

# CRI Cutting Edge

RESEARCH NEWS FROM  
CITRUS RESEARCH INTERNATIONAL

March 2018

No. 246

## Postharvest pathogen resistance monitoring in the packhouse and orchard

**Wilma du Plooy, Catherine Savage and Elaine Basson**

Citrus Research International (CRI)

Regular collection of postharvest pathogen spore samples using swabs is imperative in determining the risk for resistance development at packhouses and in orchards. Swabs need to be collected in a controlled and repeatable manner in order to accumulate trustworthy results that can contribute to compiling a risk profile for each packhouse. Furthermore, understanding the environmental conditions during sampling will assist in understanding if there are external variables that affects the risk of resistance development.

This Cutting Edge explains the collection of green mould (*Penicillium digitatum*) and sour rot (*Galactomyces citri-aurantii*) spores, which will then be sent to the Diagnostic Centre (DC) at the Citrus Research International facilities in Nelspruit, in order to test the spores for sensitivity against imazalil, thiabendazole and/or pyrimethanil. The accompanying questionnaire will become the tag for each and every batch of samples collected, and must accompany that batch when submitted for testing.

What you need for sample collection:

1. New earbuds that have not been removed from the package before, and have at least one end still perfectly **uncontaminated**.
2. NEW Ziploc bags – DO NOT reuse coin bags or Ziploc bags at all.
3. Markers to mark the Ziploc bags – normal pen markings come off too readily and may be lost by the time they reach the DC.
4. A completed list with the information as indicated below.

Method:

1. Mark each Ziploc bag individually with the sample NUMBER (1, 2, 3,... etc.) the sampling DATE and the NAME of the packhouse.
2. NEVER put more than one earbud into a bag.
3. Complete a sample information tag per batch of samples taken at the same time.

4. Use a single earbud per sampling area – this is your SWAB.
5. Handle the swab carefully at one end only – **do not touch the end you will be collecting the spores on.**
6. Swab according to the diagram in Figure 1 below if you are sampling from a FLAT SURFACE:
  - a. From top to bottom, follow a zig-zag pattern across about a 25 cm<sup>2</sup> area on the surface of interest.
  - b. Repeat the pattern, this time from left to right, over the same area as in the previous step.
7. Swab according to the diagram in Figure 2 if you are sampling from the surface of an affected FRUIT. Only swab from obviously infected areas.
8. Drop each earbud into a Ziploc bag as follows: insert the sampling end into the new, clean bag. Holding the earbud in place using the plastic bag, clip the end you have handled as indicated in Figure 3 and drop the used end into the Ziploc bag.
9. Make sure all Ziploc bags are closed tightly, in order to prevent swabs from slipping out again.
10. Drop all the Ziploc bags in an envelope and mail to the Diagnostic Centre, CRI, 2 Baker Street, Nelspruit, 1201, ATTENTION Elaine Basson.

### Notes:

1. Do not use earbuds if they have been touched or handled at both ends. Do not reuse an earbud.
2. Even if it seems like the earbud “comes out clean” after swabbing, please send it to the DC, as spores are minute and only visible with the naked eye when there are millions of spore aggregated together.
3. Please complete the sample tag below as comprehensively as possible.
4. Use a single sample tag per batch of swabs sent to CRI.
5. Make SURE that the baggies are numbered and that the numbers correspond to the sampling points indicated on the tag.
6. Approximately two weeks after receipt of the samples, the DC will send a report with the results available to the responsible person.

**YOUR LEVY WORKING FOR YOU – GROWER LEVIES ARE USED TO FUND THE ACTIVITIES OF CRI**



# CRI Cutting Edge

RESEARCH NEWS FROM  
CITRUS RESEARCH INTERNATIONAL

March 2018

No. 246

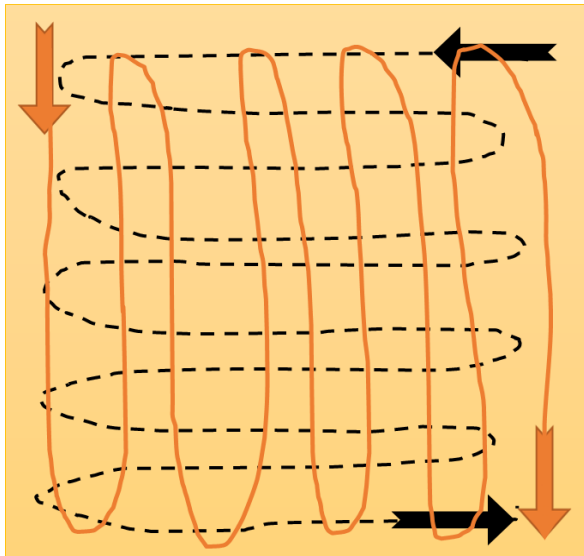


Figure 1 Diagrammatic explanation of the pattern according to which spore sampling on a flat surface is done.

At all times, touch and handle only one end, in order to minimise contamination.



Figure 2 Diagrammatic explanation of the pattern according to which spore sampling on a fruit is done. The same procedure is followed for green mould and sour rot.

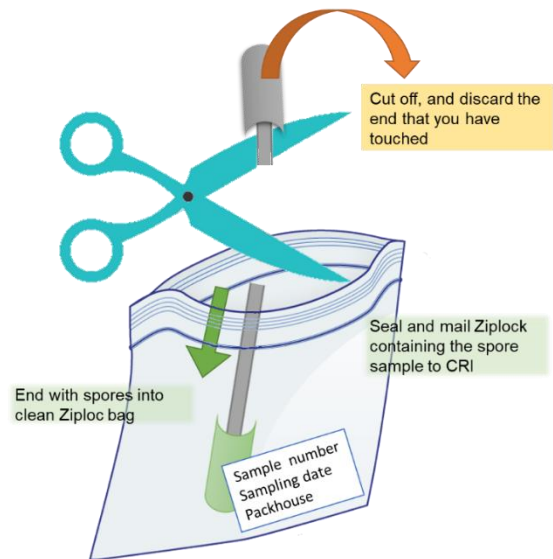
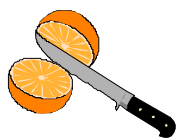


Figure 3 Diagrammatic explanation of how to put the swab into the Ziploc bag after sampling.

For the latest prices, as well as copies of the tag sheet, consult the CRI website (<http://www.citrusres.com/diagnostic-centre-0>).

For further queries, contact any of the following persons:

- Wilma du Plooy: [wilma@cri.co.za](mailto:wilma@cri.co.za)  
082 871 1669
- Elaine Basson: [elaineb@cri.co.za](mailto:elaineb@cri.co.za)  
013 759 8031
- Keith Lesar: [keithlesar@cri.co.za](mailto:keithlesar@cri.co.za)  
082 335 7543
- Catherine Savage: [catherine@cri.co.za](mailto:catherine@cri.co.za)  
072 327 0058



# CRI Cutting Edge

RESEARCH NEWS FROM  
CITRUS RESEARCH INTERNATIONAL

March 2018

No. 246

SAMPLE TAG - ONLY ONE tag per batch collected on the same day

<b>Packhouse</b>			<b>Contact person</b>		
<b>Picking date</b>			<b>Email address and contact number</b>		
<b>TESTS NEEDED</b>	IMZ <input type="checkbox"/>	PYR <input type="checkbox"/>	TBZ <input type="checkbox"/>		
<b>Sampling date</b>			<b>Cultivar</b>		
<b>Sampling point - indicate baggie numbers for the samples collected at various points</b>	Fruit from orchard		<b>Weather at time of sampling</b>	Dry	
	Fruit from waste bin			Humid	
	Retention samples			Rainy	
	Packline: before fungicide bath			Somewhat windy	
	Packline: after fungicide bath			Very windy	
	Packline: OTHER (please indicate where)			Cold	
	Bins after wash			Warm	
<b>If sampling was done in an orchard, describe the surroundings of the orchard</b>	More orchards		<b>If sampling was done in a packhouse, describe the surroundings of the packhouse</b>	Orchards	
	Natural bush			Natural bush	
	Dusty roads			Dusty roads	
	Tar roads			Tar roads	
	Animal farming (please indicate animal type)			Animal farming (please indicate animal type)	
	Mountainous			Mountainous	
	Orchard on a slope			Packhouse in a valley	
<b>Packhouse Programme</b>					
Drench				Treatment date	
Degreening protocol (if used)				Treatment date	
Sanitiser & protocol at Point of Entry				Treatment date	
Fungicide bath				Treatment date	
Sanitiser in fungicide bath (if used)					
Wax					

YOUR LEVY WORKING FOR YOU – GROWER LEVIES ARE USED TO FUND THE ACTIVITIES OF CRI



## Na-oes patogeen weerstandsmoitoring in pakhuisse en boorde

**Wilma du Plooy, Catherine Savage en Elaine  
Basson**

Citrus Research International (CRI)

Gereelde neem van na-oes patogeen spoormonsters deur middel van watte oorpluisies ("swabs") is uiters belangrik in die risikobepaling van opbou van funksiedeweerstand in pakhuisse en in boorde. Monsterneming met oorpluisies moet op 'n gereelde en herhaalbare manier gedoen word, sodat die inligting kan bydra tot die betroubare opbou van 'n risikoprofiel vir elke pakhuis. Verder sal kennis rondom heersende omgewingstoestande tydens monsterneming bydra tot insig of daar eksterne veranderlikes is wat die risiko vir weerstandsontwikkeling beïnvloed.

Hierdie Snykant beskryf die prosedure vir monsterneming van groenskimmel (*Penicillium digitatum*) en suurvrot (*Galactomyces citri-aurantii*) spore, wat dan na die Diagnostiese Sentrum (DS) by die Citrus Research International (CRI) fasiliteite in Nelspruit gestuur word, waar verdere toetse vir sensitiviteit teen imazalil, tiabendasool en/of pirimetaniel gedoen sal word. Die meegaande vraelys sal die merkvel wees vir elke besending monsters versamel. 'n Volledige antwoordlys moet dus aangeheg word by elke besending wat ingestuur word vir toetsing.

Wat nodig is vir monsterneming:

1. Nuwe oorpluisies wat nog nooit uit die verpakking verwyder was nie en waarvan een punt geheel en al ongekontamineerd is.
2. NUWE Ziploc sakkies – MOENIE geldsakkies of ou Ziploc sakkies hergebruik nie.
3. Merkpenne om die sakkies te merk – gewone penmerke kom maklik af en mag afgevee wees teen die tyd dat die monsters by die DS aankom.
4. 'n Volledige lys met benodigde inligting soos aangeheg.

Metode:

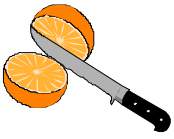
1. Merk elke Ziploc sakkie individueel met die MONSTERNOMMER (1, 2, 3,.. ens.), die DATUM van monster-neming en die pakhuis se NAAM.

2. MOET NOOIT meer as een oorpluisie in 'n sakkie plaas nie.
3. Vul die vraelys per besending monsters wat almal op dieselfde dag geneem is, in.
4. Gebruik 'n enkele oorpluisie "SWAB" per monsternemingsarea.
5. Hanteer die oorpluisie versigtig slegs aan een kant – **moet onder geen omstandighede die oorpluisie hanteer aan die kant waar die monsterneming gedoen word nie.**
6. Vee die oorpluisie volgens die patroon in Figuur 1 hieronder as die monster op 'n plat oppervlakte geneem word:
  - a. Van bo na onder, volg 'n sig-sag patroon oor 'n area van omtrent 25 cm<sup>2</sup> area op die gekose area.
  - b. Herhaal die patroon, hierdie keer van links na regs, oor presies dieselfde area as tevore.
7. ROL die oorpluisie oor die oppervlak en deur die geaffekteerde area in die geval van monsterneming op VRUGTE (Figuur 2).
8. Plaas die oorpluisie met die monsternemingskant in die nuwe, skoon sakkie as volg: Laat sak die pluisie in die sakkie en houvas met die sakkie self. Sny die kant wat hanteer was af, sodat die pluisie, met die meeste van die stokkie nog aan, los in die Ziploc sakkie val (Figuur 3).
9. Maak seker al die Ziploc sakkies is behoorlik toe, om te verseker die stokkies glip nie weer uit nie.
10. Plaas al die Ziploc sakkies van die besending, met die ingevulde vraelys in 'n koevert en stuur aan die Diagnostiese Sentrum, CRI, 2 Bakerstraat, Nelspruit, 1201, VIR AANDAG Elaine Basson

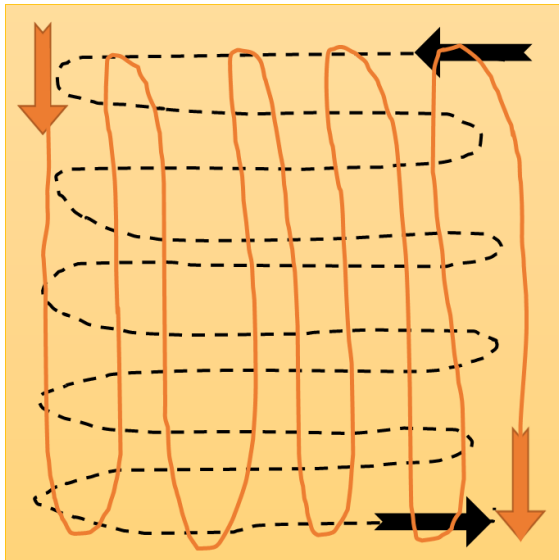
Notas:

1. Moenie die oorpluisies gebruik as hulle vantevore gebruik, aangeraak of aan beide punte hanteer was nie.
2. Al lyk dit of die oorpluisie "skoon uitkom" na monsterneming, stuur dit in na die DS, want die spore is baie klein en net sigbaar met die blote oog as daar miljoene van hulle bymekaar is.
3. Gebruik die vraelys aan die einde van hierdie skrywe – vul asseblief volledig in.

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



4. Gebruik slegs een vraelys per besending.
5. Maak SEKER dat die sakkies genommer is en dat die nommers ooreenstem met die monsternemingspunte aangedui op die vraelys.
6. Ongeveer twee weke na ontvabgs van die monsters sal die verslag met die resultate vanaf die DS beskikbaar gemaak word aan die verantwoordelike persoon.



Figuur 1 Diagram ter verduideliking van die patroon waarvolgens monsterneming van spore op 'n plat oppervlak gedoen word.

Hanteer en raak slegs aan een punt, ten alle tye, om kontaminasie te voorkom.



Figuur 2 Diagram ter verduideliking van die patroon waarvolgens monsterneming van spore op 'n vrug gedoen word.

Dieselfde werkswyse geld vir monsterneming van suurvrot.

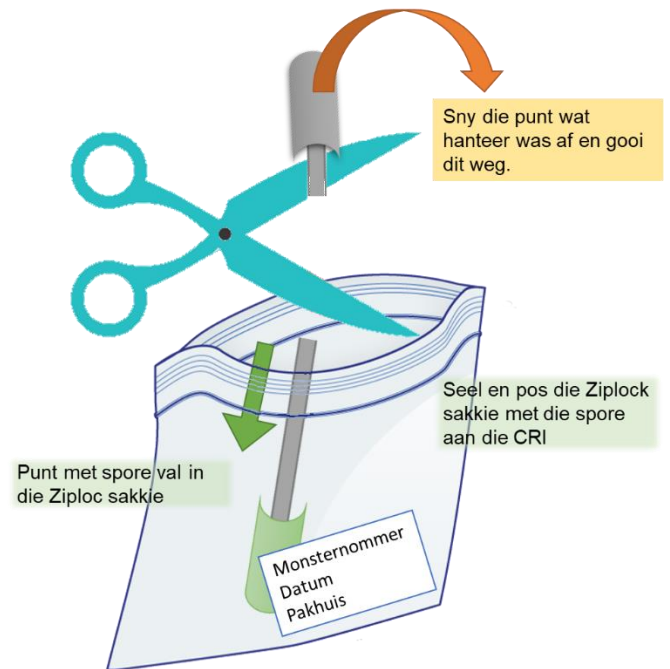


Figure 3 Diagram ter verduideliking van hoe die oorpluisie na monsterneming in die Ziplock sakkie geplaas moet word.

Vir die jongste pryse, asook verdere kopieë van die vraelys, besoek die CRI webtuiste (<http://www.citrusres.com/diagnostic-centre-0>).

Vir verdere navrae, kontak enige van die volgende persone:

- Wilma du Plooy: [wilma@cri.co.za](mailto:wilma@cri.co.za)  
082 871 1669
- Elaine Basson: [elaineb@cri.co.za](mailto:elaineb@cri.co.za)  
013 759 8031
- Keith Lesar: [keithlesar@cri.co.za](mailto:keithlesar@cri.co.za)  
082 335 7543
- Catherine Savage: [catherine@cri.co.za](mailto:catherine@cri.co.za)  
072 327 0058



MONSTERNEMINGSVRAELYS - SLEGS EEN per besending geneem op dieselfde dag.

Packhouse			Contact person		
Picking date			Email address and contact number		
TESTS NEEDED	IMZ <input type="checkbox"/>	PYR <input type="checkbox"/>	TBZ <input type="checkbox"/>		
Sampling date			Cultivar		
Sampling point - indicate baggie numbers for the samples collected at various points	Fruit from orchard		Weather at time of sampling	Dry	
	Fruit from waste bin			Humid	
	Retention samples			Rainy	
	Packline: before fungicide bath			Somewhat windy	
	Packline: after fungicide bath			Very windy	
	Packline: OTHER (please indicate where)			Cold	
	Bins after wash			Warm	
If sampling was done in an orchard, describe the surroundings of the orchard	More orchards		If sampling was done in a packhouse, describe the surroundings of the packhouse	Orchards	
	Natural bush			Natural bush	
	Dusty roads			Dusty roads	
	Tar roads			Tar roads	
	Animal farming (please indicate animal type)			Animal farming (please indicate animal type)	
	Mountainous			Mountainous	
	Orchard on a slope			Packhouse in a valley	
Packhouse Programme					
Drench				Treatment date	
Degreening protocol (if used)				Treatment date	
Sanitiser & protocol at Point of Entry				Treatment date	
Fungicide bath				Treatment date	
Sanitiser in fungicide bath (if used)					
Wax					

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