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An Overview of Citrus Virus Disease and its Control in Nigeria

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Abstract

A general overview on the status of citrus virus disease and its control in Nigeria is given based on evaluation of commercial fields and nurseries. This paper discusses the citrus viruses that are known or suspected to be transmitted by vectors. The dissemination and spread of the citrus 'virus' diseases continues under this condition, especially in the less developed citrus orchards which are not endowed with the necessary technology for the detection and elimination of most of the major citrus 'viruses' found present in propagetive budwood. Many citrus viruses exist without showing symptoms in many of the cultivars of mandarin, sweet orange, grapefruit, pumello, lemons and limes currently being grown throughout the citrus growing regions in Nigeria. The range of variability of viruses' strains, and the relationships between virus and plant hosts is discussed. The most economically important of these is Citrus tristeza, transmitted by aphids. Nevertheless attempts were justified so as to asses the relative importance of different citrus virus diseases to set priorities for research and extension activities and to evaluate the implication and success as otherwise of the cropping systems and control measure practiced. Different approaches have been adopted, but all depend heavily on personal experience on the limited literature available and a dialogue with virologist literature protection specialist. The final section reviews control methods such as improved genetic resistance to virus which can be combined into an integrated management strategy.

Introduction

Citrus, common name for several related evergreen trees and shrubs that belong to the family *Rutaceae*. The fruits they produce, include; the citron, grapefruit, lemon, lime, orange, shaddock (or pomelo), tangerine, and bergamot (a pear-shaped orange)[1]. It is a major fruit crop in numerous countries, and is grown for both domestic consumption and for export.

The Nigerian citrus industry is mostly concentrated in the southern, southwestern and middle belt regions in virtue of the suitable soil and climatic conditions for the cultivation of the various species which perfect tropical and subtropical regions, but they are highly infected by virus and virus-like diseases.

A number of graft-transmissible pathogens affect citrus. These include at least eight groups of viruses, five groups of viroids, four types of prokaryotes, and a number of uncharacterized viruslike and decline-inducing agents[2]. Several viruses and all of the prokaryotic agents are vector-borne[3]. Many citrus viruses can exist without showing symptoms in many of the citrus cultivars[4], in other words, a symptom less vigorous looking citrus plant is not an indication of the absence of viroid pathogens in the particular plant, may be

the rootstock exhibits some form of tolerance to the virus[5].

In Nigeria, the main vector for the spread of these pathologies is undoubtedly the infected propagating material. Moreover the effect has been deteriorated by continuous use of the top grafting technique, which enables to rapidly introduce new citrus varieties of great economic importance in the existing orchards. Despite the healthy status of old orchards, after a few years most of the grafted varieties show virus infection symptoms.

With increasing world population and diminishing land and water resources, the caloric energy produced by plants should not be diverted to the replication of pathogens[6]. The pathogen should be eliminated and the energy wasted in their multiplication and destructiveness should be conserved and directed towards the production of leaves and fruits for human consumption. The need for a more integrated approach to controlling citrus virus diseases has been recognized. This has promoted increased efforts to develop the necessary information on biological and molecular characteristics of citrus viruses, and also better detection methods, a better knowledge of virus/host interactions, and an improved knowledge of citrus virus epidemiology[7].

The purpose of this paper is to provide an overview of citrus viruses and virus-like pathogens on propagative budwood or suspected to be transmitted by vectors, and information from a virology standpoint that is relevant to developing a more integrated approach to citrus virus disease management.

Classification of Citrus 'Viruses'

At least eight groups of citrus viruses have now been described. These vary in distribution, economic impact and degree of characterization[8] and includes *Citrus tristeza virus* (CTV), an aphid-borne *Closterovirus*, *Vein enation* is also aphid-borne, Leprosis, a *Rhabdovirus*, is vectored by a mite, *Citrus chlorotic dwarf*, recently recognized in Turkey, which is transmitted by the bayberry whitefly (*Parabemesia myricae*), *Satsuma dwarf* is spread by an unknown, soil-borne vector and *Citrus necrotic ringspot,* a severe form of *Psorosis*[7].

Relationships between several citrus viruses and viruses of other crops have been established, and it is possible that some citrus viruses may have moved into citrus from other hosts. For example, *Tatterleaf virus* of citrus is closely related to apple stem grooving virus and a lily latent virus. While natural spread in citrus remains unconfirmed. *Tatter leaf* is widely distributed in many Asian countries. It remains a matter of concern, because most current citrus rootstocks have genetic vulnerability to it[8].

As shown in table 1, Citrus 'virus' diseases in Nigeria may be classified into four groups viz. primarily vector-transmitted; mechanically transmitted; primarily bud transmitted; and diseases of unknown aetiology. Most 'virus' diseases may also be classified as warm or cool temperature dependent. Diseases which are more destructive at cool temperatures are: *Tristeza*, *African greening*, *Vein enation* or *Woody gall*, *Infectious variegation*, *Satsuma dwarf*, *Tatter leaf*, *Concave gum*, *Psorosis* and related oak leaf pattern diseases[2].

Diseases which are more reactive under warm temperatures are: *Stubborn, Exocortis, Cachexia* and *Asian greening.* Diseases whose temperature etiology has not been determined are *Gummy bark, Blight* and *Blight* associated declines.

Warm or cool temperatures can mask the expression of symptoms of specific viruses. The symptomatic small tight compact tree induced by the *Stubborn* disease organism *Spiroplasma citri* is best expressed under warm or hot conditions but this symptom may not be apparent under cool conditions. Symptoms of gumming in the bark and cambium area caused by the *Citrus cachexia* virus can be quite pronounced in tangelo or mandarin grown in hot conditions but less so in areas where temperatures are uniformly cooler.

Conversely, severe stem pitting induced by certain strains of the Citrus *Tristeza virus* (CTV) may be quite destructive to citrus grown in a cool area, but the pitting may be totally absent on the same varieties of citrus grown in hot areas since the *Vein enation virus* is readily inactivated by warm temperatures[2].

It is important to re-emphasis that most citrus 'virus' can exist as symptom less wherever citrus can be grown. Severe strains of *Stem pitting* or *Seedling yellows* are present in mandarin, on trifoliate or other *Tristeza* tolerant rootstocks. However, if infected budwood of these cultivars is introduced, the virus can be transmitted from the leaves of the symptom less mandarin by aphid or psyllid vectors to nearby sweet orange or grapefruit. The presence of both CTV – infected budwood plus an efficient aphid vector such as *T. citicida* has the potential for destroying the sweet orange or grapefruit industry in an entire country[4]. The *Citrus exocrotis virus* can exist symptomless in sweet orange, grapefruit, mandarin and on certain rootstocks such as sour orange or rough lemon[9]. However, when viroid infected budwood is

Table 1. Classification of the major 'Virus' and Virus like diseases of citrus in Nigeria

Diseases	Organisms
Primarily vector transmitted	*Closterovirus*
Tristeza complex	
Tristeza (sweet/sour) decline	
Seeding yellows Tristeza	
Stem pitting Tristeza	
Greening disease	*Gracillicutes* bacteria
Asian	
African	
Stubborn disease	*Spiro plasma citri*
Vein enation	Probably virus
Primarily mechanically transmitted (and also bud transmitted)	
Exocortis and various citrus viroids	Viroid
Cachexia	Viroid
Infectious variegation	*Ilarvirus* (26-30nm)
Satsuma dwarf family	Virus (26-27nm)
Tatterleaf	Virus
Primarily bud transimitted (by man)	
Psorosis family	Probably virus
Psorosis-A	Virus
Ring sport	
Concave gum family (oak leaf patterns)	
Concave gum	Probably virus
Impietratura	Probably virus
Cristacortis	Probably virus
Diseases of unknown etiology	
Gummy bark and abnormal bud union	Unknown-probable virus
Blight	Unknown-probable virus
Fruits bolita (*Misiones* disease)	Unknown-probable virus

propagated on trifoliate, rangpur lime or certain trifoliate hybrid rootstocks, definitive bark shelling symptoms will appear and progeny trees may be severely stunted. Similarly, *Citrus cachexia virus* can exist symptomless in sweet orange, lemon, or grapefruit scions on many rootstocks but will induce dramatic symptoms in certain mandarin or mandarin hybrids as scions or rootstocks. Thus, since many of the viruses or viroids present in commercial citrus are symptomless, and can only be 'seen' or detected when susceptible rootstock is used, or when they are transmitted by vector, mechanically or budded to susceptible scions, propagative budwoods must be indexed if the 'virus' is to be detected.

Detection of Citrus Virus (Indexing)

Table 2 review the major citrus 'virus' diseases and their method of detection. It is evident from reviewing this table that the plants still remain primary means for the detection of most citrus 'viruses'[3]. Despite all current technology, a 'plant laboratory' i.e. a greenhouse specializing in growth of index or indicator plants and with highly trained personnel, is a necessity for any program which will have as its objectives the detection and ultimate elimination of citrus 'viruses'[10].

Elimination of Citrus from Propagative Budwood

The following methods (approaches) are applied in the citrus 'viruses' elimination process[2]:-

a. nucellar selection
b. thermotherapy
c. *in vitro* propagation – (*in vitro* shoot tip grafting)

A combination of b, c and indexing is recommended for elimination of all known major citrus pathogens. Nucellar selection is a long term process and has the following disadvantages:-

i. Juvenility
ii. Poor fruit quality – i.e. coarser, puffer and pebbled rinds e.t.c.
iii. Excessive thorniness
iv. Upright growth habit
v. Excessive tree vigour

Table 2. Major citrus virus and virus-like diseases in Nigeria and method of detection

Disease	Plant index (by Graft-transmission)	Other index
Cachexia	Parson Special Mandarin/ Rough lemon; Orlando Tangelo seedlings	Electrophoresis, Polymerase chain reaction (PCR)
Concave gum	Sweet tangor, mandarin, Sweet orange seedlings	
Exocortis and related citrus viroids	861-S-1 citron/rough lemon	Electrophoresis, PCR, Mechanical transmission to *Gynura*
Greening disease	Graft transmission to Ponkan mandarin, sweet orange, Orlando tangelo seedlings	Fluorescent markers in fruit, Electron microscopy, PCR
Gummy bark	Sweet orange/sour orange, sweet orange/ rough lemon (in field)	PCR
Psorosis-A and ring spot	Sweet orange, sweet tangor, mandarin, lemon, citron grape fruit or Mexican lime seedlings, psorosis, psorosis-B lesion, inoculums change	Mechanical transmission to *Chenopodium quinoa* for (ring spot)
Tristeza	Mexican lime seedlings, sweet orange/sour orange	ELISA, PCR, Immuno-diffusion SDA, Electron microscopy, Fluorescent antibodies, DsRNA PAGE, inclusion bodies
Seedling yellow and severe stem pitting	Grape fruit, sour ornage, lemon seedlings yellow, mandarin vinous and grape fruit seedlings for stem pitting	DxRNA PAGE, PCR

vi. Alternate bearing
vii. Unequal fruit distribution around the tree
viii. Rapid deterioration of fruit quality with certain cultivars when fruit is held on the tree.

Certification Programs

Once virus free budwood is obtained through the above methods, it is important that a means for its multiplication and distribution be developed with the objectives of getting virus-free trees into commerce as rapidly as possible. For a successful implementation of this program, a combined effort of various government and private organizations is required such as research institutes, the extension services, state or government nursery inspection services, citrus nurseryman and responsible grower representing the citrus industry.

Quarantine

Quarantine and the use of virus-free propagating stock, have been used to control some citrus virus diseases, however, preventative steps alone do not adequately control vector-borne diseases, and adequate sources of host resistance or tolerance are not available for several important diseases. Outbreaks of epidemics on citrus by importation of diseased plants or budwood, point out the need for strengthening quarantine laws to restrict movement of budwood or plants.

Since most citrus viruses exist as symptomless in many of the commercial citrus cultivars, strict quarantine laws are necessary to prevent any introduction of budwood or plants for example:- the importation of CTV-infected *Satsuma* budwood into Peru has resulted in a debilitating *Tristeza* stem pitting epidermic in both the naval and velencia orange industry throughout Peru; recent probable importation of severe seedling yellow strains have caused a severe destructive *Tristeza* of sweet orange on sour orange rootstock in certain regions of Israel[5]. The list is endless. Strict control by quarantine must be continued in order to prevent the introduction of more severe strains. Such strains may attack combinations other than those on sour orange or attack cultivars which are tolerant to strains already present. There should be sound procedures and facilities for

Table 3 Major citrus virus diseases in Nigeria and their control measures

Diseases	Causal agent	Host plants	Reactive plants
Cachexia	*Cechexia viroid*	Symptom less on most citurs species and cultivars	Highly reactive on Orlando tangelo
Concave gum	Virus	-	Sweet orange and mandarin
Exocortis	*Citrus exocortis* viroid (CEV)	-	Trifoliate orange and T. hybrid, Rangpur lime
Greening	*Fastidious bacterium* (FB)Asian form (32°C)African form (20-24°C)	-	Sweet orange and mandarin / hybrids. Less severe on grape fruit, rangpur and lemons
Psorosis	Virus-like (Ring spot, concave gum)	*Cristacortis* Most species and hybrids	Sweet orange, mandarin and sweet tangor
Tristeza (quite incline to most destructive virus disease)	*Citrus tristeza* virus (CTV)	Nearly all species	Lime, grape fruit, sweet orange and hybrids
Satsuma dwarf	*Citrus mosaic* virus (CiMV) *Natsudaidei dwarf ivirus (NDV) Navel orange infectious mottling* virus (NiMV)	-	Satsuma mandarin, Navel oranges
Cachexia	Gum impregnation and discolor-ation of bark, bumpy bark	Graft transmissible	Use of disease free budwood shoot tip graft
Concave gum	Gum deposits and concavities	-	-
Exocortis	Bark scaling, splitting, stunting, leaf epinasty	-	Tip grafting, sanitation, sterilize tools with sodium hypochlorite
Greening	Sparse foliage, extensive dieback, small chlorotic (vein) leaves/zinc deficiency symptom	Vector insect *Trioza erytrea, Diaphorina citri*	Efficient quarantine, reduce vector population, thermo therapy, tip grafting
Psorosis	Chlorotic flecks, vein banding and leaf mottling, bark scaling	Graft-transmitted, mechanical	-
Tristeza (quite incline, most des-tructive virus disease)	Stunting stem pitting, vein clearing leaf cupping, chlorosis reduced, fruit size decline	Graft-transmitted insect vactor aphid species (*T. citricida*)	Quarantine, budwood certification, replant decline orchards with CTV-tolerant stocks, rough lemon, trifoliate, rangpur lime
Satsuma dwarf	Stunting, vein clearing, mottling and curling of new leaves	-	Use disease-free budwood
Cachexia	Gum impregnation and discolora-tion of bark, bumpy bark	Graft transmissible	Use of disease free budwood shoot tip graft
Concave gum	Gum deposits and concavities	-	-
Exocortis	Bark scaling, splitting, stunting, leaf epinasty	-	Tip grafting, sanitation, sterilize tools with sodium hypochlorite
Greening	Sparse foliage, extensive dieback, small chlorotic (vein) leaves/zinc deficiency symptom	Vector insect *Trioza erytrea, Diaphorina citri*	Efficient quarantine, reduce vector population, thermo therapy, tip grafting
Psorosis	Chlorotic flecks, vein banding and leaf mottling, bark scaling	Graft-transmitted, mechanical	-
Tristeza (quite incline, most des-tructive virus disease)	Stunting stem pitting, vein clearing leaf cupping, chlorosis reduced, fruit size decline	Graft-transmitted insect vactor aphid species (*T. citricida*)	Quarantine, budwood certification, replant decline orchards with CTV-tolerant stocks,

handling introduction and quarantine of citrus from high risk countries.

Major Citrus Virus Diseases and their Control

Table 3 reviews the major citrus 'virus' diseases and measures taken to prevent and control them.

Intergrated Pest Management (IPM) of Citrus Virus Diseases

For an effective and efficient prevention and control of citrus virus diseases, the following integrated control measure should be implemented:

- Establishment of healthy foundation stocks through a modified method of shoot-tip grafting, heat therapy or nucellar line selection and a rapid propagation method of budwood certificate program.
- Elimination of virus-diseased trees to prevent re-infestation.
- Protection of healthy citrus seedlings grown in fields from re-infestation by- (1) spraying of insecticide (Dimethoate 44%EC or Malathion 50%EC) at 10-20 days intervals during critical periods to control vector-transmitted virus; (2) pre-immunization of healthy foundation stocks or mother trees with mild (avirulent) strains of CTV; and (3) sanitation-disinfection of TLV-or exocortis viroid-trasmitted pruning or budding knives, saws or scissors by dipping in 1% NaOCL (sodium hypochlorite) and rinsing with 2% summer oil + 5% acetic acid (vinegar).
- Biological control of insect vectors e.g. *Citrus psylla Diaphorine citrid* with releasing the nympha parasitoid, *Tamarixia radiate* is highly effective.

Mild Strain Cross Protection

Effort has been devoted to mild strain cross protection research. Cross protection works upon the basis that if you inoculate trees with mild strain of virus isolate (symptomless or causing only mild symptoms) and later infect with a severe isolate, the symptoms of the severe isolate would not be expressed. This approach at present appears promising in controlling stem pitting symptoms in trees on tolerant rootstock varieties. It has not been effective in controlling decline of trees on sour orange rootstocks. Before the application of any cross protection strategy, there is (1) the need to stringently exclude any introduction of severe strains, and (2) the need for mild strain protection in the future[1].

One of the best examples of the implementation of this research has been in Brazil[11]. Brazil is the largest exporter of citrus juice concentrate in the world. In the 1930's this country lost its entire industry, which is based to a large degree on the Pera sweet orange variety, because of decline due to CTV. Pera trees, even when planted on CTV tolerant rootstocks, become severely affected by the field strains of the virus. It was at this point that the pre-immunization of trees with mild CTV isolates was proposed as a means of cross-protection.

Over the years, this method has been extended in Brazil to other citrus types and strain interactions. Based upon the excellent results obtained, it is suggested now that all budwood released to growers should be pre-immunized. Increasing use of pre-immunized plant material in any given region may lead to a change in the composition of the field CTV isolates, resulting in the mild strain predominating. This in turn should lead to a decrease in infection pressure by severe isolates, thereby reducing the risk of a breakdown of the cross-protection in those citrus types that are particularly sensitive to CTV. Pre-immunization is also a standard routine for all citrus cultivars in South Africa.

Importation of new Cultivar

All new cultivar imports must now meet rigorous quarantine standards, which require that the material which passes through quarantine is virus free. These virus free trees need to be protected from infection by severe strains.

Conclusion

For many aspects of disease management, an accurate understanding of the variability and the relationships between the strains of a given virus is essential. These include detection, the development of cross protection strategies, and the breeding or genetic engineering of new cultivars for virus resistance. Most citrus viruses have isolates or strains that vary from each other to some degree.

There is need to define relationships among strains at the molecular level, and to correlate biological and molecular properties. The development of infectious clones that can be genetically manipulated has become feasible, and provides a means of systematically identifying portions of the viral genome that control disease expression and other functions.

Access to rapid, sensitive, accurate, and inexpensive assays is important in most disease

management strategies. The methods of detecting citrus viruses have continued to evolve as new information on viruses and new technology has become available, and the process is continuing.

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