Effect of Graft-Transmissible Dwarfing Agents on Lemons

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ABSTRACT. Graft-transmissible dwarfing has been successfully used in Australia to limit tree size of Washington navel and Valencia clones on citrus exocortis viroid (CEV) sensitive rootstocks. One dwarfing budline (3538) and a scaling strain of CEV (033) were used to inoculate Taylor Eureka and Prior Lisbon lemon trees on five rootstocks (trifoliate orange, Benton and Bowman citranges, rough lemon and smooth seville). CEV isolate 033 reduced tree height by 23% and width by 15% of Lisbon lemon trees on trifoliate orange. For Eureka lemon, both inoculants decreased the size of trees on rough lemon with height and width reduced by an average of 28% and 15%, respectively. These size reductions were accompanied by a slight reduction in tree health and vigour for Eureka lemon on rough lemon but were more marked for Lisbon trees on trifoliate orange.
Index words. Dwarfing budline, citrus exocortis viroid, rootstocks.

The vigour of lemon trees, particularly of trees on rough lemon rootstock, makes harvesting, spraying and general management difficult. High-density plantings offer an effective means of increasing productivity, particularly during the early years following establishment. However, without some control of tree size, high-density plantings quickly become unmanageable and yields decline as a result of crowding and competition. Frequent or severe pruning of young, vigorous citrus trees promotes excessive vegetative growth at the expense of fruit production (2).

For most scions, some reduction in tree size can be achieved when trifoliate orange is used as a rootstock. Strains of trifoliate orange used in New South Wales (NSW) have good resistance to Phytophthora root rot (*Phytophthora citrophthora* Sm. and Sm. Leon.) (3), but are incompatible with Eureka lemon (10, 15).

Further reduction of tree size can be achieved by infection with citrus exocortis viroid (CEV) when susceptible rootstocks are used (2, 5). However, bark-scaling strains of CEV generally have an adverse effect on vigour, health and productivity of trees on susceptible rootstocks (11). CEV has been associated with some dwarfing budlines in the absence of bark-scaling symptoms (18, 21), but the "dwarfing factor" remains poorly defined (4).

Field trials have been underway since 1961 to examine the commercial feasibility of using trees which have been deliberately dwarfed by inoculation, and to examine spacing and management requirements. Closely planted, dwarfed orange trees in trials at Yanco and Dareton have given higher yields per hectare in their early years than normal trees at conventional or double spacing, permitting considerable savings in management costs by more efficient use of irrigation, fertilizers and herbicides (2, 13, 14, 20).

The aim of the present study was to assess the effects of inoculation with a severe-scaling strain of CEV (033) and a nonscaling dwarfing budline (3538) upon tree size and vigour of two lemon scions on a range of rootstocks.

MATERIALS AND METHODS

Sources of CEV and dwarfing isolates. Isolates 033 and 3540 were from Washington navel trees on trifoliate orange rootstock showing dwarfing and bark-scaling symptoms on the rootstock. Isolate 3538 was from a healthy Marsh grapefruit tree on trifoliate orange rootstock, of intermediate size, and showing no bark scaling of the stock. Isolates 3637 and 3541 were from healthy but dwarfed Washington navel orange trees on

trifoliate orange. Isolate 3637 showed no bark scaling of the trifoliate orange rootstock, but isolate 3541 caused a flaky transient scaling, atypical of citrus exocortis viroid.

Trial 1. Nursery trees of Lambert nucellar Eureka lemon on trifoliate orange rootstock were inoculated by budding into the rootstock. A scaling strain of CEV (3540) and two sources of dwarfing (3637 and 3541) were used. There were five trees of each treatment.

Trial 2. Two nucellar lemon scions, Eureka cv. Taylor and Lisbon cv. Prior, were propagated onto five rootstocks: rough lemon, the Appleby selection of smooth seville, Bowman and Benton citranges and trifoliate orange. Benton citrange is compatible with Eureka lemon and a yellow ring does not develop at the budunion (12). The 'Taylor' Eureka and 'Prior' Lisbon lemon clones had been indexed free of psorosis, xyloporosis and CEV. Trees were grown in containers in the nursery. Prior to planting, each stock-scion combination received three separate treatments as follows: 1) nil inoculation (control); 2) inoculation into the stock and scion with a shield bud of Bellamy navel orange known to carry a severe scaling strain of citrus exocortis viroid (CEV) (accession 033), but free from other viruses except citrus tristeza virus (CTV); and 3) inoculation with a Bellamy navel orange bud carrying a graft-transmissible dwarfing factor (accession 3538) and CTV, but indexing negative for CEV, xyloporosis and psorosis.

When the buds had taken, the growing points of these buds were removed to prevent shoot growth. Trees were planted in December 1973 in a randomized block design consisting of three single-tree replications of 30 treatments, comprising the factorial combination of 2 scions x 5 rootstocks x 3 inoculants. Records were kept of tree size, yield, juice quality, stock-scion compatibility and tree vigour (rated on a 1-4 scale).

The site selected, with a sandy loam soil of the yellow earth group (19) was at the Somersby section of the Gosford Horticultural Research Station. This site was typical of the area, which supports a major lemon-growing industry on the central coast of New South Wales.

Measurements were subjected to univariate or repeated measures analyses of the factorial design, with orthogonal polynomials used to compare trends over time (16). The effects of inoculation and the interactions of inoculation with scions and rootstocks were examined using two single-degree-of-freedom contrasts among inoculant treatments, viz. 1 vs. 2+3 and 2 vs. 3. All tests of significance were performed at the 5% level ($P<0.05$).

RESULTS

Trial 1. All trees inoculated with CEV (3540) developed scaling of the trifoliate orange rootstock in 3 to 5 yr. When removed at the age of 16 yr, all trees inoculated with nonscaling dwarfing and with flaky (ground-level) scaling showed strong development of gummy pitting (deep pockets of gum in wood and bark of the rootstock). Bands of gum extended from the wood surface toward the center of the butt (fig. 1).

Trial 2—Tree health and vigour. Symptoms shown by lemon trees in 1980 are given in table 1 and ratings of tree vigour in table 2. Trees of both lemon scions on rough lemon and Benton citrange rootstocks were healthy and vigorous, although both inoculants slightly reduced vigour and caused minor dieback symptoms.

Eureka and Lisbon lemons on smooth seville and Bowman citrange rootstocks were not as vigorous as on other rootstocks. Both inoculants exacerbated this response (table 2), but the effect for trees on smooth seville stock was less severe when 033 was used as the inoculant. Some of the pitting of the smooth seville stocks could be attributed to CTV,

Fig. 1. Nucellar Eureka lemon on *Poncirus trifoliata* inoculated with a dwarfing budline and showing gummy pitting and the yellow-ring incompatibility.

TABLE 1
SYMPTOMS SHOWN IN 1980 BY TWO LEMON SCIONS ON FIVE ROOTSTOCKS FOLLOWING INOCULATION WITH CITRUS EXOCORTIS VIROID (INOCULANT 033) OR A DWARFING BUDLINE (INOCULANT 3538)

Cultivar/scion and inoculant	ROOTSTOCK				
	Rough lemon	Smooth seville	Bowman citrange	Benton citrange	Trifoliate orange
Prior Lisbon					
Nil	Nil	rootstock & scion pitting	bulbous union, honeycombing of scion	scion pitting	Nil
033	Nil	bulbous union, rootstock & scion pitting	honeycombing of scion	Nil	rootstock bark scaling
3538	Nil	rootstock & scion pitting	honeycombing of scion, bulbous union	Nil	gummy pitting
Taylor Eureka					
Nil	Nil	Nil	honeycombing of scion	Nil	yellow ring at budunion
033	Nil	honeycombing of rootstock & scion, bulbous union	poor growth	Nil	yellow ring at budunion & bark scaling of stock
3538	Nil	honeycombing of stock, bulbous union	poor growth	Nil	yellow ring at budunion

TABLE 2
RATINGS OF TREE VIGOUR AND HEALTH 7 YR AFTER PLANTING FOR TWO LEMON SCIONS ON FIVE ROOTSTOCKS INOCULATED WITH CITRUS EXOCORTIS VIROID (INOCULANT 033) OR A DWARFING BUDLINE (INOCULANT 3538)

Cultivar & inoculant	ROOTSTOCK				
	Rough lemon	Smooth seville	Bowman citrange	Benton citrange	Trifoliate orange
Prior Lisbon					
Nil	++++[z]	+++	++	++++	++++
033	++++	++	+	++++	++
3538	++++	+	+	+++	+++
Taylor Eureka					
Nil	++++	+++	++	++++	+++
033	+++	++	+	+++	+
3538	+++	+	+	++++	++

[z] ++++ = healthy tree with dark green, dense canopy, no dieback; +++ = healthy tree, green, dense canopy, traces of dieback; ++ = unthrifty tree, thin canopy, yellow foliage, moderate dieback; + = weak tree with sparse canopy, yellow foliage and severe dieback.

and stocks were possibly infected by aphid transmission in the nursery. Some trees of Eureka lemon and Prior Lisbon on smooth seville inoculated with isolates 033 or 3538 developed a bulbous union and pinholing or honeycombing of the rootstock, which may be attributable to sour orange rootstock necrosis (17).

Trees of both scions inoculated with the scaling strain of CEV (033) developed the typical bark scaling on trifoliate orange after 4 yr. The dwarfing budline caused gummy pitting symptoms in trifoliate orange rootstocks as occurred in trial 1, but no bark-scaling symptoms. An incompatibility developed between Eureka lemon and trifoliate orange resulting in an intermittent ring at the budunion and a decline in tree health.

Those combinations resulting in severe loss of vigour typically had sparse foliage, leaf patterns suggestive of zinc and iron deficiency and progressive twig dieback.

Tree size. Reductions in tree size due to inoculation (table 3) were usually associated with reductions in tree vigour. For Lisbon lemon, both inoculants reduced the height and width of trees on Bowman citrange, by 22% and 19%, respectively, for 033, and 44% and 34%, respectively, for 3538. The height of trees on smooth seville was reduced by an average 32%, and the width of trees on rough lemon was reduced by an average 11% by 3538. Isolate 033 reduced the height by 23%, and width by 15%, of Lisbon lemon on trifoliate orange, while inoculant 3538 reduced the width of Lisbon lemon on smooth seville by 25%. Both inoculants decreased the size of Eureka lemon trees on rough lemon, with height and width reduced by an average 28% and 15%, respectively. Inoculant 3538 reduced height, by 28%, and width, by 21%, of Eureka lemon on smooth seville.

Production. Cumulative yield (kg/tree) from 1976-1980 varied between rootstocks and in only three combinations did inoculation significantly influence total production per tree (table 4). Yield was significantly reduced for trees of Eureka lemon on rough lemon and Benton citrange when inoculated with 033 or 3538. Inoculant 3538 also significantly reduced the yield of trees of Lisbon lemon on Bowman citrange.

The trend in yield over the 1976-1980 harvests differed with scion and rootstock, and between inoculated and uninoculated trees, but there was little difference between the trends for the inoculants 033 and 3538. The increase in yield was predominantly linear up to 1979 for all rootstocks

TABLE 3
CANOPY MEASUREMENTS OF TWO LEMON SCIONS ON FIVE ROOTSTOCKS INOCULATED WITH CITRUS EXOCORTIS VIROID (INOCULANT 033) OR A DWARFING BUDLINE (INOCULANT 3538)

Cultivar & inoculant	ROOTSTOCK									
	Rough lemon		Smooth seville		Bowman citrange		Benton citrange		Trifoliate orange	
	Ht.[z]	Width[z]	Ht.	Width	Ht.	Width	Ht.	Width	Ht.	Width
Prior Lisbon										
Nil	3.8	4.1	2.6	2.7	2.3	2.6	3.1	3.2	3.0	3.4
033	3.7	3.6	1.9	2.5	1.8	2.1	3.2	3.3	2.3	2.9
3538	3.5	3.7	1.7	2.0	1.3	1.7	3.1	3.2	2.8	3.3
Taylor Eureka										
Nil	3.6	3.6	2.4	2.6	1.4	2.0	2.9	2.9	2.2	2.3
033	2.4	3.1	2.4	2.8	1.5	1.9	2.9	3.1	1.8	2.3
3538	2.7	3.1	1.7	2.1	1.1	1.8	2.7	2.5	1.8	2.5

[z]Measurements made 7 yr after planting. Height in meters, SE = 0.2; Width in meters, SE = 0.2.

other than Bowman citrange, with the rate of increase less for inoculated than uninoculated trees. While the rate of increase for inoculated trees was maintained in 1980, except for Lisbon lemon on smooth seville, the rate of increase declined for uninoculated Lisbon lemons and uninoculated Eureka lemons on Benton citrange, so that differences in yield due to inoculation for these trees were less in 1980 than in 1979. In contrast to the other rootstocks, there was little increase in the yield of trees on Bowman citrange in 1979, and, apart from uninoculated Eureka lemon, yields declined in 1980.

Cropping efficiency (kg fruit/m^2 canopy area) of inoculated trees was less than that of uninoculated trees by 1979, but in 1980 differences in efficiences due to inoculation were much reduced for rootstocks other than smooth seville and trifoliate orange, particularly for the Lisbon lemon scion. However, inoculated Eureka lemon trees on rough lemon rootstock were more efficient in 1979 and 1980 than uninoculated trees. Cropping efficiencies of both scions on trifoliate orange rootstock were unaffected by inoculation.

Average weight per fruit over the period 1976-1980 was influenced by inoculation. Inoculants 033 and 3538 reduced weight per fruit of Lisbon lemon on smooth seville by 13% and on Bowman citrange by 11%, and re-

TABLE 4
CUMULATIVE YIELD (KG/TREE) FROM 1976 TO 1980 FOR TREES OF TWO LEMON SCIONS ON FIVE ROOTSTOCKS INOCULATED WITH CITRUS EXOCORTIS VIROID (INOCULANT 033) OR A DWARFING BUDLINE (INOCULANT 3538)

Cultivar & inoculant	ROOTSTOCK				
	Rough lemon	Smooth seville	Bowman citrange	Benton citrange	Trifoliate orange
Prior Lisbon					
Nil	384[z]	141	171	284	271
033	327	81	129	308	179
3538	309	53	32	236	270
Taylor Eureka					
Nil	415	188	100	355	147
033	301	241	105	248	134
3538	294	131	70	199	136

[z]SE = 35.

duced weight per fruit of Eureka lemon on rough lemon by 9% and on Benton citrange by 12%. Also, inoculant 3538 reduced weight per fruit of Eureka lemon on smooth seville by 16%, whereas 033 reduced the fruit weight of Eureka lemon on trifoliate orange by 11%.

The distribution of the annual lemon crop between a winter main crop and spring/summer crops for the period 1976-1980 was influenced by inoculation. Lisbon lemon on Bowman citrange inoculated with 3538 produced 18% more non-winter fruit, as a proportion of total annual yield, than uninoculated trees. Both inoculants increased non-winter cropping of Eureka lemon on Bowman citrange by an average 10%, whereas 3538 increased non-winter cropping of Eureka lemon on Benton citrange by 12%.

Juice quality. Average data for 1979 and 1980 indicated that juice quality, expressed as mean percent juice and mean weight of citric acid per ton (CA/t) of fruit, was significantly affected by inoculation. For Lisbon lemon on Bowman citrange, inoculant 3538 reduced percent juice from 32.5% to 26.3%, a reduction of 19%, and reduced citric acid from 19.0 kg CA/t to 14.5 kg CA/t, a reduction of 24%. Reductions due to inoculant 033 of about 8% for both measures were not significant. For Eureka lemon on Bowman citrange, both inoculants produced similar effects, lowering juice levels by 10% and citric acid by 12%. For Eureka lemon on trifoliate orange, however, both inoculants significantly improved juice levels, by an average 8%, and citric acid, by an average 13%.

DISCUSSION

The stock/scion combination used commercially in NSW is Eureka lemon on rough lemon with minor plantings of Lisbon on trifoliate orange. CEV isolate 033 reduced tree height by 23% and width by 15% of Lisbon lemon trees on trifoliate orange. For Eureka lemon, both inoculants decreased the size of trees on rough lemon with height and width reduced by an average 28% and 15%, respectively. These size reductions were accompanied by a reduction in tree health and vigour, which was more marked in Lisbon trees on trifoliate orange. Cropping efficiency of inoculated Eureka lemon trees on rough lemon rootstock improved during the trial, and, after 6 yr, these trees were more efficient than uninoculated trees. The increase in cropping efficiency, if sustained in future years, could compensate for smaller trees in terms of total crop. There was also a 9% reduction in fruit weight but no effect of inoculation on juice quality or summer fruiting.

These results are not as promising as those obtained for Valencia and navel oranges, in which marked tree size reduction is consistent and predictable, with no adverse effects upon vigour and productivity (9).

Significant height reductions were achieved with the least vigorous rootstocks for Prior Lisbon lemon; however, these have no commercial potential. At the time of establishing this trial the potential value of smooth seville and Bowman citrange stocks was not certain. Concurrent studies have shown the variable response achieved with smooth seville as a lemon rootstock and the extremely low vigour associated with Bowman citrange (12).

The reductions in yield, on a per tree basis, for uninoculated versus inoculated trees in 1980 were due to decreased cropping efficiencies rather than reduced tree size. The small decline in cropping efficiency of trees on smooth seville stocks, however, was insufficient to compensate for the reduced tree size of inoculated trees, so that the difference in total yield per tree between inoculated and uninoculated trees in 1979 was maintained in 1980. The reductions in yield and cropping efficiency in 1980 for trees on Bowman citrange rootstock mirrored the observed loss of vigour for

trees on these stocks, which was accelerated by inoculant 3538. This particular dwarfing budline has not worked consistently across a range of stock:scion combinations. In addition to variations of tree growth and productivity, marked variations in juice yield and quality were also recorded for a number of different rootstocks.

The bark-scaling symptoms on trifoliate orange and the unthrifty appearance of trees on this rootstock, when inoculated with the CEV isolate (033), were to be expected. Isolate 033 has a moderately severe effect on trees on trifoliate orange rootstock, but does not cause scaling of citrange rootstocks.

Dwarfing budline 3538 gives a mild leaf-curling reaction in Etrog citron indicators, but CEV has not been detected by PAGE or dot-blot hybridisations to a CEV-cDNA probe (4, 18). The cause of the graft-transmissible dwarfing is unknown. Duran-Vila *et al.* (7) suggested that the "dwarfing factor" utilized in controlling citrus growth in Australia may be the result of the expression of a particular permutation of the reservoir of viroid-like RNAs apparently resident in citrus.

The production of gummy pitting symptoms in trifoliate orange rootstocks under Prior Lisbon lemon, when inoculated with dwarfing budline 3538, is consistent with the previously observed association between dwarfing and gummy pitting symptoms (8, 10). It appears that the earliest and most severe symptoms of gummy pitting occur in lemons on trifoliate orange rootstock. Fraser *et al.* (10) postulated a modification of CEV symptom expression by the presence of gummy pitting in some trees, and suggested that strains of the causal pathogens of both diseases may be distributed unevenly and independently in a tree, and be transmitted unevenly to progeny trees (9).

The successful dwarfing of navel and Valencia orange clones on trifoliate orange and citrange rootstocks in the absence of any deleterious symptoms (1, 20) has not been repeated for lemons. The poor response of some lemon scion/rootstock combinations to dwarfing budline 3538 strengthens the need for strict guidelines when releasing dwarfing budlines to the citrus industry for commercial use (6). The possible indiscriminate use of these dwarfing budlines by nurserymen for stock/scion combinations not yet evaluated may result in deleterious effects. There is a need for continued studies on the causal agent of dwarfing and the nature of the dwarfing response in order to better understand the reactions observed.

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