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The Effect of Pruning, a *Citrus tristeza virus* Isolate and a Citrus Viroid Isolate on Huanglongbing Infection

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ABSTRACT. High density planting of citrus is a common practice in South Africa. To prevent overcrowding, the trees have to be shaped and pruned. The effect of this practice on Huanglongbing (HLB) infection in an area where the disease is present was investigated. Virus-free Delta Valencia trees on Troyer citrange rootstock were grown under insect-free conditions and pre-inoculated with LMS 6 (the experimental control, a standard CTV cross protecting isolate for the industry), CD 6 (a citrus viroid dwarfing agent) and CD 4 (HLB cross-protecting CTV isolate) before planting in the field in 1996. The control treatment was planted at a 6 × 3 meter density while the other treatments were planted at 6 × 2 meters. Pruning or tree shaping treatments were applied to trees pre-inoculated with LMS 6 and CD 4 at high density. The standard vector control procedures were followed in the field. Over a 4-yr period, trees with CD 4 at high density without pruning achieved the highest production but this was not significantly better than the control trees. Pruning decreased production by 30% and 43% for trees with LMS 6 and CD 4 respectively. Production of the trees with CD 6 was 23% lower than that of the control trees but it had the lowest HLB infection (18%). HLB infection increased where pruning was applied in comparison with un-pruned trees with the same isolates (118% with LMS 6 and 46% with CD 4). CD 4 reduced HLB infection in pruned trees significantly in comparison with pruned trees with the control CTV isolate.

Index words. Cross protection, high density planting.

Escalating production costs of citrus in South Africa necessitate an increase in production per unit area. Improved genetic material and high density planting are key factors to meet the goal. High density planting at 1,000 trees/ha is currently a common practice (6). Better management practices are needed to overcome the disadvantages of high density planting. Overcrowding, which develops within the first 5 yr, makes vehicle access to control diseases and pests difficult (1, 2, 13) and it should be prevented without a loss of production (6). In the absence of suitable genetic dwarfing material and to avoid adverse effects of dwarfing by transmissible agents (7, 8), the only practical option in South Africa to control overcrowding is by pruning or tree shaping (5). However, the adverse affects of pruning on yield (3) and Huanglongbing (HLB) infection (1) have been shown. Van Vuuren et al. (9, 10, 11) identified a *Citrus tristeza virus* (CTV) isolate that reduced HLB infection. In this investigation, the effectiveness of the CTV isolate to reduce HLB was evaluated in a pruned and un-pruned situation, in an area where HLB is present.

MATERIALS AND METHODS

Virus-free Delta Valencia on Troyer citrange rootstock was grown under insect-free conditions in a greenhouse according to normal nursery practices. When the scions had developed to approximately 5 mm thickness, they were inoculated with three different isolates of graft transmissible agents (LMS 6, CD 4, CD 6) (Table 1). Six months later, plants were planted in the field.

The trees were planted in 1996 at a spacing of 6 × 2 m (6 × 3 for controls) according to a randomized block. Each treatment was replicated five times and consisted of 12-tree

TABLE 1
GRAFT TRANSMISSIBLE AGENTS AND PRUNING TREATMENTS

Treatment	Comments[1]
LMS 6 (Control)	Standard cross-protecting *Citrus tristeza virus* (CTV) isolate for sweet orange in South Africa (10)
CD 4	CTV dwarfing isolate that also gave some protection against Huanglongbing (9, 11)
CD 6	CVd-III dwarfing isolate (12)
LMS 6 + Prune	Pruning according to Joubert et al. (5)
CD 4 + Prune	Pruning according to Joubert et al. (5)

[1]Numbers in parentheses refer to literature cited.

plots (8 for controls). The site was situated in the Nelspruit district, which is in a HLB area. The standard control measures for HLB in South Africa, planting of healthy material, control of the vector *Trioza erytreae*, and the removal of infected branches, were followed in the field (4).

Pruning and tree shaping started in the first year after planting as described by Joubert et al. (5). The main aim of pruning was to keep a dominant central leader and to cut back lateral growth that exceeds one third of the leader branch. Cutting back of laterals occurred throughout the year but mainly during growth cycles. Finally the tree should have a pyramid shape to allow maximum light penetration.

The effect of the treatments on tree size, production and HLB infection were monitored over a 7-yr period of which four were production years. At harvest, fruit were inspected for HLB symptoms (olive green color, high shoulders, lopsidedness) and the crop was divided into healthy and HLB infected fruit. HLB infected fruit has no commercial value either for fresh fruit or processing since these fruit have low solids and are high in acid. The juice has a bitter taste, which is unacceptable for human consumption.

RESULTS AND DISCUSSION

Pruning and tree shaping was terminated after 4 yr in the field since yield was drastically reduced by the treatment and the aim of obtaining a high production as soon as possible after planting was not being achieved.

High infections of HLB occurred during 2001 and 2002 when unexpected high populations of the psyllid vector were present despite control measures. Tree size, production and percentage HLB infection of each treatment are shown in Tables 2 and 3, respectively. The control trees were significantly larger than trees in all the other treatments. Pruning reduced tree size signifi-

TABLE 2
THE EFFECT OF GRAFT TRANSMISSIBLE AGENTS AND PRUNING ON TREE SIZE (TRUNK CIRCUMFERENCE AND CANOPY HEIGHT) OF 7-YR-OLD DELTA VALENCIA TREES

Treatment	Circumference (cm)[1]	Height (m)
LMS 6 (Control)	33.6 a	3.5 a
CD 4	30.3 b	3.4 a
CD 6	27.5 c	2.9 b
LMS 6 + Prune	30.8 b	3.4 a
CD 4 + Prune	26.8 c	2.8 b

[1]Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

TABLE 3
THE EFFECT OF GRAFT TRANSMISSIBLE AGENTS AND PRUNING ON THE CUMULATIVE YIELD (KG/TREE) AND HUANGLONGBING INFECTION (%) OF 7-YR-OLD DELTA VALENCIA TREES OVER A 4-YR PRODUCTION PERIOD

Treatment	Yield (Kg/tree)[1]	% Greening
LMS 6 (Control)	282 a	25.5 ab
CD 4	284 a	23.2 a
CD 6	218 b	18.0 a
LMS 6 + Prune	197 bc	55.9 c
CD 4 + Prune	161 c	37.5 b

[1]Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

cantly in both treatments but more where the dwarfing CTV isolate (CD 4) was used. The size of the CD 6 treatment was similar to the CD 4 pruning treatment and the canopy height of both treatments was significantly reduced in comparison with the other treatments (Table 3).

The control trees (LMS 6) and CD 4 (un-pruned), with 282 and 284 kg of fruit per tree, respectively, achieved the highest production. The former had a 25.6% incidence of greening in comparison with 23.2% for the CD 4 trees. This result does not confirm a previous finding where greening remained low for several years in trees that were inoculated with CD 4 (11). The two pruning treatments (LMS 6 with pruning, CD 4 with pruning) showed that pruning decreased production by 30% and 43% and increased greening infection by 118% and 46%, respectively. Of the two pruning treatments, CD 4 trees had a 33% lesser incidence of greening than the LMS 6 trees. This shows that the CD 4 CTV isolate gave some protection against HLB infection (Table 3), but not as much as in a previous report (11).

The CVd-III isolate (CD 6) had a lower yield than the control trees and although the percentage of greening was lower, it was not significant (Table 3).

Two main disadvantages of pruning, a reduction of yield (3) and an increase in Huanglongbing infection (1) were shown in this study. The aim of high density planting was to maximize production per unit area and this was not achieved, and a further reduction in production occurred because of the high percentage of HLB infected fruit which are unmarketable.

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