Location Effect on Incidence of Citrus Tristeza Virus in Hawaii

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ABSTRACT. Forty-one of 45 samples from various locations on the Islands of Oahu, Maui, and Hawaii indexed positive for citrus tristeza virus (CTV) infection by enzyme-linked immunosorbent assay (ELISA). High rates of infection were also observed in young, virus-free trees planted in experimental plots in the Hilo area. In contrast, navel orange trees in a large, isolated commercial grove near South Point, Hawaii, had a low incidence of infection after 4 yr of field exposure. A gradual increase in infection was noted in the subsequent 3 yr. The isolation of the planting from large numbers of infected trees, plus lack of the efficient aphid vector, *Toxoptera citricidus* Kirk, in the grove, and prevailing on-shore winds may account for the low incidence of infection. Proper location may markedly reduce incidence of CTV in new plantings, even in areas with generally high potential for natural spread.
Index words. Citrus blight, ELISA.

Citrus is widely distributed throughout the Hawaiian Islands in numerous small backyard or semi-commercial plantings. There are also a few larger commercial plantings and several variety collections in experimental plantings. Severe strains of citrus tristeza virus (CTV) are widespread, and field trees of CTV-susceptible varieties frequently show severe CTV symptoms. Even sweet oranges, which are normally tolerant of CTV, often show CTV stem pitting symptoms. The efficient aphid vector of CTV, *Toxoptera citricidus* Kirk (10), is also abundant in many locations. These factors make Hawaii an attractive location to test the cross-protection efficiency of mild strains of CTV under field conditions. Mild strains of CTV are common in Florida, but their protective ability against stem pitting strains remains untested, due to lack of stem pitting isolates of CTV and *T. citricidus* in Florida. A program was initiated in 1978 to evaluate the protective effects of mild Florida isolates under the relatively severe challenge conditions in Hawaii.

Preliminary surveys of the islands were conducted to verify the widespread distribution of CTV and to select suitable sites for cross-protection tests. As expected, initial surveys indicated that CTV was widespread, and ELISA assays confirmed CTV infection in symptomless cultivars. Interestingly, however, several samples collected from a large sweet orange grove which had been planted with virus-free trees tested negative. These results were unexpected and tentatively ascribed to a testing error since CTV-infected trees and *T. citricidus* had been observed nearby. However, subsequent assays confirmed the original results.

This paper reports studies at this site where unexpectedly slow natural spread of CTV has occurred, including evidence that citrus blight is independent of CTV infection. The effects of location on disease development and on cross-protection are discussed.

METHODS AND MATERIALS

Sample collection and processing. Bark peeled from young shoots of new growth, the midribs from expanding young leaves, and/or the pedicel bark tissue of young fruit were collected for ELISA. Several sites were sampled on each tree. Tissues were stored at 4C until processed or dried over a dessicant for shipping and long-term storage. A sample equivalent to 0.25 to 0.50 g fresh weight was ground in 5 ml of phosphate-buffered saline containing 0.2% Tween 20 and 2% polyvinylpyrollidone (2) with a dispersion homogenizer or by a mortar and

pestle. Dry samples were rehydrated in extraction buffer for at least 1 hr prior to grinding.

Two sampling patterns were used at the South Point location. In one, samples were collected randomly from widely spaced points throughout the planting. In the other, a single 5 X 20 tree plot was sampled repeatedly.

Detection of CTV. Double antibody sandwich (DAS) ELISA (2) was used to determine the presence of CTV in field-collected samples. The immunoglobulins (IgG) used for coating and preparation of enzyme conjugates were prepared from rabbit antiserum to whole, unfixed virus of the T4 isolate (1, 2). This same CTV antiserum has been used extensively worldwide for detection of CTV and has reacted to all isolates tested (2). Microtiter plates were coated with 1 μg,/ml IgG and conjugates were used at dilutions from 1/1,000 to 1/8,000. Known sources of CTV produced strong reactions within 30 min in this system. Where possible, readings were quantified by measuring the OD_{405} of the reaction with a plate reader zeroed to a buffer reference. Reactions were considered positive where the OD_{405} value was 2X the value for healthy extracts (or the healthy extract value plus 0.10 if the healthy extract value was $\geq$ 0.10). Samples were considered positive by visual observation when wells containing the samples were clearly yellow and little or no visual reaction was present in the healthy control (a difference in OD_{405} estimated to be > 0.15). Indirect DAS ELISA (9) was used to test the reaction of selected isolates to the strain-discriminating CTV monoclonal antibody CTVMCA13 (9).

Bioassays. Several field-collected samples were indexed on a standard host range of citrus indicators (5) in quarantine facilities at Beltsville, MD.

RESULTS

Observation of citrus trees growing in most areas on the Island of Hawaii indicated that CTV was widespread. Most Mexican lime and grapefruit trees were stunted and showed obvious stem pitting symptoms. Even sweet orange trees were frequently stunted with compact canopies, short internodes, and stem pitting in small twigs. These symptoms were especially noticeable in a small planting of navel oranges near Waiohinu (fig. 1). No CTV symptoms, however, were seen in the large commercial planting at South Point which is several miles southwest of Waiohinu.

The results of ELISA assays of samples collected at different locations in seral preliminary surveys are summarized in table 1. These results confirmed that CTV was widespread in most locations. The two trees sampled at South Point were both negative for CTV in the 1979 assay. This surprising result led to more extensive tests at that location in 1982 and 1983. The 1982 and 1983 assays indicated that CTV was present at the South Point location, but only in a low percentage of the trees. Because the samples tested were collected over a wide area, it was possible that infection rates within localized areas could have been much higher than the average. Accordingly,

TABLE 1
DETECTION OF CITRUS TRISTEZA VIRUS INFECTION BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN SAMPLES COLLECTED FROM DIFFERENT LOCATIONS IN HAWAII

Date	Location	
	South Point	All other[y]
1979	0/2[z]	24/25
1982	0/25	11/12
1982	3/53	—
1982	0/12	—
1983	9/78	6/8
	12/170[x]	41/45[x]

[z]Number of trees positive/number tested. All ELISA results by visual reading. All positive samples clearly reactive. Samples in first two collections dried and stored prior to testing. All others tested fresh in Hawaii.

[y]Samples collected from different locations over the island.

[x]Totals shown are for samples, and include some duplication between years. Negative tests in the "all other" column are from a single pink shaddock tree at Waiakea.

in 1983 we sampled alternate trees in a 5 X 20 tree plot which contained a CTV-positive tree in the 1982 survey and some additional trees adjacent to other known CTV-positive trees. Four of 50 trees in the 5 X 20 plot were positive and one of 10 trees immediately adjacent to other infected trees was found infected. These results indicated that concentrated pockets of infection were probably not present. The 5 X 20 tree plot sampled in 1983 was resampled in 1986. Several trees had been removed for construction, and 41 trees were resampled. Nine of the 41 trees tested were found infected (table 2). The four positive trees from 1983 were all included in the 1986 survey. We also sampled 58 trees distributed randomly over 11 separate blocks. Eight of these 58 trees were positive for CTV.

We also checked for local movement of CTV to trees near several infected trees identified in the 1983 survey. At one location, three trees which were immediately adjacent to an infected tree in 1983, and uninfected then, were found infected in 1986. At the second site, three additional positives were found adjacent to two infected trees located in 1983. An additional infected tree was found two tree spaces away on the diagonal. Other trees in the immediate area were negative.

TABLE 2
CHANGE IN CITRUS TRISTEZA VIRUS (CTV) INCIDENCE OVER A 3-YR PERIOD IN A LOCALIZED AREA OF A COMMERCIAL NAVEL ORANGE GROVE AT SOUTH POINT, HAWAII

Row	No. of trees tested[z]	No. of trees positive for CTV[y]	
		Aug 1983	Jul 1986
18	10	0	0
19	10	0	3
20	3	0	0
21	9	1	2
22	9	3	4
	41	4	9

[z]Alternate trees sampled in each row. Same tree tested each time.
[y]CTV infection determined by ELISA.

Scattered declining trees with symptoms of citrus blight were observed in the planting, and visual diagnosis was confirmed by water injection and zinc analysis (6). ELISA assays of six blighted and six healthy trees revealed that only one of each was infected by CTV.

No stem pitting, stunting, vein clearing or leaf cupping was noted in trees found infected with CTV by ELISA at the South Point site.

Budwood from three trees at the South Point planting was sent to the USDA citrus quarantine facility at Beltsville for evaluation of isolate severity (5). All three isolates were relatively mild on Mexican lime. The mildest isolate was tested on other indicators and produced mild seedling yellows, a mild to moderate reaction in sweet orange grafted on sour orange, and a mild pitting in Duncan grapefruit. It reacted positively to the CTV monoclonal CTV-MCA13. A similar reaction was noted in an isolate collected from a naturally infected lime near Malama Ki. Most other Hawaiian sources tested have given a much stronger symptom response.

While *T. citricidus* is commonly found over much of the island, it was never observed in our surveys of the South Point planting. Scattered infestations of *Aphis citricola* were observed periodically.

Several plantings of young trees were made between 1978 and 1986, in experimental plots at Malama Ki and Waiakea to test for cross-protection effects (Fig. 1). These included virus-free Mexican lime and navel orange trees as well as lime and orange trees infected with mild Florida isolates of CTV. Existing trees carrying severe isolates of CTV were common at both sites. High rates of natural infection occurred in virus-free plants and plants infected with mild isolates. A moderate rate of natural infection occurred in a smaller test planting near Hawaii (fig. 1), where only scattered dooryard citrus was present as an inoculum source. Infection rates of 82 to 100% were observed within 6 months after exposing

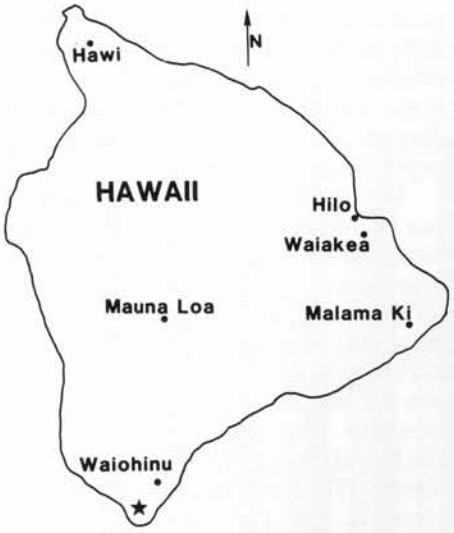

Fig. 1. Outline map of island of Hawaii showing location of sample grove near South Point (*) and other locations referred to in text.

young seedlings of rough lemon, Volkamer lemon and alemow to field infestation by aphids at Kona.

DISCUSSION

The trees planted at South Point were virus-free nursery stock from California. Our assays confirm that they were probably tristeza-free at the time of planting and indicate that CTV has spread into the planting at a slow rate. The alternate conclusion is that the trees are infected with a CTV isolate we could not detect. Presence of a CTV isolate undectable by the DAS ELISA procedure is highly unlikely (2) and not supported by any data or visual observations. It is unlikely that negative results were due to low virus titer or poor test conditions. Consistent results were obtained in separate assays, tissue samples were from prime new flush, and samples from known positives within the planting and from other locations gave strong, clear reactions. The mild subtropical weather conditions at the location are very favorable for CTV replication.

The correlation between results from random samples taken throughout the block with those from localized areas also indicates that the low rate of infection observed was not due to chance sampling error. The increase in infecting rates observed between 1983 and 1986 suggests that CTV is gradually spreading throughout the planting. Detection of new infections near known infected trees also suggests that secondary spread within the planting may become significant.

The reasons that CTV spread has been slow within this planting are not completely clear. The planting is isolated on three sides from other citrus by its location on a seacoast peninsula. Onshore winds would not bring viruliferous aphids to the planting, and the planting is also protected by windbreaks. However, isolation alone is probably not the answer. CTV-infected citrus was found within several kilometers of the planting and in a backyard planting less than 1 km away.

The vector situation within the planting may be quite important. During most of the period covered by our surveys, the planting received frequent applications of pesticides to control fruit flies, and this may have suppressed aphid populations. However, the planting also existed in a semi-abandoned state for several years prior to our initial survey in 1979 with no aphid control. While scattered infestations of *A. citricola* were observed periodically during the surveys, no massive buildups were noted. The planting was visited on numerous occasions between 1982 and 1989 by one or more of the authors, and *T. citricidus* was never observed despite deliberate efforts to locate an infestation. This aphid was also not recovered in yellow pan traps placed in the planting in 1988. Interestingly, *T. citricidus* was observed in high populations in a semi-commercial planting at Waiohinu in 1982, and this site is less than 5 km from the South Point planting and apparently under similar environmental conditions.

While aphid populations and virus movement can presumably fluctuate from year to year, the South Point planting was 17 yr old in 1986, and at

least much of that period must have been favorable for vector activity. High rates of natural infection were observed in experimental plantings at other locations between 1978 and 1988. Climatic conditions vary markedly over the island, but natural infection was observed in three separate locations and in one of these (Hawi), only limited inoculum was present.

The isolate of CTV recovered in the South Point planting has given a milder response in our CTV indicator plants than most Hawaiian sources. Its vectorability has not yet been determined, but another isolate of CTV from Hawaii with similar symptom severity has not been highly transmissible in experimental tests (11). It is possible that primary infections in the South Point location have been primarily with isolates of low vectorability and that this has affected secondary spread.

ELISA assays revealed that only one of six trees affected with blight was also infected with CTV and further confirm the lack of relationship between CTV infection and blight. Presence of blight in the absence of CTV is difficult to demonstrate in areas where CTV is endemic such as Florida, Brazil, and South Africa.

Based on the results obtained, it appears that careful selection of planting sites could markedly influence the impact of CTV on new plantings in areas where severe effects would normally be expected due to presence of severe CTV strains and efficient vectors. Even if ingress of damaging isolates eventually occurs, significant production returns can be realized before that occurs. Earlier, CTV survey results from China, where *T. citricidus* is also endemic, indicated that natural spread of CTV may be slower than expected in some areas (8).

While mild strain cross-protection can be successful against stem pitting forms of CTV (4), under severe challenge pressure cross protection with CTV, as well as with other plant viruses, may break down (7). Combining a cross-protection strategy with selection of locations with less challenge pressure may yield success where neither strategy alone would be successful (7). Even where primary ingress is not prevented, secondary spread would be reduced.

With the advent of rapid detection procedures for CTV, it is now possible to measure challenge pressure in different sites by periodic testing of virus-free trap plants put in candidate locations.

ACKNOWLEDGMENTS

The authors would like to acknowledge the cooperation of Mr. Martin Bassan who provided repeated access to the planting at South Point, the technical assistance of Carmen Henderson and Francis Zee, and the cooperation of personnel from the Hawaii State Department of Agriculture for some of the ELISA assays. This project was supported in part by U. S. Department of Agriculture Cooperative Agreement No. 58-9AHZ-1-615.

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