedlings of known parentage (two per tree). Inarches developed early symptoms of scaling at 4 to 6 years. Onset of symptoms varied between the two inarches on each tree, and between trees. The exocortis reaction has so far been the only symptom observed.

Dwarfing transmission trial — Somersby H.R.S. Bellamy nucellar navel oranges on trifoliate orange were inoculated as nursery trees with nine dwarfing budlines of Washington navel and Valencia orange and Marsh grapefruit (table 1). Four trees of each propagation were planted in 1955 at Somersby. Dwarfing was evident by 1960. The dwarfing budlines differed in tree size, shape, cropping, and vigor. To date, gummy pitting has not developed in any of these trees. One budline (3539) has been found to carry xyloporosis. Some inoculations have had little effect (e.g. 3537 — the parent tree — was found to be on an off-type stock); others (e.g. 3532, 3538, 3539) have markedly reduced tree size and total crop compared with uninoculated control trees. There has been little effect on fruit quality or fruit size. Records of cropping and tree characters are given in table 2.

Inoculum from the selections with the best crop/tree size ratios (3532, 3538, 3539) was used in replicated dwarfing trials planted at Yanco in 1961, 1963 and 1964.

THE ALLEN VALENCIA ORANGE

Variation in transmission from a single bud source is illustrated by the performance of direct propagations of Allen Valencia orange. Three selections, free of exocortis, were made from a plantation over 40 years old. Few trees in the block were affected with exocortis and some partially dwarfed trees were present. Plantings were made at Somersby, Yanco, and Dareton in 1951, 1953, 1955 and 1959. Progeny trees of each selection showed a range of sizes. Variations occurred between selections in the percentage of trees in the different size groups, in the numbers showing gummy pitting, and in the severity of the gummy pitting (table 3). Tree size and intensity of the gummy pitting symptom were not closely correlated. One tree in the Dareton planting developed a mild exocortis reaction.

WATERSHOOT BUDWOOD

A trial was initiated in 1954 to determine whether rapidly growing shoots could temporarily outgrow invasion by exocortis from the parent tree. Shamouti orange and Clementine mandarin trees on trifoliate orange, which showed typical severe exocortis, were cut back in spring to induce strong shoot growth and, before growth had ceased and the wood hardened, buds were taken from the base to the apex and propagated on trifoliate orange stock. Exocortis developed in the stocks of the Shamouti orange trees at 3 to 5 years from budding, and only 4 per cent remained free at the end of the trial after 7 years. The Clementine mandarin trees remained free of scaling and were transferred to a permanent block at Narara. No exocortis has developed, but differences in tree size started to show at 7-8 years of age. Half the trees are now showing mild dwarfing and mild gummy pitting has been detected in one tree, but none has shown bark scaling.

BEHAVIOR OF THOMPSON GRAPEFRUIT

Thompson grapefruit was introduced from California in 1938, as budsticks from a single-source tree, by Mr. R. G. Benton of the Division of Horticulture, N.S.W. Department of Agriculture. It was propagated on trifoliate orange, and trees were grown in several localities. A high proportion of the first propagations developed severe exocortis scaling and dwarfing, but some appeared normal. Subsequent propagations were made only of trees of good performance.

In a trial on a grower's property at Curlwaa (Lower Murray River, N.S.W.), second-generation progeny trees had separated into two size classes, large and vigorous and small and less vigorous, after 7 years. The size differences became more pronounced with age. No exocortis scaling had developed when the trees were removed after 32 years.

A trial at Somersby has shown that the Thompson grapefruit carries gummy pitting. Lambert nucellar Eureka lemon was propagated on trifoliate orange with Thompson grapefruit as an interstock. In this combination, gummy pitting symptoms developed in the trifoliate orange stock, indicating that gummy pitting had been introduced by the Thompson grapefruit.

INFLUENCE OF SCION VARIETY ON SYMPTOMS IN THE STOCK

Lemon scions induced earlier and more severe gummy pitting, when compared with grapefruit, on trifoliate orange stock. There is also evidence from an old trial of a nucellar lemon on trifoliate orange inoculated with several dwarfing selections and exocortis that the lemon scion had enhanced scaling and gummy pitting in the stock. In this trial, the exocortis inoculum caused scaling in all inoculated trees, but scaling was intermittent in the early years, appearing in two trees at 5 years, then disappearing and developing again at 8 years. Gummy pitting developed in all trees.

COMMERCIAL APPLICATION OF DWARFING

The first experiment to determine the transmissibility of the dwarfing agent, planted at Somersby in 1955, prompted an examination of efficiency of crop production in relation to tree size (Long *et al.*, 1972).

Trials have been underway since 1961 to examine the feasibility of using, on a commercial scale, trees which have been deliberately dwarfed by inoculation, and to examine spacing and management requirements. Close-planted, dwarfed trees in trials at Yanco and Dareton have given higher yields per hectare in their early years than normal trees planted at normal spacing, permitting considerable savings in management costs by more efficient use of irrigation, fertilizers and herbicides (Long *et al.*, 1972; Stannard *et al.*, 1975; Bevington and Bacon, 1977).

Five trials are current:

- Nucellar Washington navel orange trees on trifoliate orange were planted at Yanco in 1961. Two sources of dwarfing were compared — exocortis (033) and moderate dwarfing (3538). Trees were inoculated in the nursery and at 1, 2, 3, and 5 years. The dwarfing effect has been greatest on trees inoculated in the nursery, and it appears that tree size will stabilize in five classes according to age at inoculation. Exocortis has caused greater size reduction than has the moderate-dwarfing budline (Stannard *et al.*, 1975).
- Nucellar Bellamy navel orange trees on trifoliate orange rootstock were planted at Yanco in 1963. Five sources of dwarfing were compared — exocortis (budline 033) and four sources of moderate dwarfing (budlines 3531, 3532, 3538, 3539) in high- and low-density plantings. Cumulative yields per hectare of high-density dwarf trees have been higher than those of normal trees at low density. Fruit quality remains unchanged.
- Nucellar and old-line Valencia orange, on trifoliate orange, Troyer and Carrizo citrange at high density and normal spacing, were planted at Yanco in 1964. Sources of dwarfing used were exocortis and four dwarfing selections. Yield and size of individual trees on the citrange are greater than twice that on trifoliate orange. With adjustmust for spacing, production is satisfactory for the smaller trees. Trifoliate orange stocks inoculated with exocortis have normally scaled at 4 to 5 years, but no scaling has yet appeared on the citranges. Fruit size and quality are unaffected by stock and dwarfing inoculations.
- A trial comprising nucellar Bellamy navel orange on trifoliate orange stock was planted at Yanco in 1966. Sources of dwarfing used were four lines of Unshiu mandarin. No dwarfing has occurred to date, but cropping has been enhanced (Bacon and Bevington, 1977).
- A trial comprising nucellar Bellamy navel orange on trifoliate orange, Troyer citrange, Carrizo citrange, Rangpur lime, Cleopatra mandarin, sweet orange, and rough lemon was planted at Dareton in 1966. Two sources of dwarfing budlines used in previous trials — 3539 and 3532 — were compared with uninoculated controls. Percentage of reduction in canopy surface area and yield has been 51, 25, 25 and 19 for trifoliate orange, Troyer and Carrizo citranges, and Rangpur lime, respectively. To date no significant difference has developed in trees on sweet orange or rough lemon (Bevington and Bacon, 1977).

RESULTS OF INDEXING

Seven dwarfing budlines (one Marsh grapefruit, five Washington navel, one Valencia orange), showed positive exocortis reactions on Etrog citron (Broadbent *et al.*, 1971). One dwarfing budline (grapefruit) was also positive for xyloporosis. All were negative for psorosis. All carry the strains of tristeza virus normal for the variety. Most of the dwarfing budlines cause creasing of the wood of Orlando tangelo trees, but the cause of this is unknown.

DISCUSSION

The constant association in old commercial blocks of trees with exocortis scaling, pustules, dwarfing, and gummy pitting, the range of field symptoms of intermediate type and the variation in transmission from single sources suggest that one highly variable agent is involved in this disease complex, with components causing the exocortis reaction at one end of the spectrum and gummy pitting at the other. There is evidence of strain mixture in field trees and of competition between strains. Variable transmission of the symptoms from single sources and from single budsticks indicate uneven distribution in the tree and uneven concentration of virus strains in different tissues within the plant. Budwood may carry few or many components. In the inarching trial, trifoliate orange inarches grafted onto trees with a variety of symptoms, as yet, have shown only the exocortis scaling reaction.

Challenge by tissue inoculation of trees carrying nonscaling dwarfing, with exocortis, usually results in dominance of exocortis symptoms at first, though there are indications that with time exocortis scaling becomes less, and flaky scaling and pustules tend to predominate. A degree of competition between strains is also shown by the results of challenge inoculation and in the variations in visible symptoms with time which have been a feature of the older trials.

The purification and characterization of the viroid causing exocortis by Semancik and Weathers (1968) pointed the way for a further study of the disease complex. If, as seems likely, exocortis and gummy pitting are expressions of different strains and combinations within a complex, it might be possible to purify a dwarfing isolate for consistency in performance for commercial use. This would also eliminate the chance of interaction with mild, symptomless, as yet undetectable, strains of other viruses. The work of Weathers (1963) with citrus vein enation virus and citrus yellow vein virus has indicated that inter-

TABLE 3

VARIATION OF PROGENY TREES IN THREE BUDLINES OF ALLEN VALENCIA ORANGE ON TRIFOLIATE ORANGE ROOTSTOCK

Budline, location and date of planting	No. of trees in trial	Tree size and incidence of pitting: Large		Medium		Small		Trees with gummy pitting (%)
		NP*	P+	NP	P	NP	P	
2045, Yanco, 1953	14	0	2	3	6	0	3	78
2045, Yanco, 1951	9	0	2	2	4	0	1	71
2048, Yanco, 1953	15	10	3	2	0	0	0	20
2048, Yanco, 1951	15	10	4	1	0	0	0	26
3018, Somersby, 1955	105	7	7	57	26	6	2	33
3018, Yanco, 1959	18	1	2	6	2	4	3	38
3018, Dareton, 1955	51	0	14	0	17	2	18	96

* NP — Number of trees without gummy pitting symptoms.

+ P — Number of trees with gummy pitting symptoms.

action between viruses can have far-reaching effects on the host. Such a possibility has commercial significance for dwarfing propagations where, of necessity, tissue inoculation is used. The problem could be avoided if purification of the chosen inoculum can be achieved. But, having accepted the place of purposeful virus dwarfing, could there be other virus reactions or interactions which may be worth consideration for future improvement of the commercial tree? A trial with grapefruit stem-pitting strains at Narara indicates improved control of stem pitting in trees on trifoliate orange stock infected with a mild strain of gummy pitting. An as yet unexplained crop enhancement factor has been detected, in a dwarfing trial, in trees inoculated with Unshiu mandarin budwood. This factor is under further study. Other viral interactions could perhaps improve fruit quality or size. Should we be looking to a future where virus-free citrus varieties are inoculated with a selection of interacting viruses to produce a particular result as specified by the grower?

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