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Kennisgewing rakende geïnfekteerde sitrus voortplantingsmateriaal

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Opsomming

Die Sitrus Verbeteringskema (SVS) maak 'n belangrike bydrae tot die welwaart van die suider-Afrikaanse sitrusbedryf deur deurgaans te streef om die kwaliteit (insluitend die afwesigheid van skadelike organismes) van voortplantingsmateriaal wat in die bedryf gebruik word, te verbeter. Skadelike, of moontlik-skadelike, organismes (virusse / viroïede) is moeilik opspoorbaar. Periodieke hertoetsing van materiaal is dus nodig en diagnostiese metodes word deurgaans verbeter om sodoende die kans van opsporing te verbeter. Viroïede en 'n virus in onlangs in Cambria C, Royal Late, Witkrans, Limpopo SL en Henderson Mutante 13 en 17 gevind. Enthout-voorsiening na kwekerye is tydelik gestaak, totdat risiko-bepalings gedoen en/of skoon bronne verskaf kan word. Cambria C is met die nie-patogeniese *Sitrus Viroïed-IIa* besmet, en gegewe die verlaagde risiko, is enthout-voorsiening tydelik voortgesit tot 'n skoon bron beskikbaar is. Royal Late en Witkrans is met *Sitrus Viroïed –III* besmet wat 'n sitruspatogeen is, en daarom word enthout-voorsiening gestaak totdat 'n skoon bron beskikbaar is, of nuwe inligting die hersiening van die risiko-bepaling ondersteun. *Sitrus Psorosis virus*, 'n skadelike sitruspatogeen, is in Limpopo SL en Henderson Mutante 13 en 17 waargeneem. Hoewel hierdie diagnotiese resultaat eers bevestig moet word, is dit as 'n voorsorgmaatreël aanbeveel dat die kultivars onder nie-amptelike kwarantyn geplaas word. Hierdie voorvalle is veral problematies vir die betrokke partye, maar die SVS moet in die langtermyn belang van die algehele bedryf optree. Na deregulasie van die industrie is 'n periode van onvoldoende beskikbaarheid aan die nodige hulpbronne ervaar. Besteding aan virologie ondersteuning vir die SVS het egter oor die afgelope jare aansienlik verbeter, en sal voortgaan ter ondersteuning en verbetering van die SVS. Verskeie veranderinge is reeds aan die SVS riglyne gemaak om die fitosanitêre risiko beter te bestuur. Hierdie artikel beskryf die tegniese agtergrond, veranderings aan SVS riglyne asook aanbevelings aan produsente en kwekerye.

Agtergrond

Die doel van die Sitrus Verbeteringskema (SVS) is om die winsgewendheid van die suidelike Afrika sitrus-industrie te verhoog, deur te verseker dat sitrusprodusente met kwekerybome van die hoogste moontlike gehalte voorsien word, gemaak van geneties gesonde sitrusmateriaal, vry van enige skadelike patogene. Laasgenoemde ideaal is egter uiters uitdagend aangesien verskeie ent-oordraagbare patogene (sekere bakterieë, virusse en viroïede) sitrus kan infekteer en, om sake te vererger, vir baie jare simptoomloos in plante kan bly voordat dit óf meganies, óf deur insekvektore oorgedra kan word.

Hoewel virus-verwyderingstegnieke (groeipunt-enting – GPE en indeksering) op alle materiaal toegepas word voordat dit in die SVS opgeneem word, is hierdie tegnieke nie onfeilbaar nie. 'n Program van periodieke hertoetsing van kultivars in die skema word dus gevolg. Die tegnieke wat gebruik word vir die opspoor van sulke patogene, is ook onder konstante internasionale ontwikkeling, en verbeterde tegnieke, veral polimerase kettingreaksie (PKR) tegnologie, verhoog die moontlikheid om kriptiese patogene op te spoor. Hierdie sisteem is hulpbron-intensief en benodig veral ervare tegnici en temperatuur-beheerde glashuise. Na deregulasie van die industrie is die beskikbaarheid van hulpbronne beperk wat tot 'n mate van agterstand in die hertoetsing, spesifiek vir sitrus viroïede (CVd), gelei het.

Hierdie agterstand is die afgelope 3 jaar uitgewis, en deur hertoetsing en ontwikkeling en gebruik van verbeterde diagnostiese tegnieke (spesikfiek PKR), beide plaaslik en internasionaal, is die voorkoms van virusse / viroïede is in 'n aantal kultivars waargeneem. Die geaffekteerde kultivar is, in elke geval, onder streng "kwarantyn" by die Sitrus Grondvesblok (SGB) geplaas en geen enthout is uitgestuur voordat 'n deeglike risiko-bepaling deur die industrie se viroloë voltooi is nie. Hierdie

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gevalle word as prioriteitstake hanteer om die verskaffing van patogeenvrye materiaal voort te sit. Hierdie verantwoordelikheid en die tydsraamwerke betrokke, is ongelukkig nie altyd in lyn met die korttermyn kommersiële doelwitte nie. Dit mag dan soms 'n frustrerende dinamika tussen die beginsels van die skema, soos uitgedruk in die advies van die viroloë wat die industrie in sulke tegniese sake ondersteun, en die kommersiële entiteite betrokke, skep. Die SVS speel 'n kritieke rol in die biosekuriteit van die industrie en sal nie onverantwoordelik optree in 'n poging om die proses te verkort nie.

Die doel van hierdie kennisgewing is om die tegniese punte, prosedures en tydsraamwerke betrokke in die hantering van geaffekteerde kultivars, te verduidelik.

Die volgende ent-oordraagbare agente/patogene is waargeneem, grootliks as gevolg van verbeterings in konvensionele diagnose of molekulêre diagnostiese prosedures (PKR):

- ***Citrus Viroid IIa*** **(CVd-IIa)** in Cambria C

CVd-IIa is nog nooit met 'n siekte geassosieer nie en nadat die risiko-bepaling voltooi is, is verskaffing van Cambria C enthout vanaf SGB op advies van die SVS Advieskomitee tydelik voortgesit, totdat 'n skoon bron beskikbaar gestel kon word. Virus-verwydering is herhaal en 'n skoon bron is na SGB gestuur vir vermeerdering. Kommersiële hoeveelhede van die skoon bron behoort vanaf Desember 2009 beskikbaar te wees.

- ***Citrus Viroid III*** **(CVd-III)** in Witkrans, Royal Late, Owari en Arufatina

In Spanje (1,6) het CVd-III verlaging in boomgrootte, opbrengs, en wortelsisteem van bome op Pomeroy trifoliaat of Carrizo citrange onderstamme veroorsaak, terwyl hierdie effekte in die teenwoordigheid van ander CVd vererger is; in hierdie gevalle met opbrengste van 30-40% laer in CVd-besmette bome en daarmee saam ook swakker vrugkwaliteit. CVd-III is met "Gum Pocket" siekte in Suid-Afrika geassosieer, wat ernstige verdwerging en agteruitgang as gevolg van vergomming in die geaffekteerde onderstam veroorsaak (Fig. 1). Terwyl trifoliaat onderstamme gevoelig is vir "Gum Pocket" siekte, was trifoliaat hibriede verdraagsaam. Nietemin, aanduidings van 'n klimaatseffek op siekte-uitdrukking is in Suid-Afrikaanse navorsing (3,4,5) waargeneem. Geen simptome is op Rubidoux trifoliaat of Swingle citrumelo in die koeler Nelspruit proefperseel waargeneem nie, terwyl hierdie onderstamme "Gum Pocket" simptome in die warmer Malelane perseel gewys het.

Daar is nog nie aangeteken dat CVd-III "Gum Pocket" siekte in ander dele van die wêreld veroorsaak nie, gevolglik is die gebruik van sommige CVd-III variante in sekere lande vir biologiese verdwerging voorgestel, alhoewel hierdie praktyk deur Roistacher in 1992 (2) gekritiseer is. Siende dat geïnfekteerde bome meer stres-gevoelig is, is hierdie praktyk nie algemeen toegepas nie. Hierdie verskil tussen lande verras nie aangesien viroïede onstabiel is, en daar aansienlike variasie in die patogenisiteit van variante is. Die effek van viroïede varieer ook relatief tot bo- en onderstam. Waar dit dus in 'n spesifieke bo-/onderstam kombinasie nie opgemerk word nie, kan dit patogenies in ander, of onder stres-toestande wees. CVd is meganies hoogs oordraagbaar en besmette bome (wat selfs geen simptome wys) voorsien 'n inokulumbron vanwaar CVd na ander meer sensitiewe bome versprei kan word. In Suid-Afrika, het die bewyse van patogenisiteit wat met variante van CVd-III teenwoordig in SA geassosieer word, en die onbekende eienskap van toekomstige uitdrukking van patogenisiteit, daartoe gelei dat die beginsel "geen gebruik van viroïede om wenslike hortologiese kenmerke in kultivars uit te druk nie" deur die skema aangeneem is.

'n Risiko-bepaling rakende die CVd-III besmette kultivars is uitgevoer en die SVS Advies Komitee het aanbeveel dat verskaffing van enthout van hierdie kultivars opgeskort word totdat: (1) óf verdere navorsing die aggressiwiteit van die variante in die geaffekteerde kultivars uitklaar, wat moontlik tot tydelike hervatting in verskaffing van geïnfekteerde enthout mag lei; (2) of 'n viroïed-vrye bron beskikbaar word. Bome wat egter alreeds

geplant of in kwekerye gemaak is, kan voorsien word, op voorwaarde dat die ontvanger produsent behoorlik geadviseer word. Sulke bome is sertifiseerbaar, maar die sertifikate sal kwalifiseer dat die bome CVd-III kan bevat. Geaffekteerde industrie-partye is diensooreenkomstig in 2008 geadviseer. Voorsiening van 'n viroïed-vrye bron vir hierdie kultivars is 'n dringende en top-prioriteit en die huidige status is as volg:

- **Royal Late.** Skoon kernbron-materiaal is opgespoor, is gepre-immuniseer vir CTV kruisbeskerming en aan SGB verskaf. Daar word verwag dat enthout-verskaffing in Maart 2010 hervat sal word.

- **Witkrans.** Ongelukkig was geen skoon kernbron bome beskikbaar nie, en die kultivar moes weer vir virus-verwydering ingedien word. Hierdie proses is in die finale stadia van afhandeling; 'n skoon bron behoort in Desember 2009 aan SGB verskaf te word en die beskikbaarheid van kommersiële hoeveelhede word teen die einde van 2010 verwag.

- **Owari.** Alle kernbron bome was geïnfekteer, en virus-verwydering is begin. Kommersiële enthout voorraad word vir Januarie 2011 beraam, maar daar is slegs beperkte kommersiële belang in hierdie kultivar.

- **Arufatina.** Alle kernbron bome was geïnfekteer, en virus-verwydering is begin. Daar is geen kommersiële aanvraag na hierdie kultivar nie.

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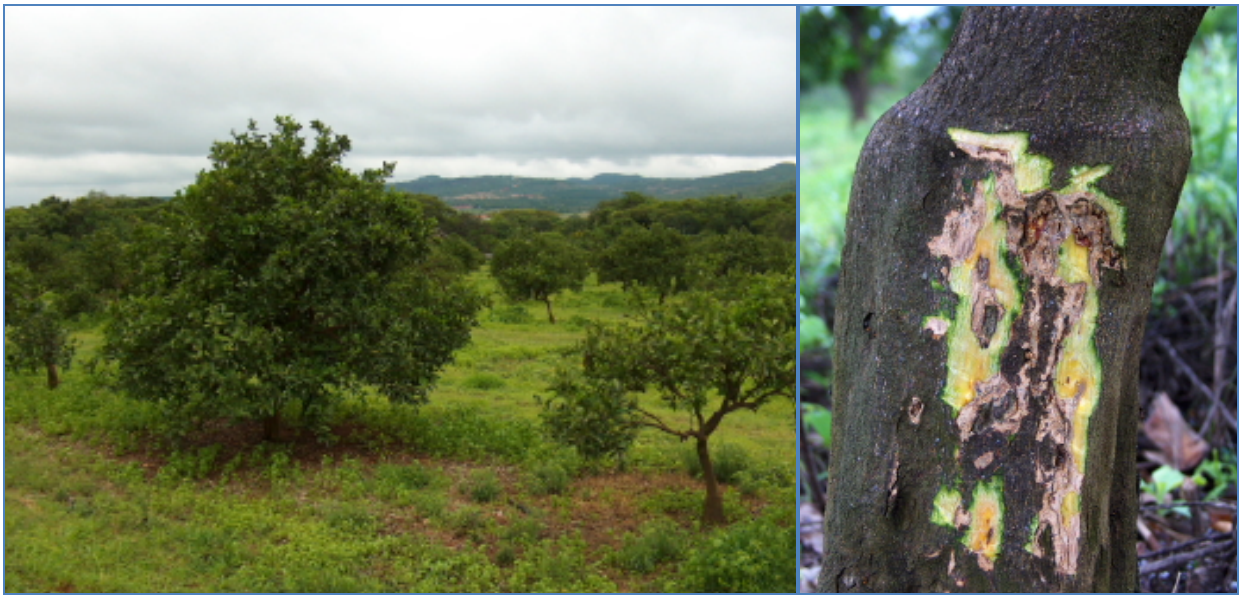

Figuur 1. Ernstige verdwerging en "Gum Pocket" siekte veroorsaak deur aggressiewe variante van *Citrus Viroid III.*

***Citrus psorosis virus* (CPsV) in Henderson Mutant 13, Henderson Mutant 17 en Limpopo Seedless**

CPsV is een van die oudste bekende ent-oordraagbare siektes van sitrus en kom wêreldwyd voor. Dit was die eerste sitrussiekte wat as ent-oordraagbaar bewys is, en het tot die eerste uitwissingsprogram (in Suid-Afrika), en die eerste sertifiseringsprogram (in Kalifornië) gelei. Kenmerkende veldsimptome van hierdie siekte sluit bas-afskilfering op die stam en hooftakke in, houtverkleuring in die takke, en soms word chlorotiese vlekke, kolle of ringkolle in jong blare van die lente stuwing waargeneem (Fig. 2). Ander siektes soos "concave gum", "impietratura" en "cristacortis" kan soortgelyke blaarsimptome veroorsaak en diagnose van CPsV, gebaseer op die teenwoordigheid van bas-afskilfering alleen, kan ook misleidend wees soos wat aangetoon is met 'n afwyking genaamd Bahia bas-afskilfering. Bas-afskilfering kom gewoonlik voor wanneer die bome meer as 10 jaar oud is, maar CPsV-geïnfekteerde bome kan simptoomloos bly. Alle sitruskultivars is vatbaar vir die siekte. Geen insekvektor is bekend nie, maar verslae vanaf Argentinië en Texas rakende die toename in die voorkoms van die siekte, dui op moontlike natuurlike oordraging van die virus. Daar was ook verslae van moontlike saad-oordraging. Meganiese oordraging van CPsV deur sny-gereedskap is eksperimenteel deur die gebruik van gesuiwerde virus as inokulum gedemonstreer. Die siekte was feitlik heeltemal gedurende 1927 tot 1967 in Suid-Afrika uitgewis (Psorosis Wet No. 427).

- **Henderson Mutant 13 en Henderson Mutant 17.** In 1987 is klaarblyklike virusvrye Henderson (LNR-ITSG # 1284) materiaal vanaf

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die Universiteit van Kalifornië, Riverside, ingevoer. Die materiaal is deur GPE gesit en die standaard biologiese indeksering prosedures is gevolg, ten einde vas te stel of die materiaal skoon was van enige bekende virus of viroïed. Materiaal van hierdie bron is later in 'n bestralings-teelprogram gebruik waaruit Henderson Mutant 13 (ARC-ITSG # 1671) en Henderson Mutant 17 (ARC-ITSG # 1672) ontstaan het. Die naam Flamingo is deur LNR-ITSG aan 1671 gegee, maar dieselfde naam is later deur die kultivar-agent vir 1672 gebruik, wat verwarring veroorsaak het; gevolglik moet die onderskeie LNR-ITSG nommers gebruik word totdat die name vir die bronne uitgesorteer is. Die materiaal van 1671 en 1672 is in 2005 geïndekseer ten einde hul viroid-status te bepaal. Onlangs is 1671 na Australië uitgevoer, en CPsV is in die materiaal waargeneem. Met gebruik van 'n nuut-ontwikkelde PKR-tegniek in Suid-Afrika, is CPsV onlangs in die kernbronne en SGB materiaal van beide 1671 en 1672 waargeneem. ***Neem egter asseblief kennis*** *dat hierdie PKR-diagnostiese resultate in die proses is om bevestig te word, en afdoende bevindinge word nie voor die einde van September verwag nie.* Konvensionele diagnose (indeksering) neem gewoonlik 'n periode van 12 maande om voltooi te word, terwyl hierdie nuwe PKR-toetse in 'n paar dae voltooi kan word. In die lig van die potensiële ekonomiese implikasies, is dit belangrik dat ons seker maak dat hierdie resultate nie 'vals positiewe' is nie. Addisionele toetsing is dus onderweg ten einde die aanvanklike bevindinge te bevestig.

- **Limpopo Seedless.** Hierdie nuwe kultivar is, ná virus-verwydering, in veldproewe geëvalueer waar swak boomprestasie en suspisieuse simptome opgemerk is. Hierdie het Dr Barry Manicom (LNR-ITSG) aangespoor om PKR-diagnose te doen, beide die kernbron en SGB moederbome het positief vir CPsV getoets. Dit is onduidelik waar hierdie kultivar besmet kon raak. Daar is tans beperkte kommersiële belang in hierdie kultivar. ***Neem asseblief kennis*** *dat hierdie PKR-diagnostiese resultate in die proses is om bevestig te word, en afdoende bevindinge word nie voor die einde van September verwag nie.*

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Figuur 2. Bas-afskilfering, blaarsimptome en interne houtsimptome veroorsaak deur *Citrus psorosis virus.*

Onlangse veranderinge aan SVS riglyne

Bogenoemde voorvalle het aandag op aspekte in die SVS riglyne wat verbeter kan word, gevestig. Die volgende veranderinge is gemaak:

- Duplisering van komponente van die virusvrye kernbron.
- Herindeksering vir CTV en CVd elke 2 jaar deur die Etrog indikator op die 'Mexican' lemmetjie indikator na CTV indeksering te ent. Hierdie is in 2006 alreeds begin.
- PKR diagnostiese toetse word vir implementering in SVS ontwikkel. Hierdie sal nie biologiese indeksering as primêre diagnostiese tegniek vervang nie, maar sal vir bevestiging van resultate gebruik word.
- Alle kernblokmateriaal sal voor verskaffing aan SGB herindekseer word, en geen material sal voor finale diagnose vrygestel word.
- Samewerking tussen viroloë van CRI en LNR-ITSC het verbeter.
- 'n Dringende CRI-befondsde projek is begin om alle kultivars in die CRI en ITSC kernblokke vir

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CVd, Psorosis en CTV te toets om hul fitosanitêre status te bepaal.
- Voortaan sal alle resultate van biologiese indeksering met PKR of relevante alternatiewe tegniek bevestig word.
- Twee molekulêre laboratoria is nou in werking (CRI en ITSC) wat dit moontlik maak om mekaar se resultate te bevestig.
- Daar was ‘n sterk groei in die toewysing van hulpbronne (fasiliteite, toerusting, personeel en operasionele begroting) aan SVS oor die afgelope paar jaar. Tans is die hulpbron toewysing aan SVS die grootste persentasie van totale industrie besteding ooit. Die hoë prioriteit van die SVS vir die bedryf sal voorts ook verseker dat hierdie besteding net slegs volhou sal word nie, maar dat dit ook sal groei waar en wanneer nodig.

Samevattings en Aanbevelings

- **CVd-IIa en CVd-III geïnfekteerde bome.** Geen ernstige simptome is waargeneem tydens ‘n opname van bome, hoewel swak opbrenste wel ervaar word. Entlas “onverenigbaarheid” in CVd-III geïnfekteerde bome, wat ook met ernstige CTV geïnfekteer is, is waargeneem. Aangesien CVd-III boomverdwerging veroorsaak, moet hoër-digtheidsaanplantings in hierdie gevalle oorweeg word ten einde die impak van verminderde opbrengs per boom te verminder. Blootstelling van hierdie bome aan stres moet so ver as moontlik vermy word, aangesien hierdie bome meer sensitief as viroïed-vrye bome weens ‘n verminderde wortelsisteem is.
- **CPsV.** Met die afhandeling van die diagnostiese proses (verwag teen die einde van September 2009), sal spesifieke kennisgewings uitgegee en aanbevelings gemaak word. Intussen word kwekerye en produsente aangeraai om hierdie bome onder kwarantyn te hou ten einde moontlike siekteverspreiding te voorkom.
- Meld alle gevalle van duidelik verdwergde bome en bome wat bas-kraking of -afskilfering toon, aan.
- Produsente moet as ‘n standaard praktyk, die snykante van alle snoei- en oes-gereedskap ontsmet wanneer tussen boorde beweeg word.
    - Ontsmet gereedskap met ‘n 30% huishoudelike bleikmiddel (bv. Jik) oplossing (5% natrium hipochloriet) deur die snykante in die ontsmettingsmiddel te doop of deur die ontsmettingsmiddelmiddel met ‘n handspuit toe te dien. Ten einde roes, veroorsaak deur die natrium hipochloriet, te verminder, kan lemme met die volgende oplossing aan die einde van elke dag behandel word: 1 deel spuit-olie: 10 dele asyn: 39 dele water.
- Die SVS dien die suidelike Afrika sitrus-industrie en die verantwoordelike bestuur van fitosanitêre status van voortplantingsmateriaal word deur die skema as top-prioriteit opgeneem ten einde die lang-termyn mededingendheid van die suidelike Afrika sitrusprodusente te verseker. Die beginsels van die skema en aanbeveling van die SVS Advieskomitee is op wetenskaplik korrekte risiko-bepaling gebaseer en moet, ten einde die industrie as ‘n geheel te beskerm, gehandhaaf word. Prosesse in die skema moet nie gekortwiek word in die belang van die korttermyn kommersiële belang van individue en partye nie.
- Die SVS het ten doel om deurentyd die status van die voortplantingsmateriaal wat aan die industrie beskikbaar gestel word, te verbeter. Voortdurende ontwikkeling van diagnostiese tegnieke en herhaaldelike hertoetsing ondersteun hierdie doelstelling. Die periodieke ontdekking van kriptiese kontaminasies is deel van die suksesvolle werking van ‘n uitstekende skema. Hierdie gevalle is demonstratief van die sukses van die skema ten einde sy doelstellings te haal en die gevolglike veranderinge aan die SVS riglyne sal die skema verder verbeter. Die SVS bly die mees betroubare bron van sitrus voortplantingsmateriaal in suidelike Afrika. Produsente moet dus slegs bome vanaf SVS-geakkrediteerde kwekerye aankoop, wat van gesertifiseerde enthout vanaf SGB gebruik maak.

JOU HEFFING WERK VIR JOU – PRODUSENTE SE HEFFINGS WORD AANGEWEND OM DIE AKTIWITEITE VAN DIE CRI TE BEFONDS

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Cutting Edge
RESEARCH NEWS FROM CITRUS RESEARCH INTERNATIONAL
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Notice regarding infected citrus propagation material

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Summary

The Citrus Improvement Scheme (CIS) plays an important role in the profitability of the southern African citrus industry by constantly striving to improve the quality (including the absence of harmful organisms) of propagation material used in the industry. Harmful or potentially harmful organisms (viruses / viroids) are often difficult to detect. Periodic re-testing of material is undertaken and diagnostic procedures are continuously improved to increase the likelihood of detecting the presence of such organisms. Viroids and a virus were recently detected in Cambria C, Royal Late, Witkrans, Limpopo SL and Henderson Mutants 13 and 17. The supply of budwood to nurseries was suspended, pending the completion of risk assessments and/or provision of a clean source. Cambria C was infected with the non-pathogenic *Citrus Viroid IIa* and given the reduced risk, supply of budwood was temporarily resumed until a viroid-free source becomes available. Royal Late and Witkrans were infected with *Citrus Viroid-III*, which was found to be pathogenic to citrus, and suspension of budwood supply is being maintained until a viroid-free source becomes available or there is new information to support a revision of the risk assessment. *Citrus Psorosis virus*, a harmful citrus pathogen, was detected in Limpopo SL and Henderson Mutants 13 and 17. However, the diagnostic results for Psorosis still need to be verified, but as a precaution it was recommended that these cultivars be placed under unofficial quarantine. It is recognised that these incidents are problematic for affected parties, but the CIS acts in the long term interest of the overall industry. Whereas a period of inadequate resource availability was experienced after deregulation of the industry, the resources allocated to virology support of the CIS have increased considerably over the past few years and will continue to receive the allocations required to meet the CIS requirements. Various CIS procedures have also been amended to improve management of phytosanitary risk. This article explains the background and technicalities, and describes changes made to the CIS and recommendations to growers and nurseries.

Background

The objective of the Citrus Improvement Scheme (CIS) is to increase the profitability of the southern African citrus industry, by ensuring that citrus growers are supplied with nursery trees of the highest possible quality made from genetically sound citrus material and being free from harmful pathogens. The latter ideal, however, is extremely challenging as various graft transmissible pathogens (certain bacteria, viruses and viroids) can infect citrus and, to make matters worse, remain symptomless in plants for many years before becoming evident or being transmitted, either mechanically or by insect vector.

Although virus elimination techniques (shoot tip grafting - STG and indexing) are applied to all material prior to uptake in the CIS, these techniques are not failsafe. Therefore, a programme is followed whereby cultivars in the scheme are periodically re-tested. The techniques used for detecting such pathogens are also under constant international development and improved techniques, particularly polymerase chain reaction (PCR) technology, increase the likelihood of detecting cryptic pathogens. Such a system requires a fair amount of resources, most pertinently skilled technicians and temperature-controlled glasshouse space. Post deregulation of the industry, the availability of resources came under challenge and led to some back-log in the periodic re-testing, specifically for citrus viroids.

Over the past 3 years, the back-log was addressed and through a combination of this action and the development and adoption of improved diagnostic techniques (specifically PCR technology), both locally and internationally, the occurrence of viruses / viroids has been detected in a number of cultivars. In each instance, the affected cultivar is placed under strict “quarantine” at the Citrus Foundation Block (CFB) and no budwood is handed out, until a thorough risk assessment has been completed by the industry’s virologists. These cases are treated as priority tasks to ensure the rapid resumption in supply of pathogen-free material. Unfortunately,

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this responsibility and the time-lines involved are not always aligned with short-term commercial objectives. This gives rise to a frustrating dynamic between the principles of the scheme (as expressed in the advice of the virologists supporting the industry on such technical issues) and the commercial entities involved. Given that the CIS has a critical role to play in the biosecurity of the industry, the CIS will not act irresponsibly by short-circuiting proper process.
The purpose of this notice is to explain the technicalities, procedure and time-lines involved in dealing with affected cultivars.
The following graft transmissible agents/pathogens were detected, largely due to improvements in conventional diagnosis or PCR-based diagnostic procedures:

- ***Citrus Viroid IIa* (CVd-IIa)** in Cambria C

CVd-IIa has never been associated with a disease and after completing the risk assessment, on advice of the CIS Advisory Committee, supply of Cambria C budwood from CFB was temporarily resumed until a clean source becomes available. Virus elimination was repeated and a clean source was sent to CFB for multiplication. Commercial quantities of the clean source should be available as of December 2009.

- ***Citrus Viroid III* (CVd-III)** in Witkrans, Royal Late, Owari and Arufatina

In Spain (1,6), CVd-III caused reduced tree size, reduced yield and reduced root system on trees grafted to Pomeroy trifoliate or Carrizo Citrange rootstock and the detrimental effects were exacerbated by co-infection with other CVd's (yields were 30-40% with impaired fruit quality). CVd-III has been associated with Gum Pocket disease in South Africa, which causes severe stunting and decline as a result of gum pockets in the affected rootstock (Fig. 1). Even though trifoliate selections appear to be highly susceptible to gum-pocket disease, trifoliate hybrid selections appear to be tolerant to the disease. Nonetheless, from the research conducted in South Africa (3,4,5), indications were observed that climate might influence disease expression. No symptoms on Rubidoux trifoliate selection or Swingle citrumelo were observed in the cooler climate Nelspruit trial site, but both these rootstocks exhibited gum pocket symptoms in the warmer climate Malelane trial site.

CVd-III has not been reported to cause Gum Pocket disease in other parts of the world and the use of some CVd-III variants have been proposed for biological dwarfing in some countries, although this practice has been criticised by Roistacher in 1992 (2). This practice has not been widely adopted and infected trees are reported to suffer from heightened sensitivity to stress. The regional differences are not surprising since viroids are unstable and there is considerable variation in the pathogenicity of variants. The effect of viroids also varies relative to scion and rootstock. So whereas it may be unseen in a particular scion rootstock combination it may be pathogenic in others or under stress conditions. Viroids are highly transmissible through mechanical means and hence infected trees (even if they are not severely affected themselves) provide an inoculum source for inadvertent transmission to other trees that may be more susceptible. In South Africa, evidence of pathogenicitiy associated with variants of CVd-III present in SA, and the unknown nature of future expression of pathogenicity, led to the scheme historically adopting the principle of "not using viroids to express desirable horticultural characteristics in cultivars".

A risk assessment was conducted on the situation with the recently affected cultivars and the CIS Advisory Committee recommended that the supply of budwood of these cultivars be suspended until: (1) either further research clarifies the aggressiveness of the variants in the affected cultivars, potentially enabling temporary resumption in supply of infected budwood; (2) or a viroid-free source becomes available. However, trees already planted or made in nurseries can be supplied, on condition that the receiving grower is properly advised. Such trees will still be certifiable, but the certificates will be qualified to indicate that they may contain CVd-III. Affected industry parties were accordingly advised of this in 2008. Provision of a viroid-free source for these cultivars is an urgent and top priority and the current status is as follows:

- **Royal Late.** Clean gene source material was located, which was pre-immunised for CTV cross-protection and supplied to CFB. Budwood supply is estimated to resume in March 2010.

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- **Witkrans.** Unfortunately, no clean gene source trees were available, and the cultivar had to be resubmitted for virus elimination. This process is in the final stages of conclusion; a clean source should be supplied to CFB in December 2009 and availability of commercial quantities is expected by end 2010.
- **Owari.** All gene source trees were infected, and virus elimination is in progress. Commercial budwood supply is estimated for January 2011, but there is very limited commercial interest in this cultivar.
- **Arufatina.** All gene source trees were infected, and virus elimination is in progress. There is no commercial demand for this cultivar.

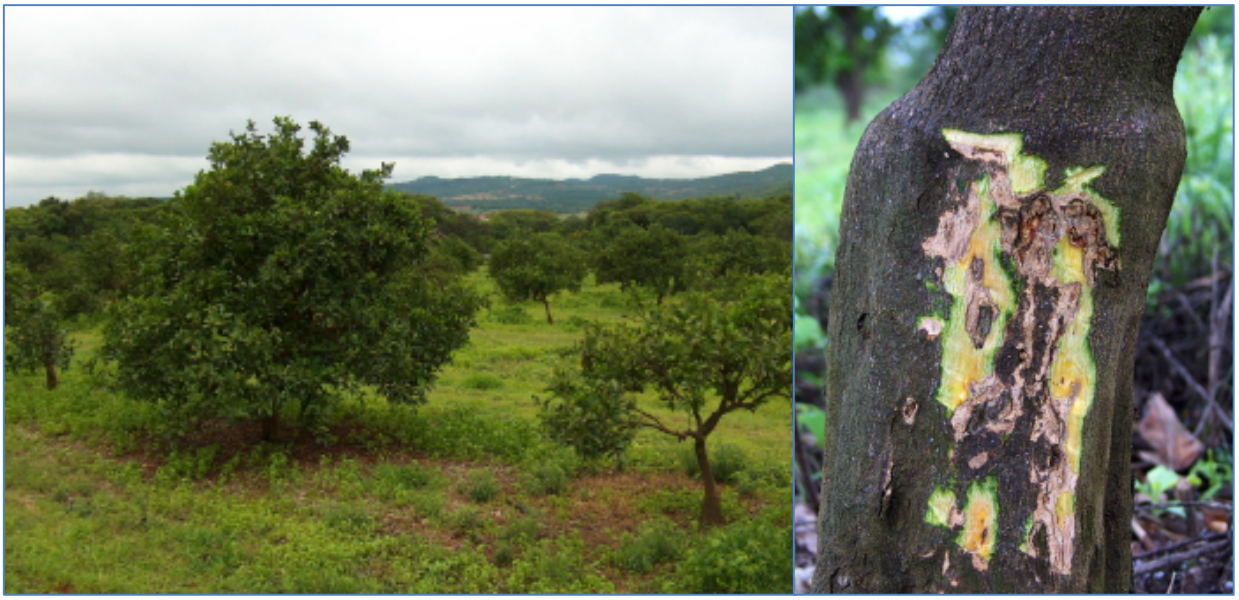

Figure 1. Severe stunting and Gum Pocket disease caused by aggressive variants of *Citrus Viroid III.*

Citrus psorosis virus (CPsV) in Henderson Mutant 13, Henderson Mutant 17 and Limpopo Seedless

CPsV is one of the oldest known graft transmissible diseases of citrus and occurs worldwide. It was the first citrus disease proven to be graft transmissible and this led to the first eradication programme (in South Africa) and the first certification programme (in California). Characteristic field symptoms of the disease include bark scaling on the trunk and main branches, wood staining in the branches and sometimes chlorotic flecks, blotches or ring spots are observed in young leaves of the spring flush. Other diseases such as concave gum, impietratura and cristacortis can cause similar leaf symptoms and diagnosis of psorosis based on the presence of bark scaling alone can also be misleading as was shown with a disorder called Bahia bark scaling. Bark scaling usually appears when the trees are more than 10 years old but CPsV-infected trees can remain symptomless. All citrus cultivars are susceptible to the disease. No insect vector is known but reports from Argentina and Texas on the increase in the incidence of the disease suggest possible natural transmission of the virus. There were also reports of seed transmission. Mechanical transmission of CPsV by cutting tools was demonstrated experimentally using purified virus as inoculum. The disease was almost completely eradicated from South Africa during 1927 to 1967 (Psorosis Act No. 427).

- **Henderson Mutant 13 and Henderson Mutant 17.** In 1987, apparently virus-free Henderson (ARC-ITSC # 1284) material, that was imported from the University of California, Riverside, was introduced into the CIS for STG. The material was put through STG and the standard biological indexing procedures were followed to establish if the material was clean from any known virus or viroid. Material from this source was later used in an irradiation breeding programme from which Henderson Mutant 13 (ARC-ITSC # 1671) and Henderson Mutant 17 (ARC-ITSC # 1672) was derived. The name Flamingo was given to 1671 by the ARC-ITSC but the same name was used by the cultivar agent for 1672, which caused confusion, therefore the respective ARC-ITSC numbers should be used until the names for the sources are sorted out. The material of both 1671 and 1672 was tested in 2005 to determine its virus and viroid status. Recently, 1671 was exported to Australia, and CPsV was detected in it. Using a newly developed PCR technique in SA, CPsV was recently detected in the gene sources and CFB material of both 1671 and 1672. ***However, please note*** *that these PCR diagnostic results are in the process of being validated, and conclusive findings are not expected before end-September.* Conventional diagnosis (indexing) normally takes a period of 12 months to conclude and is notoriously fickle, while these new PCR tests (once optimised) can be completed in a few days. However, in light of the potential economic implications, it is essential to ensure that these results are not 'false positives'. Therefore, additional testing is under way to validate the initial findings.
- **Limpopo Seedless.** After virus elimination, this new cultivar was being evaluated in field trials where poor tree performance and suspicious symptoms were observed. This prompted Dr Barry Manicom (ARC-ITSC) to conduct PCR diagnoses and both the gene source and CFB mother trees have tested positive for CPsV. The point of contamination of this cultivar is unclear. There is limited commercial interest in this cultivar. ***Please note*** *that these PCR diagnostic results are in the process of being validated, and conclusive findings are not expected before end-September.*

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Figure 2. Bark scaling, leaf symptoms and internal wood symptoms caused by *Citrus psorosis virus.*

Recent changes to the CIS procedures

These incidents have drawn attention to aspects of the scheme's procedures that can be improved to reduce the risk of recurrences. The following are some of the changes recently made.

- Duplication of components of the virus-free nucleus block.
- Combining the re-indexing for CTV and CVd every two years by budding the Etrog indicator on the 'Mexican' lime indicator after the CTV indexing was done. This was implemented in 2006.
- PCR diagnostic protocols are being optimised for implementation in the CIP procedures. This will not replace biological indexing as primary diagnostic technique, but will be implemented as a validation phase to confirm all results following indexing.
- All nucleus block material will in future be re-indexed at the time of supply to CFB or for export purposes and no material will be released until full indexing (diagnosis) results are known.
- Collaboration between the virologists and diagnosticians at CRI and ARC-ITSC has been improved.
- An urgent CRI-funded project was initiated to diagnose all cultivars in the CRI and ITSC nucleus blocks for CVd, Psorosis and CTV to determine their phytosanitary status.
- In future, results from biological indexing will be verified by PCR or relevant alternative test.

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- Two molecular diagnostic laboratories are now in operation (CRI and ITSC) enabling them to cross-check each other's results and verify results.
- There has been a strong growth in the allocation of resources to the CIS over the past few years. This has been in the form of facilities, equipment, personnel and operational budget. As a consequence the CIS today receives a greater proportion of industry resource allocation than ever in the past. The high priority attached to the CIS in the industry will ensure that this is not only sustained, but also continues to grow as required.

Conclusions and Recommendations

- **CVd-IIa and CVd-III infected trees.** A field survey of trees revealed reports of unsatisfactory yields but no severe symptoms; except for bud union creasing in CVd-III infected trees that were also infected with severe CTV. As CVd-III causes tree dwarfing, higher density plantings should be considered in these cases to reduce the impact of reduced yield per tree. Avoid as far as possible exposure of such trees to stress as they can be expected to be more sensitive than viroid-free trees because of a reduced root system.
- **CPsV.** Following the conclusion of the diagnostic process (expected by end September 2009), specific notification and recommendations will be made. In the meantime, nurseries and growers are advised to keep these trees under quarantine to prevent possible disease spread.
- Report any cases of obviously stunted trees and trees exhibiting bark cracking or scaling.
- As a standard practice, growers should disinfect the cutting edges of all the pruning, hedging and harvesting implements when moving between orchards.
    - Disinfect implements with a 30% commercial household bleach (i.e. Jik) solution (5% sodium hypochlorite) by dipping the cutting edges into the disinfectant or applying the disinfectant with a hand spray. To reduce corrosion caused by the sodium hypochlorite, the blades can be treated with the following solution at the end of each day: 1 part spray oil: 10 parts vinegar: 39 parts water.
- The CIS serves the southern African citrus industry and the responsible management of the phytosanitary status of propagation material is undertaken by the scheme as a top priority in ensuring the long term competitiveness of the southern African citrus growers. The principles of the scheme and recommendations of the CIS Advisory Committee are based on scientifically sound risk assessment and must be upheld to protect the industry as a whole. The scheme must not be short-circuited in the interest of short term commercial interest of individuals.
- The CIS aims to continuously improve the status of the propagation material made available to the industry. Ongoing development of diagnostic techniques and recurrent re-testing supports this objective. The periodic discovery of cryptic contaminations is part of the successful operation of a superior scheme. These incidents are a demonstration of the success of the scheme in achieving its objectives and the consequent changes made will result in ongoing improvement into the future. The CIS remains the most trusted source of citrus propagation material in southern Africa. Growers should therefore only purchase trees from CIS accredited nurseries that made use of certified budwood supplied by the CFB.

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