

Research Application Summary

**Incidence and severity of citrus quick decline virus in Abura-Asebu-Kwamankese district, Ghana**

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**Abstract**

*Citrus tristeza virus* (CTV) is one of the most important causal agents of citrus diseases and has varying strains of the tristeza virus. Production of sweet orange in Abura-Asebu-Kwamankese District is highly constrained by quick decline and stem-pitting variants of the Tristeza virus. For better cognition of CTV prevalence, a survey on incidence and severity of CTV was conducted between May 2012 and February 2013 in the six major citrus growing communities in Abura-Asebu-Kwamankese District, Ghana. A total of 4200 sweet orange trees from 60 farms in six communities, were systematically observed for CTV disease incidence and severity during the survey. Symptoms such as stunted growth, leaf cupping, vein clearing, leaf curling, leaf chlorosis, twig die-back, leaf defoliation, reduced fruit size and stem back breaks were used at assessing incidence. Rough lemon were the most preferred rootstock used by farmers in all the locations in establishing sweet orange farm. A severity scale of 1–5 was used. The analysis of variance for both incidence and severity showed a significant difference at across all locations. Disease incidence was highest in Asebu, Amoanda and Edumifa at 75%, 73.5% and 74.6% respectively. There was a significant difference in the severity index between and across four locations; Asebu (3.5), Amoanda (2.7), Abakrampa (3.1) and Nsanfo (2.8) with the exception of Edumifa (2.1) and Bantanya (2.0). The use of CTV free (clean) bud-wood and resistant rootstocks in establishing *Citrus* farms will help the management of the disease.

Key words: *Citrus Tristeza Virus*, incidence, severity

**Résumé**

Citrus tristeza virus (CTV) est l'un des plus importants agents causals des maladies des agrumes et a différentes souches du virus de la Tristeza. La production d'orange douce dans le district d' Abura-Asebu-Kwamankese est fortement limitée par le déclin rapide et les variantes de souches du virus Tristeza. Pour une meilleure connaissance de la prévalence CTV, une enquête sur l'incidence et la gravité de CTV a été menée entre mai 2012 et Février 2013, sur les six principales communautés d'agrumes qui poussent dans le District d' Abura-Asebu-Kwamankese au Ghana. Un total de 4200 arbres d'oranges douces de 60

fermes dans six collectivités, ont été systématiquement observée pour l'incidence des maladies CTV et de sa gravité lors de l'enquête. Les symptômes tels que des retards de croissance, feuille bombement, veine compensation, l'enroulement des feuilles, chlorose des feuilles, Brindille dépérissement, défoliation, la taille réduite des fruits et des tiges de retour des pauses ont été utilisées à l'évaluation de l'incidence. Le citron rugueux était le rhizome le plus préféré et utilisé par les agriculteurs dans tous les endroits dans l'établissement de la ferme d'orange douce. Une échelle de gravité de 1-5 a été utilisé. L'analyse de la variance pour l'incidence et la sévérité a montré une différence significative à travers tous les emplacements. L'incidence de la maladie était plus élevée dans Asebu, Amoanda et Edumifa à 75%, 73,5% et 74,6% respectivement. Il y avait une différence significative dans l'indice de gravité entre et à travers les quatre emplacements; Asebu (3.5), Amoanda (2.7), Abakrampa (3.1) et Nsanfo (2,8), à l'exception des Edumifa (2.1) et Bantanya (2.0). L'utilisation des tiges libre de CTV (propre) et porte-greffes résistants dans l'établissement des fermes de citrus peut aider à gérer la maladie.

Mots clés : *Virus Citrus Tristeza*, incidence, sévérité

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## Background

*Citrus* is the most economically significant fruit crop and a valuable fruit in international trade with over 110 million metric tonnes produced globally and estimated over \$14 billion value (FAO, 2010). *Citrus* fruits are produced worldwide, with Oranges constituting more than two thirds of the world *Citrus* production (FAO, 2010). With over 140 countries globally engaged in the *Citrus* industry, Brazil is the leading producer of *Citrus* followed by the USA, China and Mexico in the second, third and fourth place respectively. Ghana ranks fifth as the leading *Citrus* producing country in Africa with an annual production capacity of 0.6 million tonnes (FAO, 2010). South Africa, Egypt and Algeria are the three leading Orange producing countries in Africa contributing 4.54 million tonnes representing 66% of the total orange produced in Africa (FAO, 2010). The world *Citrus* production has been increasing steadily to accommodate growing demands of domestic and international markets. *Citrus* is not only economically important for generation of income and foreign exchange but also very important in terms of nutrition.

There are many edible species mostly within the genus *Citrus* which are economically very important. The centre of diversity for *Citrus* ranges from north-eastern India through the Malay Archipelago and to South Australia (Reiger, 2006). *Citrus* fruits were introduced to the new world in the late 1400s and early 1500s (Reiger, 2006), and has since been extensively cultivated and consumed in almost every part of the world. *Citrus* collections were established around the world for the purposes of breeding and cultivation which later lead to the introduction of *Citrus* viruses including *Citrus tristeza virus* (CTV) (Korkmaz, 2008). CTV is the most devastating and economically important viral disease in most *Citrus* producing regions of the world (Bar-Joseph *et al.*, 1989; Rocha-Peña *et al.*, 1995). By 1991, over a 100 million trees had been destroyed by CTV quick decline in Argentina, Brazil, Spain, California, Venezuela and other areas (Roistacher, 1992).

Despite the positive economic and nutritional importance *Citrus* plays in Abura-Asebu-Kwamankese district, its production is equally challenged by a myriad of problems of which *Citrus tristeza* disease is the most important (MoFA, 2006). *Citrus* vulnerability to *Citrus tristeza* closterovirus and lack of details on CTV for *Citrus* growing regions augment the necessity to carry out a detailed study for the identification and characterization of CTV strains. CTV causes multiple disease symptoms in sweet orange depending on the virus strain and host susceptibility, but far alarming is the extreme economic losses it causes as a result of reduction in fruit yield, fruit size and fruit quality.

### Literature summary

*Citrus* is one of the leading fruits in many countries worldwide and produced for export and domestic consumption. Many diseases effect its production and quality. Besides fungal and bacterial diseases, other copious graft-transmissible pathogens effect the *Citrus* production (Whiteside *et al.*, 1988). Several types of fastidious prokaryotes, spiroplasma, viruses, viroids and virus-like pathogens of vague etiology are graft-transmissible (Roistacher, 1991). Among the known *Citrus* viruses, *Citrus tristeza closterovirus* (CTV) is the most important and devastating pathogen affecting *Citrus* species worldwide. The diversity of CTV, the crop and ecosystems where *Citrus* is grown create a challenging and complex disease control problem (Garnsey *et al.*, 2005). It is the major cause of decline of *Citrus* trees and infects all species, hybrids and varieties of family Rutaceae (Guzman *et al.*, 2001). The CTV is most likely to have originated from Asia and through the movement of infected plant material it has been disseminated to almost every region of *Citrus* cultivation (Anonymous, 2004). It seems that *Citrus*, CTV and the brown *Citrus* aphid (*Toxoptera citricida* Kirkaldy) all were originated in Asia (Garnsey *et al.*, 2005). It is assumed that the distant past CTV was originated in China (Koizumi, 2001). Tristeza was first thought to be a rootstock-scion incompatibility, or a nutritional problem or a root disease. In Ghana, CTV disease was first reported in 1938 in Lime production (Hughes and Lister, 1949). By 1947, limes could not be grown in Ghana from seeds due to the devastating nature of the disease.

Hughes and Lister (1949) found symptoms including twig die-back, vein flecking of the young leaves and severe stem pitting of the trunks and branches. In 1946, Meneghini transmitted the disease by aphids and experimentally proved it to be of viral origin (Bar-Joseph *et al.*, 1989; Lee and Rocha-Peña, 1992). Earlier reports of incompatibility, nutritional problems and quick decline are now all recognized as different forms of the same viral disease caused by *Citrus tristeza* virus (CTV) (Grant *et al.*, 1951; McClean 1950). Some other disorders found later in *Citrus*, such as stem pitting and seedling yellows (Roistacher, 1982) are also associated now with CTV. Tristeza is now present in most part of the world, with the exception of some Mediterranean and Central American countries, and some isolated islands (Lee and Rocha-Peña, 1992).

### Study description

The study was conducted in Abura-Asebu-Kwamankese district which is geographically located between latitude 5°05'N and 5°25' N and longitude 1°5' W and 1°20' W found in the

Central Region of Ghana. Two experiments were conducted during this study, in the first experiment, a purposive sampling technique proposed by Gray *et al.* (2007) was used to select the major citrus growing communities in the district from which 10 citrus farms were randomly selected from each community for CTV incidence and severity survey. In each farm 70 citrus plants were systematically samples for CTV incidence and severity assessment. A cross sectional survey was further carried out using a structured questionnaire alongside interview schedules to obtain data from sweet orange farmers whose farms were randomly selected for the incidence and severity assessment. The questionnaire was made up of both open and closed ended questions. Farmers were asked series of question such as how long they have been into sweet orange farming, the type of cultivar/variety of sweet orange they cultivate, type of root stocks used, the cropping pattern being practiced by them, the types of crop included in the cropping pattern, major diseases and pests, source of their planting material, major disease symptoms seen on the farm and any management practice(s) put in place to reduce the incidence of pest, prevention and control mechanism used for those pest and diseases. Symptoms such as growth stuntedness, leaf (cupping, chlorosis, defoliation and curling), fruit size as well as twig die-backs were use as key indicators whiles are severity scale of 1 – 5 was used.

## Results and discussion

Results showed that farmers were ignorant and unaware of the prevalence and high incidence of CTV in their farms. Typical and characterized symptoms of CTV such as leaf curling, quick decline, chlorosis, twig die-back, defoliation and reduced number of fruit set were commonly observed (Table 1) in all the farms where the survey was carried out. Twig die-back, defoliation and quick decline symptoms were frequently prominent and more predominant symptoms seen in Asebu and Edumifa (Table 1) and these symptoms were also notably reported by Garnsey *et al.* (1987). These symptoms make citrus production

**Table 1. Rootstocks used in the six citrus locations and reported symptoms**

Location	Rootstock	Frequency	Symptoms reported
Asebu	Rough lemon	10	Twig die-back, defoliation, quick decline
Abakrampa	Rough lemon	10	Twig die-back, defoliation, quick decline
Amoanda	Rough lemon	10	Twig die-back, defoliation, quick decline
Edumfa	Rough lemon	7	Twig die-back, defoliation, quick decline, chlorosis and leaf curling
Nsanfo	Sour orange	3	
	Rough lemon	8	Twig die-back, defoliation, quick decline, chlorosis and leaf curling
Batanya	Sour orange	2	
	Rough lemon	8	Twig die-back, defoliation, quick decline, chlorosis and leaf curling
	Sour orange	2	
Total		60	

uneconomical due to the resultant poor tree vigour and small fruit. The prevailing symptoms in Nsanfo, Abakrampa, Amoanda and Batanya followed a similar trend as those in Asebu and Edumifa (Table 1). However chlorosis and leaf curling symptoms were more prevalent in these locations. These observed symptoms confirm a similar report by Lee *et al.* (2002), describing the diversity of symptoms that are associated with CTV regardless of *citrus* cultivar.

Another factor that can also be linked to the prevalence of some key observable symptoms like vein clearing, die-pick, stem pitting, stunting and leaf corking are related to rootstock/scion combination which has also been reported by Lbida (2005). There was variation in incidence and severity of CTV in sweet orange. Both incidence (Table 2) and severity (Table 3) varied significantly ( $P < 0.05$ ) between locations. Asebu recorded the highest mean CTV incidence of 75.0 % but was not significantly different from Amoanda and Edumifa which recorded a mean incidence of 73.5 and 74.6 respectively (Table 4). Conversely, farms across locations showed no significant variation ( $P < 0.05$ ) in both incidence and severity for sweet orange CTV disease as shown in Tables 2 and 3. The highest CTV severity score were recorded in Asebu (3.5) and Abakrampa (3.1) as shown in Table 4.

The significant differences amongst these locations can be attributed to the transmission efficiency of the aphid species, the plant source (bud-wood and graft materials), pathogenic state of the plant source, grafting infected plant materials on rootstocks or vice versa, temperature variations and differences in the virus titre. These contributory factors are in

**Table 2. Analysis of variance for CTV incidence**

Source of variation	d.f.	Sums of square	Mean square	F. value
Farms	9	3874	430.5	2.99
Location	5	3368.6	673.7	4.68*
Error	45	6475.3	143.9	
Total	59	13718.0		

\*\* = significant at ( $P < 0.05$ )

**Table 3. Analysis of variance for CTV severity**

Source of variation	d.f.	Sums of square	Mean square	F. value
Farms	9	6.5684	0.7298	4.42
Location	5	16.4162	3.2832	19.90**
Error	45	7.4259	0.1650	
Total	59	30.4105		

\* = significant at ( $P < 0.05$ )

**Table 4. Mean separation for incidence and severity at the different locations**

Location	Incidence (%)	Severity
Asebu	75.0a	3.5d
Edumifa	74.6a	2.1a
Amoanda	73.5a	2.7b
Abakrampa	68.2ab	3.1cd
Batanya	59.2bc	2.0a
Nsanfo	56.3c	2.8bc
LSD	10.8	0.37
CV%	12.1	15.2

Mean Values with the same alphabet within a column are not significantly different at described probability level ( $P < 0.05$ ); CV = coefficient of variation; LSD = Least significant difference

positive coherence with Roistacher *et al.* (2002), also reported that the spread and movement of tristeza depends on the distribution of infected citrus bud-wood, the different species of the vector and their abundance, strain of the virus present, the citrus variety infected and the environmental temperatures. Cambara *et al.* (2000) also stated that the initial distribution of CTV in Spain was due to the propagation of uncertified and infected but symptomless plant material.

### Conclusion

The high severity and incidence suggest that citrus tristeza disease is a major threat to production in Abura-Asebu-Kwamankese of Ghana. Moreover, the high severities are likely due to a combination of factors such as; the use of CTV susceptible rootstocks and CTV infected bud-wood in establishing *Citrus* farms. No evidence was found for a source of virus-free citrus seedlings for farmers and perhaps accounting for the high disease incidence recorded in the study sites. The combination of susceptible rootstock and good weather (warm-humid) perpetuates high CTV disease severity and incidence. It is therefore obvious that deployment of virus-free (clean) buds and the use of resistant rootstock will go a long way in management of Citrus tristeza virus disease.

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