



Kennisgewing rakende geïnfekteerde sitrus voortplantingsmateriaal: DuRoi Valencia

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, en vry van skadelike patogene. Laasgenoemde ideaal is egter uiters uitdagend aangesien verskeie oordraagbare patogene (sekere bakterieë, virusse en viroïede) sitrus kan infekteer en vir baie jare simptoombloos in plante kan bly voordat siekte veroorsaak word, of voordat dit óf meganies, óf deur insekvektore oorgedra kan word.

Hoewel virus-verwyderingstegnieke (groeipunt-enting; GPE) 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 vir die opspoor van sulke patogene gebruik word, is ook onder konstante nasionale en internasionale ontwikkeling, en verbeterde tegnieke, veral polimerase-ketting-reaksie tegnologie (PKR), verhoog die moontlikheid om kriptiese patogene op te spoor.

Gedurende die tweejaarlikse sitrus viroïed (CVd) indeksing van die Sitrus Grondvesblok (SGB) moederbome, is positiewe simptome op die Etrog biologiese indikatoren van drie uit vyf DuRoi Valencia moederbome waargeneem. PKR toetse het CVd infeksie bevestig en CVd-IV is geïdentifiseer. Dit was die eerste waarneming van CVd-IV in Suid-Afrika. CVd-IV, ook bekend as die "bark cracking CVd", is 'n betreklik nuut-beskryfde CVd (1) en nie baie is bekend rakende sy effek op boomgroei en produksie nie. CVd-IV word met 'n ernstige kraak van die bas in trifoliaat onderstamme geassosieer (2), maar viroïed simptoombeskrywings beskikbaar, is meestal waar CVd-IV saam met ander CVds (3),(4) geïnfekteer is.

In daaropvolgende toetse, het moederbome PO4 en PO5, wat aanvanklik negatief was, positief getoets, asook die vermeerderingsblokke, wat aandui dat CVd-IV oneweredig in die bome voorgekom het. Dit kan óf op 'n onlangse infeksie dui, sonder genoegsame tyd om sistemies binne

die plante te versprei, en gevolglik word opsporing so vertraag, óf 'n natuurlike ongelyke verspreiding van die viroïed. Al die kernbron bome in die SGB is vernietig. Vorige waarneming van hierdie viroïed is belemmer aangesien CVd-IV slegs kortstondige en gematigde tot geen simptome op die biologiese indikator "Etrog" getoon het.

In 'n geval soos hierdie, word die geïnfekteerde kultivar onder streng kwarantyn in die SGB geplaas en geen enthout word uitgegee nie, totdat 'n deeglike risikobepaling deur die industrie se viroloë voltooi is. Hierdie gevalle word as prioriteit behandel, in 'n poging om die bron van kontaminasie vas te stel, asook om die verskaffing van patogeenvrye materiaal so spoedig moontlik voort te sit.

PKR toetse op die kernblok (KB) bome het aangetoon dat hulle vry was van CVd-IV. Dit beteken dat die DuRoi Valencia geïnfekteer is nádat hulle by die SGB vrygestel is. Pogings om 'n beter prentjie te kry van wanneer infeksie plaasgevind het: monsters van DuRoi Valencia bome is kwekerye geneem (geënt op 16/3/09 en 8/11/09), asook van twee veld-aanplantings (4- en 9-jaar-oud). Die twee kwekerymonsters het positief getoets, terwyl die twee veld-aanplantings negatief getoets het, wat aandui dat infeksie ná 2006 plaasgevind het.

Die doel van hierdie kennisgewing is om die tegniese aspekte, prosedure en tydsraamwerke, betrokke in die hantering van die geïnfekteerde kultivar, te verduidelik.

Gevolgtrekkings en Aanbevelings

- Gedurende roetine her-indeksing by die SGB, is CVd-IV in moederbome en vermeerderingsbome van DuRoi Valencia waargeneem. Hierdie is die eerste aantekening van CVd-IV in Suid-Afrika, en word met bas-kraking van trifoliaat onderstamme geassosieer.
- Enthout-verskaffing van DuRoi Valencia vanaf SGB is opgeskort totdat 'n skoon bron beskikbaar kom.
- Groeipunt-enting is weer op die KB boom toegepas en gepreïmmuniseerde materiaal, vry van CVd-IV, is aan die SGB op die 26ste Oktober 2010 verskaf. Enthout-verskaffing vanuit die SGB behoort teen die einde van 2011 voortgesit te kan word.
- Produsente moet as 'n standaardpraktyk, die snykante van alle snoei-, 'hedging'- en oes-

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



implemente disinfekteer wanneer tussen boorde beweeg word.

- Disinfekteer implemente met 'n 30% Jik-oplossing (5% natriumhipochloried) deur die snykante met 'n lap wat die disinfekteermiddel bevat, af te vee. 'n Vars oplossing moet daagliks opgemaak word en in 'n donker houer geberg word. Ten einde korrosie, veroorsaak deur die natriumhipochloried, te verminder, kan die lemme aan die einde van elke dag met die volgende oplossing behandel word: 1 deel spuit-olie: 10 dele asyn: 39 dele water.
- Huidig wil dit voorkom of infeksie van die SGB materiaal ná 2006 plaasgevind het; verdere toetse sal gedoen word om die tyd en vlakke van infeksie te bepaal. 'n Deeglike opname van bome wat van geïnfekteerde enthout gemaak is, behoort gedoen te word. Tot dusver is geen geïnfekteerde boorde gevind nie.
- Rapporteer alle gevalle van abnormale simptome op onderstamme en DuRoi Valencia bome, wat baskraking of -afskilfering toon, aan Dr Fanie van Vuuren of Glynnis Cook (Tel. 013-7598000).

Verwysings

1. Putcha H, Ramm K, Luckinger R, Hadas R, Bar-Joseph M, Sanger HL. 1991. *Nucleic Acids Research* 19: 6640
2. Vernière C, Perrier X, Dubois C, Dubois A, Botella L, et al. 2006. *Phytopathology* 96: 356-68
3. Malfitano M, Barone M, Duran Vila N, Alioto D. 2005. *Journal of Plant Pathology* 87: 115-21
4. Mohamed ME, Bani Hashemian SM, Dafalla G, Bové JM, Duran-Vila N. 2009. *Journal of Plant Pathology* 91: 185-90.



Notice regarding infected citrus propagation material: DuRoi Valencia

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 remain symptomless in plants for many years before causing disease or before it can be transmitted, either mechanically or by insect vector.

Although virus elimination techniques (shoot tip grafting; STG) are applied to all material prior to uptake in the CIS, these techniques are not failsafe. Therefore, a programme of periodic re-testing of cultivars in the scheme is followed. The techniques used for detecting such pathogens are also under constant national and international development and improved techniques, particularly polymerase chain reaction (PCR) technology, increase the likelihood of detecting cryptic pathogens.

During the biennial citrus viroid (CVd) indexing of the Citrus Foundation Block (CFB) mother trees, positive symptoms were observed on the Etrog biological indicators of three out of five DuRoi Valencia mother trees. PCR tests confirmed CVd infection and CVd-IV was identified. This was the first detection of CVd-IV in South Africa. CVd-IV, also known as the bark cracking CVd, is a fairly newly described CVd (1) and not much is known of its effects on tree life and production. CVd-IV is associated with a severe bark cracking in trifoliolate rootstocks (2), but viroid symptom descriptions available are mostly where CVd-IV is co-infected with other CVds (3),(4).

In subsequent tests, mother trees PO4 and PO5, which were initially found to be negative, tested positive as well as the multiplication blocks indicating that CVd-IV occurred unevenly in the trees. This could indicate either a recent infection without sufficient time to spread systemically within the plants and thus delaying the detection, or a naturally erratic spread of the viroid. All the mother trees at the CFB have been destroyed. Previous detection of this viroid was hampered as CVd-IV on the biological indicator "Etrog" displays only transient and mild to no symptoms at times.

In an instance such as this, the affected cultivar is placed under strict quarantine at the 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, in the attempt to determine the point of contamination, as well as to resume the supply of pathogen-free material as soon as possible.

PCR tests on the nucleus block (NB) trees showed that they were free of CVd-IV. This meant that the DuRoi Valencia got infected after release to the CFB. Attempts were made to get a clearer picture of when infection took place: samples from DuRoi Valencia trees were taken at nurseries (budded on 16/3/09 and 8/11/09) as well as from two field plantings (4- and 9-years-old). The two nursery samples tested positive while the two field plantings tested negative, indicating that infection took place after 2006.

The purpose of this notice is to explain the technicalities, procedure and time-lines involved in dealing with the affected cultivar.

Conclusions and recommendations

- During routine re-indexing at CFB, CVd-IV was detected in mother trees and multiplication trees of DuRoi Valencia. This is the first report of CVd-IV in South Africa, and is associated with bark-cracking of trifoliolate rootstocks.
- Budwood supply of DuRoi Valencia from CFB has been suspended until a clean source is available.
- Shoot tip grafting was again employed on the NB tree and pre-immunised material free of CVd-IV was supplied to the CFB on the 26th of October 2010. Budwood supply from CFB should resume toward the end of 2011.
- 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% Jik solution (5% sodium hypochlorite) by wiping the cutting edges with a cloth containing the disinfectant. A fresh solution should be made up daily and kept in a dark container. 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.



Cutting Edge

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- At present, it appears that infection of CFB material occurred after 2006; further testing will be done to determine the time and extent of infection. A thorough survey of trees made from infected budwood should be made. So far no infected orchards were found.
- Report any cases of abnormal symptoms on rootstocks and DuRoi Valencia trees exhibiting bark cracking or scaling to Dr Fanie van Vuuren or Glynnis Cook (Tel. 013-7598000).

References

1. Putcha H, Ramm K, Luckinger R, Hadas R, Bar-Joseph M, Sanger HL. 1991. *Nucleic Acids Research* 19: 6640.
2. Vernière C, Perrier X, Dubois C, Dubois A, Botella L, et al. 2006. *Phytopathology* 96: 356-68.
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