Evaluation of a Sixteen-Year Citrus Tristeza Virus Cross-Protection Trial in Florida

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ABSTRACT. The Indian River citrus-growing region is an area of Florida where many citrus trees are planted on sour orange rootstock. Many of these trees are currently experiencing rapid decline due to citrus tristeza virus (CTV). Three non-decline-inducing (NDI) isolates of CTV were evaluated to determine their effectiveness as cross protecting agents against decline-inducing strains of CTV. The three isolates were inoculated separately into grapefruit scions on sour orange rootstocks. Trees inoculated with NDI isolates of CTV as well as uninoculated trees were planted in an area with a high incidence of trees affected by CTV decline. Sixteen yr after planting, 86%, 90%, and 90% of trees inoculated with the three NDI isolates of CTV were unaffected by severe CTV as determined by negative reaction to the CTV MCA 13 monoclonal antibody. By the same criteria, only 33% of the unprotected trees were free of severe CTV.

Citrus tristeza virus (CTV) causes economically important disease wherever citrus is grown (1, 3). The virus is genetically and biologically diverse and can cause stunting, slow decline, quick decline, stem pitting, or no symptoms depending on the virus isolate, citrus cultivar, rootstock, time of infection, and environmental conditions (2, 4). In Florida, CTV isolates are of two general types: those that cause no detectable symptoms and those that cause stunting, and/or decline of citrus on sour orange or Alemow rootstock. CTV isolates that cause stem-pitting of grapefruit and sweet orange scions regardless of the rootstock are not present in commercial citrus in Florida at this time. Therefore, CTV control strategies in Florida have focused on use of tolerant rootstocks to protect against endemic decline isolates and budwood certification to avoid distribution of any stem-pitting isolates that may be introduced by illegal importation of budwood or that may already be present in small homeowner plantings.

There are still many productive grapefruit groves on sour orange rootstock in the Indian River region of Florida that are at a high risk from CTV-induced decline. One possible means of maintaining these groves in the presence of decline-inducing CTV inoculum is mild strain cross-protection (6, 7, 8). In 1981, a field experiment to test this hypothesis was initiated at the Univ. Florida Indian River Research and Education Center (IRREC) at Fort Pierce, FL. The experiment tested the ability of three mild isolates of CTV to protect Valencia sweet orange and Ruby Red grapefruit on sour orange rootstock from local decline-inducing CTV isolates. The data collected from the sweet orange block indicated that most of these cross-protected trees became superinfected with decline-inducing isolates of CTV and declined within 8 yr (5). The data from the adjacent grapefruit block, reported herein, suggest that the same mild isolates prevented superinfection of most of the grapefruit trees for 16 yr.

MATERIALS AND METHODS

Virus isolates and tree propagation. The three mild isolates of CTV: DD 102bb; Guettler HS; and DPI 1-12-5-X-E were described in an earlier report on cross-protection of sweet orange on sour orange rootstock (6). Each of these isolates originated from a surviving sweet orange tree on sour orange rootstock where most of the nearby

trees were in decline. Each of the three isolates were initially transferred by grafting from the field tree into several CTV-free Valencia sweet orange trees on sour orange rootstock using three scion bark chips per tree and then were maintained in an aphid-free greenhouse. The presence of CTV in these trees was confirmed by ELISA and the mild phenotype of each of the transferred isolates was verified by indexing on Mexican lime seedlings and sweet orange on sour orange rootstock indicators.

Budwood from the greenhouse trees containing each of the three mild isolates of CTV was used to inoculate CTV-free Ruby Red grapefruit on sour orange rootstock. Control grapefruit were mock-inoculated using buds from a virus-free source. The presence or absence of CTV in the grapefruit was verified by ELISA prior to transplanting the trees into the field.

Field plot design. Three trees of each of the four treatments (DD 102 bb, Guettler HS, DPI 1-12-5-X-E, or CTV-free buds) were randomized within each of seven plots. Each plot contained 12 trees on two single-row raised beds with six trees on one bed and six on the adjacent bed. The seven plots were planted adjacent to each other in an east/west direction at the IRREC at Ft. Pierce, FL, in 1981. The experimental area, therefore, consisted of two rows with 42 trees per row. The experimental area was adjacent to the plots that were used to evaluate the CTV isolates for cross-protection of sweet orange on sour orange rootstock (5). The Ruby Red grapefruit blocks were irrigated as needed with a microsprinkler system. Insects and fungus diseases were controlled as needed by application of pesticides according to University of Florida recommendations.

Data collection. In 1997, 16 yr after the blocks were planted, trees were evaluated for virus content, decline symptoms, tree condition, and height. Virus content was evaluated by indirect DAS-ELISA using monoclonal antibodies that react either with only severe CTV isolates or all CTV isolates as previously reported (5), except monoclonal antibody 17G11 was used as the nonspecific antibody instead of 3DFI.

Trees were evaluated for decline in the spring of 1997. A tree was rated in decline if its foliage was visually thinner than healthy trees. Tree ratings were taken at the same time using a 0 to 3 scale, with 0 being no symptoms and 3 being severe decline (near death). Tree heights (m) were measured in the fall of 1997 with a telescoping measuring rod.

RESULTS

After 16 yr of field exposure to CTV, only two of the uninoculated control grapefruit trees remained uninfected (Table 1). Sixty-seven percent of these uninoculated trees were infected with severe isolates of CTV as determined by reaction with monoclonal antibody MCA13. This compares with severe CTV infection levels of 14%, 10% and 14% for grapefruit trees cross-protected with DD 102bb, Guettler HS, and DPI 1-12-5-X-E mild isolates of CTV, respectively. Three indicators of disease (percentage of trees in decline, tree ratings, and tree height) indicated that grapefruit trees cross-protected with the DD 102bb mild isolate of CTV were significantly better than unprotected control trees.

DISCUSSION

All three of the mild isolates of CTV reduced superinfection of Ruby Red grapefruit trees with severe (MCA13 positive) isolates of CTV for 16 yr. These results suggest that

TABLE 1
CONDITION OF GRAPEFRUIT TREES PROTECTED WITH MILD ISOLATES OF CTV AFTER 16 YR[z]

Mild isolate[y]	% CTV[x]	% Severe CTV[w]	% Decline[v]	Tree rating[u]	Tree height (m)
DD 102bb	100	14 a	19 a	0.69 a	3.33 a
Guettler HS	100	10 a	29 ab	0.86 ab	3.06 a
DPI 1-12-5-X-E	100	14 a	33 ab	0.88 ab	3.00 ab
None	90	67 b	43 b	1.17 b	2.83 b

[z]Data are the average of measurements from 21 trees in seven randomized complete blocks (three trees of each treatment per block). Numbers within a column followed by different letters are significantly different ($P \leq 0.05$).
[y]Each of 21 trees was propagated on sour orange rootstock using buds from Ruby Red grapefruit that had been previously infected with a specific mild isolate of CTV or left uninfected by mock inoculation.
[x]Percentage of trees positive for CTV using monoclonal antibody 17G11.
[w]Percentage of trees positive for decline isolates of CTV using monoclonal antibody MCA13.
[v]Percentage of trees with moderate decline (foliage visibly thinner than healthy trees).
[u]Rating on a scale of 0 to 3, with 0 = no symptoms, 1 = slight decline, 2 = moderate decline, and 3 = severe decline (near death).

these mild isolates can be used to slow loss of grapefruit on sour orange rootstock from severe CTV isolates prevalent in the Indian River region of Florida. The reduction in infection levels with severe isolates of CTV by two of the mild isolates Guettler HS and DPI-1-12-5-X-E did not result in significant differences in the health of the trees. However, isolate DD 102bb did result in the trees being significantly healthier than the control trees. There are at least two reasons for the lack of greater differences in tree health. First, grapefruit trees on sour orange rootstock that became positive for severe CTV may not express symptoms for several years and, thus, lessen the contrast with unprotected controls (Powell, unpublished data). Second, some of the trees were beginning to decline due to causes other than CTV in all treatments. The most notable additional cause of decline was damage from the citrus root weevil, *Diaprepes abbreviatus*.

An adjacent experiment in which the same mild CTV isolates were used with Valencia sweet orange on sour orange rootstock showed that cross-protection from severe CTV isolates broke down within eight years (6). Therefore, the ability of the mild isolates of CTV to prevent superinfection with severe isolates was highly species dependent. The reason why cross-protection was successful in grapefruit, but failed in sweet orange is not known. These results suggest that close attention must not only be paid to the protecting isolate and potential challenging isolates, but also to the citrus scion species or cultivar when developing cross-protection control strategies for CTV (5, 6, 7, 8).

Within the last year, the experimental area has experienced periodic infestation with the brown citrus aphid, *Toxoptera citricida*, an efficient vector of CTV that was not present during the 16 yr period when this experiment was conducted. Trees will continue to be monitored for CTV infection and decline to determine the impact of this new vector on cross-protection of grapefruit from severe CTV isolates.

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