

## The Occurrence of Citrus Tristeza Virus in the Republic of Yemen

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### Abstract

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A survey for citrus tristeza virus (CTV) was conducted in the main citrus producing areas of the Republic of Yemen. 190 samples from 24 locations in eight areas were collected and tested for CTV. Of these samples, 48 were identified positive for CTV by ELISA tests. CTV was found with varying disease incidence in five of the eight areas surveyed. The virus strain prevalent in the Republic of Yemen seems to be a mild one.

**Key words:** Citrus, ELISA, quarantine, Tristeza, quick decline, Yemen.

### Introduction

Citrus production in the Republic of Yemen in 1991 was about 21558 tons of fruits (3). The value of the crop amounted to 569 millions Yemeni Rials, corresponding to 3.2% of the total value of agricultural crops of the country.

Most of the citrus production is in the northern part of the country, which accounts for 87% of the total production. The best producing regions are around Marib, Harib, Sa'dah, Hodeidah, Taiz and Hamman Ali (8). In the southern and eastern parts of the country only 641 hectares are grown to citrus, mainly in the district Abyen and around Lawdar and Mudia. Only few citrus orchards can be found in Al-Jawf (Bayhan), Hadramout (Seyun) and Lahj.

Citrus tristeza virus is an economically important virus disease of citrus. It is regarded as one of the major disease problems affecting citrus production worldwide (10) and serious losses have been reported (6, 13).

In a survey on crop virus diseases in Yemen between 1985 and 1988, several viruses on different vegetable crops were identified (15). However, citrus was not included in that survey. Dimitman (8) identified trees with CTV symptoms at Surdud in the Tihama and at Taiz. These trees have been imported from India and Japan. Bové and Garnier (5) tested several citrus samples from the Taiz area. Electron microscopy revealed the presence of mycoplasma-like organism (MLO) and the pathogen of the greening disease. ELISA tests, performed on these samples were all negative for CTV. From samples taken from the Sa'dah area in 1988, some were found positive for CTV in ELISA tests performed in England (Moghni, pers. comm.). CTV was reported for the first time in Yemen by EPPO (9).

In 1992, an ELISA laboratory was established in the Yemeni-German Plant Protection Project (YGPPP) at Sana'a. The laboratory was functional since 1993, and the Department of Plant Protection has now the capacity to identify virus diseases.

The main objective of this work was to obtain more quantitative data on the occurrence and distribution of CTV in the Republic of Yemen.

### Material and Methods

The survey was conducted in November and December of 1993, when temperatures are low, which facilitates virus detection in trees (2). The survey and sampling data are presented in Table 1.

**Table 1.** Dates and locations, where citrus samples were collected.

Date	Region	No. of Locations	No. of samples
03/11/93	Hamman Ali	3	35
17/11/93	Marib	4	34
21/11/93	Sa'dah	4	25
01/12/93	Taiz	4	30
04/12/93	Sana'a	1	16
05/12/93	Mudia	3	15
11/12/93	Seyun	4	20
15/12/93	Lawdar	1	15
<b>Total</b>		<b>24</b>	<b>190</b>

For sampling, only trees with symptoms suggestive of CTV infection were selected. Depending on location visited, about one out of 50-100 trees displayed potential symptoms of CTV; stem pitting, top-decline, yellowing and stunting. These trees were marked for sampling by a numbered ribbon and their position was fixed in a small design for easier re-identification of the tree after testing. Each sample taken from a marked tree consisted of four small twigs with young leaves cut from four different sides within the tree. The combined sample from one tree was kept in a plastic bottle, marked with the same number as the tree. The bottles with the samples were transported in an ice-chest to the laboratory, and stored in a refrigerator until tested by ELISA, 10 days after sampling, at the latest.

For the preparation of one individual test sample, one leaf of each of the four twigs was taken, so that leaves from all four quadrants of the tree were included in the test. The

leaves were ground in a leaf press (MEKU Wannigsen/Germany). The leaf samples was diluted with a grinding buffer in the ratio 1:1.5 (W/V).

In the ELISA test, the double antibody sandwich (DAS) technique was used (7, 12). The CTV antiserum was obtained from Loewe laboratories (Otterfing/ Germany). Dilution's used for antiserum coating and conjugate were 1:400. Each sample was tested in two wells for more accurate determinations. To monitor the performance of the ELISA-test, the microtiter plate was provided with two CTV positive and negative controls each. Reactions were read at 405 nm in a spectrophotometer (Anthos II). A sample was considered CTV positive, when absorbancy readings were at least three times of the values of the negative (Healthy) control, and readings of both wells were similar.

## Results

Disease incidence of CTV differed between samples from the various areas surveyed. The results of the ELISA tests are presented in Table 2.

**Table 2.** Number of samples taken and number of CTV-positive samples detected from different locations.

Area	Samples positive/ Samples taken				Total
	Location 1	Locati. 2	Locati. 3	Locat. 4	
Hammam Ali	1/09	1/12	0/14	-	2/35
Marib	0/22	0/04	0/03	2/05	2/34
Sa'dah	1/08	0/04	6/10	3/03	10/25
Taiz	5/11	3/07	2/07	2/05	12/30
Sana'a	0/16	-	-	-	0/16
Mudia	2/05	3/05	4/05	-	9/15
Seyun	0/06	0/06	0/05	0/03	0/20
Lawdar	13/15	-	-	-	13/15
<b>Total</b>	<b>22/125</b>	<b>7/38</b>	<b>12/44</b>	<b>7/16</b>	<b>48/190</b>

No CTV was found in Sana's, where samples from only one location were tested. Also none of the samples collected from the four locations around Seyun in Hadramout was infected with the virus. On the national scale, Seyun and Sana'a are of minor importance for citrus production.

In the other areas surveyed, CTV incidence varied. Low disease incidence of around 5% was found in Hammam Ali, where CTV was present in samples collected from two out of three locations, and in Marib, in samples collected from one out of three locations were positive.

40% of the samples from Sa'dah and Taiz were infected with the virus. CTV was detected in samples collected from three out of four locations from Sa'dah, and in all four locations from Taiz.

In the Mudia/Lawdar region, samples from all three locations of the Abyen Governorate in the south were positive

for CTV. 60% and 87%, respectively, of the samples tested from this region were infected, which was the highest CTV incidence found in Yemen.

## Discussion

This survey showed presence of CTV in the main citrus producing areas, North and South of Yemen. Although negative test results were obtained from samples of Seyun and Sana'a, it cannot be concluded that CTV is not present in that area, as sample size and number of locations surveyed in this region was small.

On the basis of this limited survey, a tentative extrapolation of CTV incidence in Yemen suggest that four out of 1000 trees may have the disease. However, actual disease incidence may be higher, as trees with no obvious CTV symptoms were assumed to be disease free, which might not be the case.

In this study, CTV was detected in a low incidence in most citrus producing areas of the country. Disease spread is either by insect vectors or by man through propagation, mainly in nursery multiplication. Concerning insect vectors, the tropical citrus aphid *Toxoptera citricidus*, which is the most efficient CTV vector (14), has not been observed yet in the Republic of Yemen (van Harten, Pers, communication). However, among different *Aphis* species found in Yemen (8) identified *Aphis gossypii*, which is as well an effective vector of many isolates (4). Therefore, it can be presumed, that CTV could become a problem of greater magnitude in the future.

Pitting was used as the main selection criterion for sampling trees during the survey. As this symptoms was never strongly expressed and it was observed only at a low degree, it was assumed that the strain of CTV prevalent in the Republic of Yemen may be a mild one. The situation hence may resemble that of India, where mild strains are more prevalent than severe and devastating ones (1). Differentiation between mild and aggressive strains can be done by biological indexing, but was not pursued in this work.

To insure, that no severe strains are imported, the Quarantine Department of the Yemeni Plant Protection will further reinforce strict phytosanitary measures, where the FAO/IBPGR technical guidelines for the safe movement of citrus germplasm are being followed (11). The ELISA laboratory provides rapid virus tests with high reliability. This is needed, as identification of CTV by symptoms alone is not reliable; stunting, yellowing and top-dieback can be caused by several disease agents. Stem pitting, besides CTV, can also be caused by *Spiroplasma citri* (10). Biological indexing on the other hand is expensive and time consuming. Therefore, the ELISA technique is a very valuable tool for quick virus detection.

So far, propagation and distribution of citrus seedlings is not controlled and no certification program for CTV-free planting material exists in Yemen at present, but it is hoped to be operational soon. The survey results support the need of a national certification program as an important future task in the Republic of Yemen.

## الملخص

دربر، و. ج.، ل. أ. الخميسي و ح. م. الأكبري. 1996. الكشف عن فيروس التدهور السريع (تريستيذا) على الحمضيات/ الموالح في الجمهورية اليمنية. مجلة وقاية النبات العربية. 14(1): 54-56.

تم القيام بحصر لفيروس التدهور السريع (تريستيذا) في بيارات الحمضيات/ الموالح الرئيسة في الجمهورية اليمنية. وتم جمع 190 عينة من 24 موقعا في ثماني مناطق. واختبرت العينات لمعرفة إصابتها بالفيروس. وأظهرت النتائج تفاعل 48 عينة إيجابياً لفيروس التدهور السريع بواسطة اختبارات الإليزا. ووجد الفيروس بشدة مرضية مختلفة في خمس من المناطق الثماني الممسوحة. ويبدو أن سلالة الفيروس السائدة في اليمن هي من النوع المتوسط الضراوة.

كلمات مفتاحية: حمضيات/ موالح، إليزا، حجر زراعي، فيروس التدهور السريع (تريستيذا)، اليمن.

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