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TRISTEZA

The Worldwide Threat from Destructive Isolates of Citrus Tristeza Virus-A Review

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ABSTRACT. This paper reviews the effects of extremely destructive forms of citrus tristeza virus (CTV) which poses serious threats to citrus industries worldwide. These include Capao Bonito CTV in Brazil, navel orange stem pitting CTV in Peru, stem pitting 12B CTV found in the university orchards in Southern California, severe grapefruit stem pitting CTV found in South Africa, recent forms of CTV responsible for decline of sweet orange on sour orange rootstock in Florida and Israel and other severe CTV isolates reported from Spain and elsewhere. Many of these destructive CTV isolates are transmitted by *Toxoptera citricidus* but most can be transmitted by *Aphis gossypii* at relatively high levels of efficiency. The impact of recent changes in aphid transmissibility and population dynamics, and the threat of movement of *T. citricidus* into new regions of the world are reviewed. The appearance and impact of new strains or mutants of CTV differing in pathogenic capacities or in aphid transmissibility are discussed. Methods for the identification of new or destructive isolates of CTV are also reviewed. Concepts for prevention which include quarantine, eradication and education are presented. The immediate need is to test for presence of CTV in those countries where sour orange is the predominant rootstock. Also, to test for and eliminate very destructive forms of CTV, to strengthen quarantine laws and regulations, and to educate scientists, nurseryman and growers to the dangers involved in budwood importation and virus or vector spread.

Tristeza, caused by the citrus tristeza virus (CTV) remains today as one of the most destructive of all diseases of citrus. It is an international disease recognizing no borders. The potential for spread of severe forms of the virus, and the movement of its principal vector, *Toxoptera citricidus*, into new areas, remains a constant threat. The destruction of many tens of millions of trees by tristeza in the 1930's through the 1950's was a major incentive for the formation of the International Organization of Citrus Viroloists (IOCV). Publications on tristeza are in the thousands and predominate in each of the eleven proceedings of the IOCV, signifying its importance and the extent of worldwide interest and research.

There are still many citrus producing countries in the Mediterranean region, in Central America, in Mexico or in the Caribbean Islands where the predominant rootstock is sour orange. The citrus industries in these countries face a real threat from the potential ravages of tristeza once it begins to spread. Whenever a search is made in countries where citrus is successfully grown on sour orange rootstock, CTV is usually found. The amount of CTV found is in direct proportion to the energy put into the search. Unaware growers and nurserymen traveling abroad continue to bring in foreign budwood. Some of these introduced cultivars contain severe CTV isolates which eventually can be transmitted efficiently by local aphids (6). In California and Israel, a continuing battle is being fought to keep certain citrus growing areas free of tristeza by intensive tristeza eradication programs. In many countries where tristeza is now endemic, exotic and destructive CTV isolates are a constant threat and some of these can debilitate and even destroy the citrus industry regardless of the rootstock used. Also, many countries where *T. citricidus* is not present face the real threat of its possible introduction.

The objective of this review is to report the serious threat posed by new and severe isolates of CTV; to review changes in transmissibility of the virus and to review some of the new techniques for detection of CTV. Also, to re-emphasize the importance for strengthening quarantine regulations and to encourage campaigns to educate and inform the public; especially those in the citrus industry on this most serious threat.

NEW DESTRUCTIVE ISOLATES OF CTV.

Several severe and potentially destructive isolates of CTV have been reported. Some attack the scions directly and are not dependent on sensitive rootstooks. Some isolates may be new to a region and can severely affect the bud unions of citrus on CTV-sensitive rootstocks. Examples are:

-- Grapefruit stem pitting isolates in South Africa (15, 40), Australia (14), and Japan (42).
-- The Capao Bonito isolate attacking sweet orange in Brazil (12, 45, 46).
-- The severe 12-B sweet orange stem pitting isolate reported from California (11, 57).
-- The sweet orange stem pitting isolate destroying navel orange production in Peru (59).
-- Recently appearing destructive isolates of CTV affecting sweet orange on sour orange rootstock in Florida (10).
-- A recent development of a new CTV isolate destroying trees in Israel (8, 9).
-- A new stem pitting and destructive isolate affecting navel oranges in Australia. (This outbreak is very recent and occurred in 1990, after presentation of this review).

In South Africa, grapefruit is difficult to grow free of stem pitting, small fruit size and tree decline. Da Graca (15) reported that the problem of decline of grapefruit associated with stem pitting is widespread. "Because of this decline there are few productive grapefruit trees in South Africa over 20 years old and the industry has had to gear itself to this phenomenon by the regular removal of declining trees and the replanting of entire orchards after they reach 16 to 20 years in age". One puzzling development was the bringing of trees of a registered Redblush grapefruit into one area in Natal where they developed severe stem pitting. Trees from the same parent propagated in other areas showed few symptoms. Climatic differences may be a factor in this differential symptom development.

At the variety collection in Kuchinotsu, Japan, several trees of virus-free Marsh grapefruit imported from California were grown at the experiment station. During the first few years, the fruit was normal and large sized. However, after the third fruiting year, the fruit was considerably smaller. Many fruit were not larger than small lemons and most were very small (Fig. 1). Apparently, local severe isolates of CTV were transmitted by *T. citricidus* to these virus-free grapefruit, inducing the smaller sized fruit.

In the extensive variety collection at the Citrus Research Station at Riverside, California, yearly surveys between 1970 and 1977 showed dieback, decline and death of many trees. Many of these were seedling trees. The number of declining trees increased exponentially each year and indexing showed presence of CTV-SY isolates in many trees showing branch dieback. This suggested that seedling yellows tristeza was spreading in the variety collection (54). At this same time a very severe form of CTV was discovered spreading in field 12B near the variety collection (11). This '12B' CTV isolate was found to severely stem pit and stunt all inoculated sweet orange varieties. Transmission studies with *A. gossypii* showed the 12B isolate was 100% transmissible (55, 56). Also, when used as challenge inoculum in a cross protection experiment to sweet orange, there was no protection by 18 of 20 California field isolates of CTV (61). Because of the serious potential for destruction of this and possibly

other seedling yellows or stem pitting isolates, an extensive indexing program was undertaken to test for and destroy all severe forms of CTV in the Citrus Research Station orchards.

In Capao Bonito County, San Paulo State, Brazil. a severe CTV isolate was discovered by Muller *et al.* (45, 46). "This particular strain induced stunting, stem pitting and poor and sometimes misshapened fruits on practically all sweet orange scions budded on sweet orange or Rangpur lime rootstocks. Most sweet orange citrus types previously had been considered tolerant to the commonly occurring CTV strains in Brazil". It is believed that the Capao Bonito strain was introduced from Japan or East Asia (60).

In Peru, navel oranges grown primarily on rough lemon rootstock were a profitable crop domestically and for export. Navel orange trees have been declining since the 1960s. In a consultancy visit to the citrus producing regions of Peru in August 1988 under the sponsorship of concerned citrus growers and nurserymen, the senior author visited the major citrus growing regions in the country. Declining trees of navel orange showing small crops of very small fruit were observed in all coastal citrus growing areas and in all of the orchards visited. Growers were in the process of pulling large blocks of declining navel orange trees and replanting them with mandarins. Leaves were curled (Fig. 2a) and often mottled. When peeled, stems of all sizes were often severely pitted. The pits were very small but extremely abundant (Fig. 2b). The bark was somewhat thick and cheesy and stems would easily break at a node when lightly bent. Severe pitting was observed in the wood of navel, Valencia and tangelo scions and on citrumelo rootstock. In some locations, new plantings of satsuma mandarin were adjacent to navel orange orchards. These satsumas were propagated and increased from budwood imported from Japan. Importations of budwood from Japan by local growers was common and had been going on for years.

The symptoms of stem-pitting and small fruit size found in Peru appeared similar to the symptoms of Capao Bonito CTV-infected trees seen in Brazil. Also, the easy breakage due to excessive stem pitting and the puffed and cheesy bark were the same as that observed in sweet orange seedlings inoculated with the 12B isolate found in California. Indexing of the Peru isolate at the USDA facilities at Beltsville, Maryland showed exceptionally severe reactions on indicator plants (26). Reactions were comparable to those of the Brazil Capao Bonito and the California 12B isolates.

In Florida, Brlansky *et al.* (10) reported the decline of sweet orange trees on sour orange rootstock in a 100 sq. mi. area west of Ft. Pierce and in a 200 sq. mi. area south of Labelle. Losses greater than 50% occurred in some plantings. They reported that severe isolates of CTV causing quick decline and stunting were becoming more abundant in Florida. In the LaBelle area, 90% of the young trees were found infected with a severe stunting isolate. It is apparent from this report and others that new isolates of tristeza are posing a serious threat to citrus plantings on sour orange rootstock in many areas in Florida.

In a visit to Israel in 1988, the senior author observed destruction and decline of Shamouti orange, Minneola tangelo and Ortanique tangor trees on sour orange rootstock in the Morasha Junction area near Tel Aviv. This decline, was first noted in 1985. Israeli scientists reported erratic results in their early index tests for detection of CTV. (8, 9). The destruction of citrus by this Morasha CTV isolate was reminiscent of the description of the death of millions of sweet orange trees on sour orange rootstock reported by Knorr and Ducharme in Argentina in 1951 (35). The decline observed in Israel was devastating with few surviving trees and appeared to spread at a rapid rate. An unusual aspect of this decline was the apparant absence of CTV in many declining trees. In ELISA and biological tests, Ben Zeev

et al. (9) reported many negative responses even with ten samples from around each tree. Later findings by these workers suggested that a unique isolate of CTV might be involved. Possibly, the virus or some toxin moved rapidly from the infection site to the bud union area and induced death of the tree before the virus became distributed in the tree. In indexing tests to lemon and sour orange, the virus was noted as a seedling yellows tristeza. Another factor relating to the rapid spread of tristeza in the Morasha Junction area was probably cultural. The practice of heavy pruning in the winter months produced a vigorous young leaf flush in the spring and resulted in a proliferation of aphids (7). Thus, in Israel there is now a new and very destructive type of tristeza which can kill a tree on sour orange rootstock even before the virus can be detected by rapid indicator methods.

FACTORS WHICH CAN INFLUENCE EPIDEMIOLOGY OF TRISTEZA

The introduction and potential movement of *Toxoptera citricidus*. The potential for rapid movement and spread of *T. citricidus* was described by F. Geraud, an entomologist in Venezuela who conducted an extensive survey for this aphid from 1974 to 1981. In a personal communication he writes "the rapid movement of this vector from one area to another was observed in Venezuela in 1976 to 1978 by myself and collaborators. *T. citricidus* was first found in the southern Lake Maracaibo region of western Venezuela in the Andes foothills in early 1976, apparently spreading from Colombia where the vector was endemic. At about the same time, the aphid was also found localized near the Brazilian border in southwest Venezuela. Within this two-year period the vector moved throughout Venezuela and could be detected almost everywhere in the country where citrus was growing. I feel it was carried long distances by the upper winds and efficiently found citrus relatives to feed on and establish colonies. The first dramatic tristeza disease outbreaks were observed within two years of colonization by *T. citricidus* in the north-central region of Venezuela. There, evidence was found of scattered small endemic foci of diseased trees that had survived for about 20 years (without incurring decline) previous to the colonization of the area by this vector."

Since the first appearance of *T. citricidus* in Venezuela, over 6 million trees on sour orange rootstock have died from tristeza disease (41). A similar very rapid spread of this aphid throughout Argentina was described in the early 1950s by Ducharme *et al.*, (24).

There is no reason why *T. citricidus* could not flourish in any country where citrus is grown. The aphid exists in the colder regions of Japan, in the subtropical regions of South America, and in the dry regions of South Africa and Australia. Climatic conditions in the citrus growing regions of the Mediterranean, Central America, the Caribbean Islands, Mexico or in the USA do not differ from many regions where *T. citricidus* is now endemic. With modern air travel and the ability of *T. citricidus* to move across large distances in a relatively short time (as shown for Venezuela or Argentina) there is the ever continuing danger that this aphid will show up in new areas. The aphid is now reported in Panama, Costa Rica, El Salvador and Honduras (36). If *T. citricidus* becomes well established in Central America, it may be just a matter of time before it will spread north to Mexico and to the United States. Not only is this aphid the primary vector for many severe isolates of CTV but it is a major pest of citrus. The danger from the introduction of this serious pest by air travel is a continuing concern.

Changes in transmissibility of CTV. Bar-Joseph and Loebenstein (5) first showed a change in transmissibility of CTV by *A. gossypii* from 2-5% in existing isolates to 40.7% in a CTV-SY isolate in the Hibet Zion region of

Israel. In California, comprehensive transmission tests by Dickson *et al.* (17) in the 1950s with *A. gossypii* showed a transmission rate for CTV of not more than 5-6%. This has now changed dramatically. In extensive transmission studies between 1976-79, transmission rates were shown to be at 100% for a large number of CTV and CTV-SY isolates obtained from trees in southern and central California (55, 56). This change in transmissibility of CTV was probably responsible for the spread of CTV-SY and the decline and death of trees in the university variety collection at Riverside. Recent studies in Spain and in Florida have shown relatively high transmission rates of CTV by *A. gossypii*. Hermoso de Mendoza *et al.* (28) tested two CTV isolates in Spain and found transmission rates of 28 and 78%. Studies by Yokomi and Garnsey (63) with four CTV isolates in Florida showed transmission rates of CTV by *A. gossypii* of 11 to 53%.

A. *citricola* was shown to be a vector for CTV in Florida, Spain and Israel, but not in California. Early studies with this aphid in Florida showed it to be a relatively poor vector for CTV (47). Studies by Yokomi and Garnsey (63) showed 11 of 38 CTV isolates were transmitted by *A. citricola* compared to 29 of 38 by *A. gossypii*. Comparative studies in Florida with four CTV isolates transmitted by *A. gossypii* and *A. citricola*, showed transmission rates of 17.9 and 6.3% respectively (63). In a similar comparison with T-300, a common Spanish isolate, Hermoso de Mendoza *et al.* (28), obtained 78% transmission with *A. gossypii* but only 6% with *A. citricola*. In the citrus area of Valencia (Spain), *A. citricola* was about 45 times more abundant than *A. gossypii* but only 13 times less efficient in transmitting the CTV isolate T-300 (29). The rate of CTV spread achieved by vectors in a given citrus area will depend not only on the transmission efficiency of aphid species present but also on the relative abundance of each species. Although *A. gossypii* is a more efficient vector then *A. citricola*, population dynamics must be considered. *A. citricola* is far more abundant than *A. gossypii*. Yokomi (personal communication) reports: "in Florida the Spirea aphid is extremely abundant and exhibits much alate activity. This is of great epidemiological importance, even though it has a low vector efficiency".

Variations in the balance of aphid fauna. Changes in the composition of the aphid fauna may also occur as a consequence of several factors such as selective resistance to certain pesticides, variations in the population of natural enemies or changes favoring population increase of efficient vector species may have implications in the movement of CTV. An example of variation in the balance of aphid fauna seems to have occurred in the citrus area of La Plana (Spain). A survey in 1986-87 showed that *A. citricola* accounted for 40-58% of the total aphid population and *A. gossypii* for 35-50% (30). Previous surveys in 1974-78, 1980-81, and 1984-85 showed that *A. citricola* was about 90% of the total aphid population and *A. gossypii* less than 2%. Consequently the great difference in vector efficiency between both species plus the aphid population dynamics in La Plana could accelerate CTV spread in this citrus area.

Introduction of exotic CTV isolates and increase in vector transmissibility. Severe CTV isolates have been introduced into many countries but have remained quiescent for lack of transmission. This was certainly true at the University of California variety collection in Riverside where known CTV and CTV-SY isolates were present in the original introductions as cuttings or grafted scions. Between 1910 and 1963, trees on sour orange rootstocks adjacent to CTV-infected introductions did not become infected (57). CTV isolates were known to be present in Spain and in Israel 25 yr before outbreak of the disease. There are reports from Texas (48), Turkey (3), Italy (13) and from Iran (25) of CTV-infected trees surrounded by trees on CTV susceptible rootstocks which showed little or no infection. The

presence of these quiescent CTV isolates represent a real danger if efficient vectors should move in or changes should occur in their transmissibility. Examples illustrating both situations are the movement of CTV in Venezuela following the introduction of *T. citricidus* and the spread of severe CTV-SY by *A. gossypii* at the University of California variety collection in Riverside. These examples should encourage the implementation of intensive programs for CTV detection and eradication of infected trees in citrus areas where the incidence of virus is low. Potentially destructive isolates should be indentified in areas where CTV is already endemic.

Once a new CTV isolate begins to spread by vector it may be very difficult to prevent an epidemic. The dynamics and equations in temporal spread of CTV by aphids, and the relationship of aphids and CTV are comprehensively reviewed by Raccah *et al* (52) and Bar-Joseph *et al.* (7).

Certain cultural practices may increase the rate of natural spread of CTV by aphids, e.g., heavy pruning of citrus trees and topworking to other cultivars, common practices in Spain and Israel. Severely pruned trees produce a vigorous young leaf flush in the spring resulting in a proliferation of aphids. This factor was probably related to the rapid spread of CTV in the Morasha Junction area in Israel and has been a primary factor in the introduction and spread of the virus in many citrus areas in Spain.

Possible change in virulence of the virus and the invisible virus. The presence of different strains or mixtures of strains of CTV hidden in tolerant varieties and the eventual appearance or segregation of mutants differing in their pathogenic capabilities or transmissibilities may be paramount to understanding the epidemiology of tristeza. In most cases CTV exists symptomless in sweet orange or mandarin. It is conceivable that CTV, as with other viruses, does not have a repair mechanism to correct transcription errors during the replication. Thus, any

change or mutation occurring during replication will remain and accumulate. Since CTV has one of the largest genomes of any virus, a certain number of errors are likely to occur and these punctual mutations may be undetectable or result in certain changes in biological or biochemical properties.

Biological variability for CTV is well documented. Large variations exist in symptom expression on field trees as well as on indicator plants. Changes in aphid transmissibility as occurred in California and in Israel are also examples of variability of CTV within a host. Raccah, *et al.* (51) showed segregation of isolates of CTV with variable transmissibility and suggested "that tristeza-infected trees could harbor more than one variant and that trees from a location where limited tristeza spread is observed could contain CTV variants that are highly transmissible but are quantitatively suppressed by a dominating poorly transmissible variant".

Biochemical variability is perhaps less documented due to inherent difficulties. However there is some recent evidence of differing dsRNA patterns in plants infected with CTV isolates having similar biological properties (43, 44). Differences in peptide maps of CTV isolates having similar biological properties and the same dsRNA patterns (27) is another indication of this variability.

Direct evidence has been obtained for the presence within an isolate from a single source of several virus strains differing in serological properties or in the dsRNA pattern induced on host plants. Kano *et al.* (34) observed that some aphid-inoculated CTV-infected plants failed to react with the strain-specific monoclonal antibody MCA-13 even though the source of inoculum did react with this same antibody. Moreno *et al.* (44) obtained segregation of variants having different dsRNA profiles from a single source by graft-inoculating several citron plants with bark inoculum from a lime plant recently inoculated by aphids. Both findings suggested the coexistence of different

CTV mutants irregularly distributed in the source plant.

Under these circumstances it is not difficult to conceive natural segregation of mutants by aphid transmission, by budwood propagation or by changes in the balance of variants present within a plant, due to changes in the environmental conditions. Thus, the "invisible virus" present in symptomless or tolerant hosts may show up and give rise to new severe strains or to highly transmissible severe variants when propagated as new varieties. These strains may be vector transmitted at high rates of efficiency or show new and extensive symptoms under different environmental conditions.

DETECTION PROCEDURES FOR IDENTIFYING SEVERE CTV-SY AND CTV-SP ISOLATES

Due to the wide biological diversity of CTV, any strategy to control damage caused by severe variants of this pathogen requires accurate procedures to identify virus strains. At the present time, the only consistently reliable method for identification of severe CTV-SY and CTV-SP isolates is by biological indexing. This is a very costly and time consuming process and new technology is urgently needed to replace plant indexing. The California seedling yellows eradication program is one example of an intensive program designed for the detection and eradication of severe CTV-SY isolates using biological indexing. Over 20,000 trees in the Citrus Experiment Station orchards were indexed for the presence of both CTV-SY and severe stem pitting isolates using grapefruit, sour orange and sweet orange indicators. The procedures involved the collecting and labeling of budsticks and graft-inoculation to grapefruit seedlings. These were held for 3-4 months for observation and reading for seedling yellows and stem pitting. Positive controls were always included. Budwood from all infected and suspect trees were recollected and further inoculated to sour orange and sweet orange

seedlings to determine the severity of the CTV-SY isolate. Seedlings required 9-12 months of care from seed planting to inoculation, and another 3-4 months for reading after inoculation. Although this is a difficult and long term process, it was the only available and secure technology for determining the presence of severe CTV isolates.

In addition to biological testing, there is currently new technology for differentiating the more severe from the non-severe reacting CTV isolates. The four techniques cited below depend on different properties of the virus and offer some promise for strain identification.

Analysis of dsRNA in CTV infected plants. This technique is based on the fact that healthy plants do not contain detectable amounts of dsRNA whereas CTV-infected plants contain a dsRNA band of 13.3×10^6 kd, corresponding to the full length replicative form of the virus plus several subgenomic bands. The number and position of these bands are constant for each CTV isolate when assayed in the same host. The current procedure for dsRNA analysis consists of a phenol extraction of nucleic acids from young bark tissue, dsRNA purification by CF-11 cellulose column chromatography and separation of dsRNAs by polyacrylamide gel electrophoresis (18, 19, 21). This is a quick and simple method for identification of CTV strains, but it has the following limitations: i) dsRNA patterns of some isolates may change depending on the host and on the season (32, 43); ii) dsRNA patterns are not necessarily related to pathogenic capabilities of CTV isolates. Some isolates having similar biological characteristics may differ in their dsRNA profiles, whereas some others having the same dsRNA pattern may differ widely in biological behavior (43); iii) The presence of low molecular weight dsRNA band (0.5×10^6) has been associated with severe CTV isolates inducing a seedling yellows reaction and/or stem pitting in grapefruit (19, 32). However, in other citrus areas such as Spain or Corsica

this dsRNA band can also be found in some mild reacting CTV isolates (1, 43); iv) Under field conditions, dsRNA recovery has strong seasonal variations and sampling has to be carefully timed to detect the complete dsRNA profile (21, 43).

Hybridization with complimentary DNA (cDNA) probes to the virus genome. This technique is based on the specific hybridization of nucleic acids with complimentary strands. The viral RNA is usually attached to a membrane and the cDNA probe, labelled with P_{32}, hybridizes on the membrane whereas the unreactive probe is washed off. Reaction is detected by autoradiography of the membrane using a special film and an amplifying system. Since biological properties of the virus are encoded by the viral genome, in theory, any variation in the nucleotide sequence of the genome should be detectable by this system, as far as sufficiently specific cDNA probes would be available. In practice, the system has not been widely exploited although there are some indications of its increased usage.

The major limitations of this technique are: i) A wide range of highly specific probes would be necessary for unequivocal classification of CTV strains. ii) At present, the necessity of radioactive labeling of cDNA probes for sufficient sensitivity in the assay is a major drawback of the technique. A number of laboratories are presently trying to develop sensitive assays with non-radioactive probes to transform cDNA hybridization into an easy and sensitive assay similar to ELISA.

Strain-specific monoclonal antibodies. Recently, strain discriminating antibodies have been obtained in Florida (31, 49, 50) and in Taiwan (62). These enable a quick and reliable classification of CTV isolates in different serotypes by ELISA (33, 62). In Florida there has been good correlation between CTV isolates reacting with monoclonal antibody MCA-13 and those inducing decline of sweet on sour orange rootstocks (31, 50, 53, 64). This procedure has great potential for grouping CTV isolates and for monitoring movement of certain types of CTV in the field or within a plant, even when other non-reactive isolates are already established (53, 64). The major limitation of strain discriminating monoclonal antibodies are: i) A wide battery of monoclonals to different epitopes will be necessary in order to get an accurate strain identification. ii) At present, CTV variability detected by monoclonal antibodies is only in the viral coat protein, which barely represents 3% of the genome of CTV. In fact, pathogenic capabilities of any CTV variant may not be necessarily linked to variations in the coat protein. In the future, monoclonal antibodies could develop to noncapsid virus-induced proteins in the infected plants. This would widen the possibilities for detecting genetic variability by serological methods.

Peptide map analysis of virion coat protein. This procedure consists of purifying the viral coat protein, making a partial digestion with endoproteases and separating the resulting peptides by electrophoresis (27,39). Small changes in the amino acid sequence of the coat protein may result in a differing set of peptides. Peptides can be further characterized by Western blot using different monoclonal antibodies. By this procedure CTV isolates differing in biological properties and dsRNA as well as some having similar biological behavior and the same dsRNA pattern could be distinguished (27). The major limitations of this procedure are: i) The method is long and tedious and consequently not practical for routine identification of field strains. ii) As in serological identification, peptide maps only reflect variability of the coat protein which may not be related to pathogenicity.

CONCEPTS FOR PREVENTION

Quarantine. In those countries where tristeza is not endemic, strict quarantine measures are necessary if entry of CTV isolates is to be prevented. This may be a very difficult

problem. For example, in California, movement of tristeza-infected budwood into the central valley has been quarantined since 1947. Yet, despite legislation and cooperation by most growers, over 25,000 tristeza-infected trees were detected in over 400 properties in the central valley. Uninformed growers continue to bring infected material from southern California, where tristeza is endemic, into the area. Recently, heavy fines were levied and severe penalties were given to a citrus nurseryman who broke quarantine regulations and illegally brought budwood into the state (2). This incident was primarily responsible for new legislation which now sets very high fines for any illegal introduction of budwood into California. Unless there is strict enforcement plus a program for education on the importance of not bringing in trees or budwood into any region except via specific certification programs, quarantine will break down. Yet, quarantine remains one of the most important means for preventing the introduction of new, exotic and potentially destructive CTV isolates.

In countries such as Peru, where apparently no quarantine program has been enforced, importation of satsuma and other mandarin budwood directly from Japan was undoubtedly responsible for introduction of severe CTV isolates resulting in the debilitation of their navel orange industry (59).

Eradication. Once a substantial number of trees are found dying due to infection by tristeza, it may be very difficult to prevent an epidemic. If temperature conditions are optimal, CTV isolates are transmissible and aphid vectors are abundant progressively rapid decline may be inevitable. In southern California, the fortuitous circumstances of the early detection of severe CTV isolates in the variety collection at UCR, plus the massive infusion of money and energy into an eradication program, may have prevented an epidemic. The combined efforts of the University of California and the citrus industry enabled the building of several greenhouses suitably staffed.

This made surveys, collection of budwood and indexing possible. This program, though considerably diminished in scope, continues on a statewide level (23).

The tristeza eradication program in the central valley of California is perhaps the oldest in the world for detection and elimination of tristeza-infected trees. This program began when tristeza-infected trees were accidentally moved from southern California (where tristeza is endemic) to the central valley of California (where tristeza is precluded) (58). Initially, the inspection for CTV-infected trees involved search for these accidentally introduced trees. However, once testing began, the number of trees found infected with CTV was alarming. Since its inception in 1963, over 4 million trees have been indexed and tristeza is still being detected. This testing and eradication program in the central valley continues. It is primarily grower funded with direction and support from the California Department of Food and Agriculture. Some CTV isolates appeared to be spreading more rapidly in certain orchards. The isolates were tested for transmissibility using *A. gossypii* and were found to be 100% transmissible (55, 56). The long term success of this most important tristeza eradication program will depend on the continued support by the growers and by the California State Department of Agriculture. New ELISA technology has help speed up detection.

The Israeli citrus tristeza eradication program is perhaps the most efficient and completely computerized of any program. Bar-Joseph *et al.* (7) reports that between 1970-1977, more than 300,000 tests were made by indexing to Mexican lime and by electron microscopy. Since that time many millions of trees have been indexed by ELISA. However, despite the ability to index millions of trees per year by use of ELISA in this most efficient facility, tristeza continues to spread. In the excellent and comprehensive review on the continued challenge of control of CTV, Bar-Joseph *et al.* (7) listed

four main factors contributing to the continuing epidemic: "1) allocation of limited resources 2) failure to impose regulations and lack of grower cooperation in timely removal of infected trees 3) low rates of CTV detection by ELISA in certain groves carrying CTV isolates that caused a rapid CTV decline (9); and 4) the recent adoption of the horticultural practice of topping and hedging mature citrus trees which caused both increased aphid vector populations and acceleration in the spread of the virus." The reviewers further reported: "The eradication efforts which so far involved some six million tests at an estimated cost of US $5 million, were not effective in suppressing the disease, but undoubtedly extended citrus production on the sour orange rootstock for 5-10 years."

In Spain, a severe CTV isolate inducing seedling yellows and stem pitting in grapefruit and other citrus species was discovered in an early satsuma cultivar illegally introduced, probably from Japan (4). Since this severe isolate was initially restricted to this particular cultivar and this cultivar was new in Spain, an eradication program was established in the Valencian Community on the following basis: Growers who had propagated the early satsuma cultivar had to make a mandatory statement declaring plots in which they had made propagations. The Plant Protection personnel took samples of these plots and indexed for CTV by ELISA. If any of the tests were positive the entire planting was destroyed and the grower was compensated. Current estimates are that 80% of propagated trees have been removed. Plantings surrounding these satsumas will be surveyed by ELISA for CTV and a percentage of infected trees will be indexed for presence of severe strains by biological indexing or by any of the procedures described herein.

It thus appears that eradication can be effective under some circumstances, but may be difficult under certain environmental conditions and

without sufficient government and grower support.

Education. This remains as a very powerful and effective tool for limiting import of destructive CTV isolates (or any other citrus pathogens). This is particularly true for areas of the world (such as countries of the Mediterranean basin, Mexico, Central America or the Caribbean islands) where tristeza is not endemic and not causing decline of sweet orange on sour orange rootstocks. Knowledge of the destructive capacity and rapacity of tristeza has been known for many years. It is estimated that over 50 million trees were killed by tristeza and the virus continues to destroy and debilitate citrus in many countries. The IOCV was founded on this need to learn about why tristeza destroys and, through the Organization and its publications, there is an excellent opportunity for members to share and use this information to publicize the many dangers of tristeza. Publicity must be *repeatedly* given to extension personnel, to local citrus journals, to the press and television, at airports and to travelers. Growers, and especially nurseryman, must be informed and made aware that bringing untested budwood into their citrus growing area can be potentially dangerous and destructive to their orchards as well as to their industry. Educational programs should be stepped up to meet the ever increasing ease of bring in budwood via modern air transport. It is not enough for informed scientists to exchange information amongst themselves at meetings and through technical publications, but they have a duty to inform the public and publicize their findings, and the findings of others. They must take leadership and write for popular journals on the dangers of tristeza. These warnings should be frequent and constantly repeated. Informed and educated growers or nurserymen can be forearmed and they can materially aid in not only preventing introduction of new pathogens, but in supporting programs for education and research.

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