

## Australia's Khapra Beetle Response

#### 2021 Quarantine Regulators Meeting



### What is a Khapra Beetle?

Khapra beetle (Trogoderma granarium) is a tiny pest that infests stored produce such as grain shipments or silos, eating the produce and making it inedible.







Larvae typically:

- appear very hairy, forming distinctive tufts over the body and giving the appearance of a short tail
- range in size from 1.6 to 4.5 millimetres long
- are initially pale yellow and become golden-brown when they grow.

#### Adult beetles are:

- light yellowish brown to dark brown in colour
- oval shaped
- tiny, just 1.6 to 3 millimetres long.

### **Difficult to detect**



Khapra beetle larval skins vacuumed from the floor of a container



Larvae skins in the corner of a container after the floor was removed



Packaging infested with khapra beetle larvae.

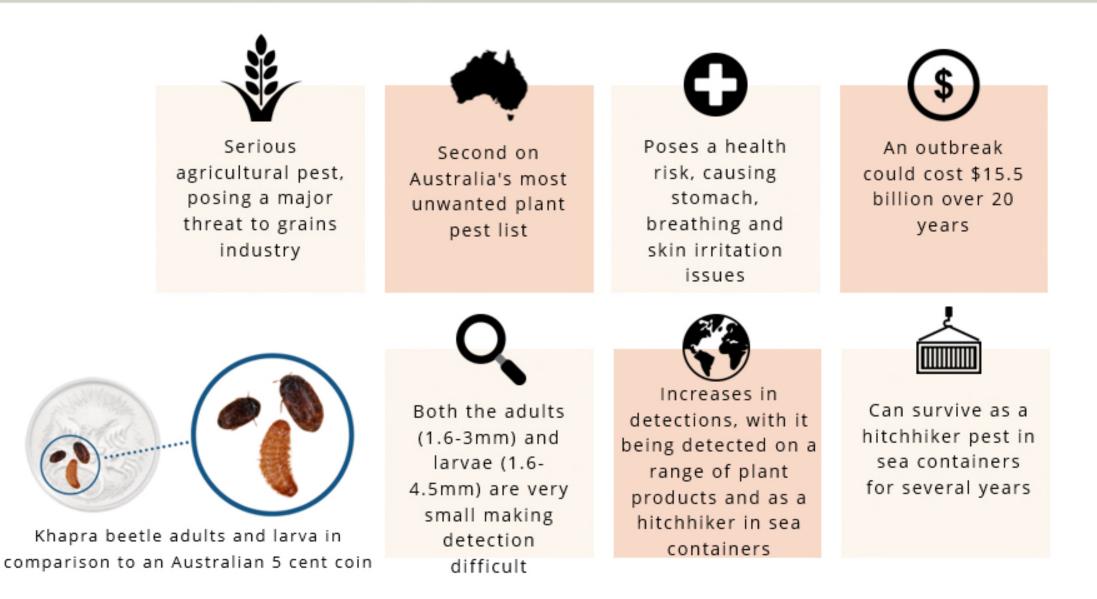


Khapra beetle larvae in shipping container after the floor was removed.



Larvae behind rubber door seal





#### **KHAPRA BEETLE INTERCEPTIONS IN AUSTRALIA**



URGENT ACTIONS TO PROTECT AGAINST KHAPRA BEETLE

| <b>3</b><br>SEPTEMBER 2020 | Ban on high-risk plant products within unaccompanied personal effects and low value freight                   |
|----------------------------|---|
| <b>15</b><br>OCTOBER 2020  | Ban on high-risk plant products within accompanied baggage or via<br>international travelers or mail articles |
| <b>12</b><br>APRIL 2021    | New measures for sea containers   |
| Mid 2021                   | Revised phytosanitary certification and new offshore treatment requirements for high-risk plant products      |
| Late 2021                  | Revised phytosanitary certification and new offshore treatment requirements for other risk plant products     |
| Late 2021                  | Introduction of phytosanitary certification for all seeds for sowing  |
|                            |   |

I\_ AWE.GOV.AU/KHAPRA-URGENT-ACTIONS

### **Target Risk Containers**

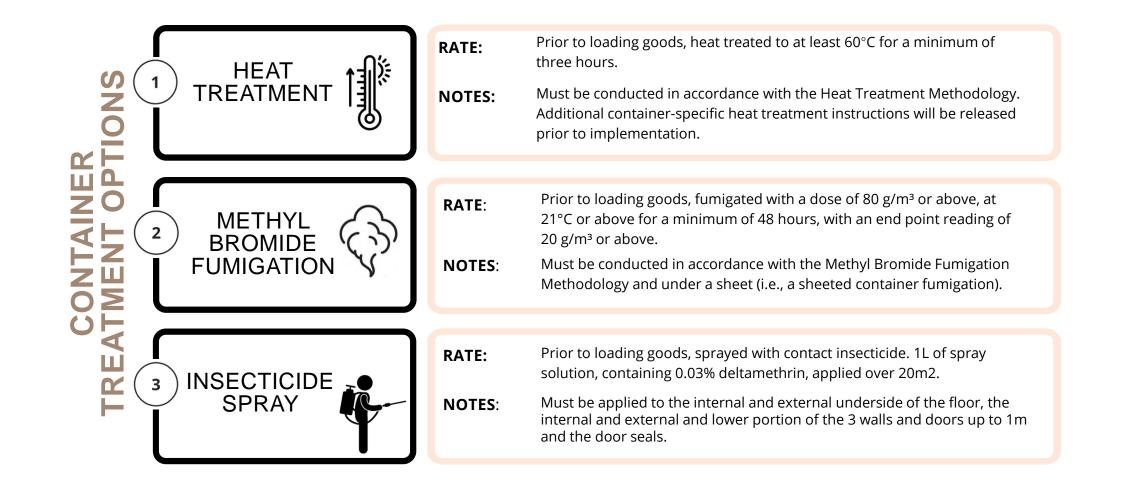
A **'target risk container'** is defined as a Full Container Load/Full Container Consolidated (FCL/FCX) where:

- high-risk plant products are packed into the sea container in a khapra beetle target risk country - 12 April 2021
- other goods are packed into the sea container in a khapra beetle target risk country and destined to a rural grain growing area of Australia – 12 July 2021

**Note:** ISO tanks, reefers, flat racks, LCL /FAK and containers that will be shipped as empty containers are excluded from the measures.



#### PHASE 6A: OFFSHORE TREATMENT OPTIONS & RATES



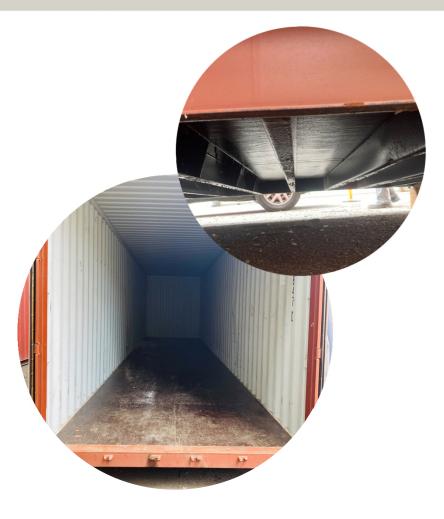
Failure to comply with these requirements will result in export of the container upon arrival in Australia2

## Phase 6A: Insecticide Spray

How did we come up with our insecticide treatment requirements?

- Scientific literature review
- Engagement with global experts (government and industry)
- Engagement with insecticide chemical manufacturers
- Industry treatment information sessions
- Open consultation on draft requirements

**Important note:** the current measures are interim, and likely to change as we build our understanding.

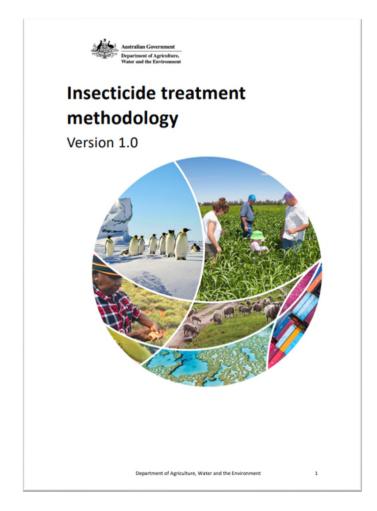




Prior to loading goods, sprayed with a contact insecticide, containing deltamethrin as a suspension concentrate. Sprayed with 1 litre spray solution, with a concentration of 0.03% of deltamethrin or above, per 20m2, applied as a coarse spray of 350 to 400 microns.

Must be applied to the internal and external underside of the floor, the internal and external and lower portion of the 3 walls and doors up to 1m and the door seals.

Must be conducted in accordance with the Insecticide Treatment Methodology.

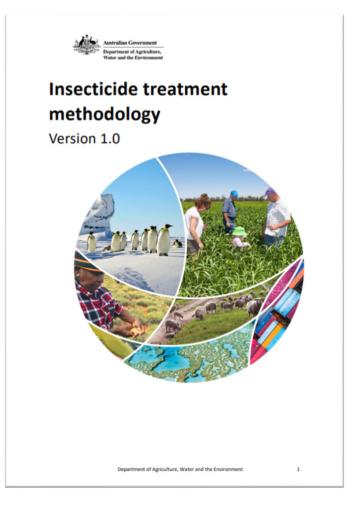


Failure to comply with these requirements will result in export of the container upon arrival in Australia.



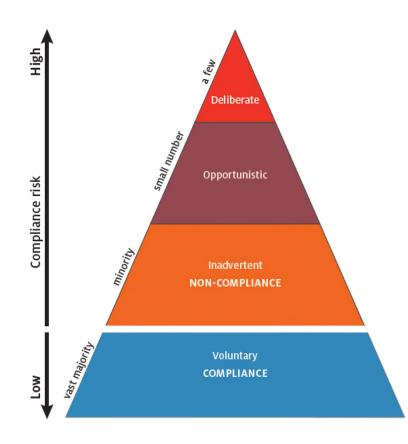
Key requirements of the insecticide methodology:

- Follow product label requirements
- Clean the container prior to treatment
- Calibrate equipment weekly
- Calculate the dose correctly
- Spray to the point of run off
- Capture relevant details in the Record of Treatment and Treatment Certificate



Failure to comply with these requirements will result in export of the container upon arrival in Australia.

### Assurance

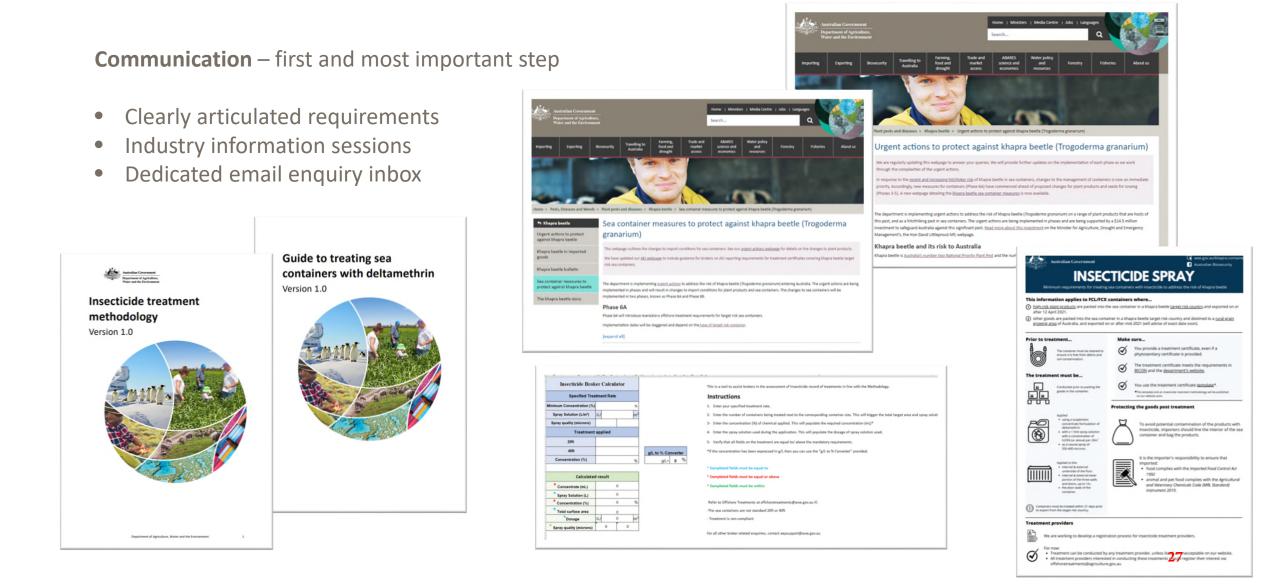


Refresher on Australia's Compliance Model:

- Majority of people want to do the right thing
- Of those that want to do the right thing, some will inadvertently (accidently) do the wrong thing
- A small number of people will try to cut corners where they think they can get away with it
- A very small number of people will deliberately do the wrong thing

We want controls in place that can prevent or detect issues across the spectrum.

### **Assurance - current**



### **Assurance - current**

#### **Documentary assessment**

- Transactional assessment of every treatment certificate for all imports subject to khapra measures
  - By industry
  - By the department
- Desktop audit verification of targeted treatment providers

#### **Border inspection**

• Targeted inspection of a percentage of imports

|   | COMPANY<br>(Including physical add | LETTERHEAD<br>ress and contact detai            | ls)                                       |
|---|------------------------------------|---|---|
| INSEC   | TICIDE TREAT                       | MENT CERT                                       | IFICATE                                   |
| Certificate Number:   |                                    | Registration Number:                            |   |
|   | CONSIGNM                           | ENT DETAILS                                     |   |
| Target of treatment Cor   | nmodity Packa                      | ging Empty o                                    | ontainer                                  |
| Target surface(s) description:  | nal & external walls & de          | oor up to 1m 🗌 inter                            | nal & external underside floor 🗌 door     |
| seals 🗌 other:  |                                    |   | Total area:m <sup>2</sup>                 |
| Consignment link or container number                                    | (s) and size:                      |   |   |
| Country of origin:  | Port of loading:                   |   | Country of destination:                   |
| Name and address of exporter:   |                                    | Name and address of importer:                   |   |
|   |                                    |   |   |
|   |                                    |   |   |
|   |                                    |   |   |
|   | INSECTICI                          | DE DETAILS                                      |   |
| Insecticide(s) active constituent:                                      | Concentration of ac                | tive constituent:                               | Product name(s):                          |
| 1   | 1                                  | or%   | 1   |
| 2   | 2g/l                               | or% 2   |   |
|   | TREATME                            | NT DETAILS                                      |   |
| Specified treatment rate:   |                                    | Treatment applied:                              |   |
|   |                                    | Concentrate:                                    |   |
| Spray solution:   | L                                  | Spray solution: L                               |   |
| Minimum concentration:  |                                    | Concentration:%                                 |   |
| Per   | m²                                 | Total target area: m <sup>2</sup>               |   |
| Spray quality:microns to  | microns                            | Dosage per 20m <sup>2</sup> :L/20m <sup>2</sup> |   |
|   |                                    | Spray quality: microns to microns               |   |
| Date of treatment:  |                                    | Location of treatment:                          |   |
|   |                                    |   |   |
|   | DECLA                              | RATION  |   |
| I declare that all information on<br>in accordance with the Insecticion | this certificate is true, co       | omplete, and accurate                           | , and that the treatment has been applied |
| Signature: Name:  |                                    |   | Company stamp                             |
|   |                                    |   |   |
|   |                                    |   | -   |
|   |                                    |   |   |
|   |                                    |   |   |

### **Assurance - future**

#### More communication products

- Instructional videos
- Increased community awareness

#### **Treatment provider assurance scheme**

- Registration and approval process
  - Knowledge and equipment assessment
- Regular auditing and compliance verification
  - Physical and desktop
- Non-compliance sanctions
  - Suspension and reapproval mechanism

#### **Better detection technology**

• eDNA/eRNA detectors

The future of assurance is continued improvement through maturation and understanding.



### **Questions?**



Australian Government

Department of Agriculture, Water and the Environment

## Hitchhiker pests

### **Quarantine Regulators Meeting**



May 2021

附件2

## Rama Karri

Director Department of Agriculture, Water and the Environment AUSTRALIA

Department of Agriculture, Water and the Environment









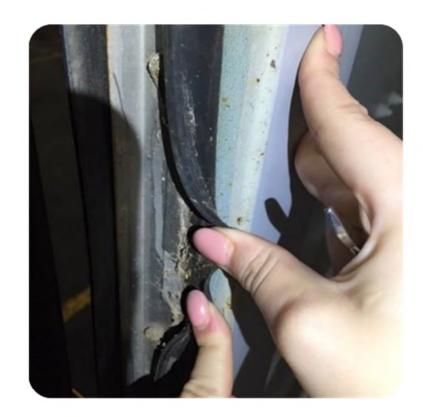






















# **CONSEQUENCES (over 20 years)**

### Khapra beetle \$15.5 billion



Giant African snail \$1.5 billion



Gypsy moth \$1.7 billion

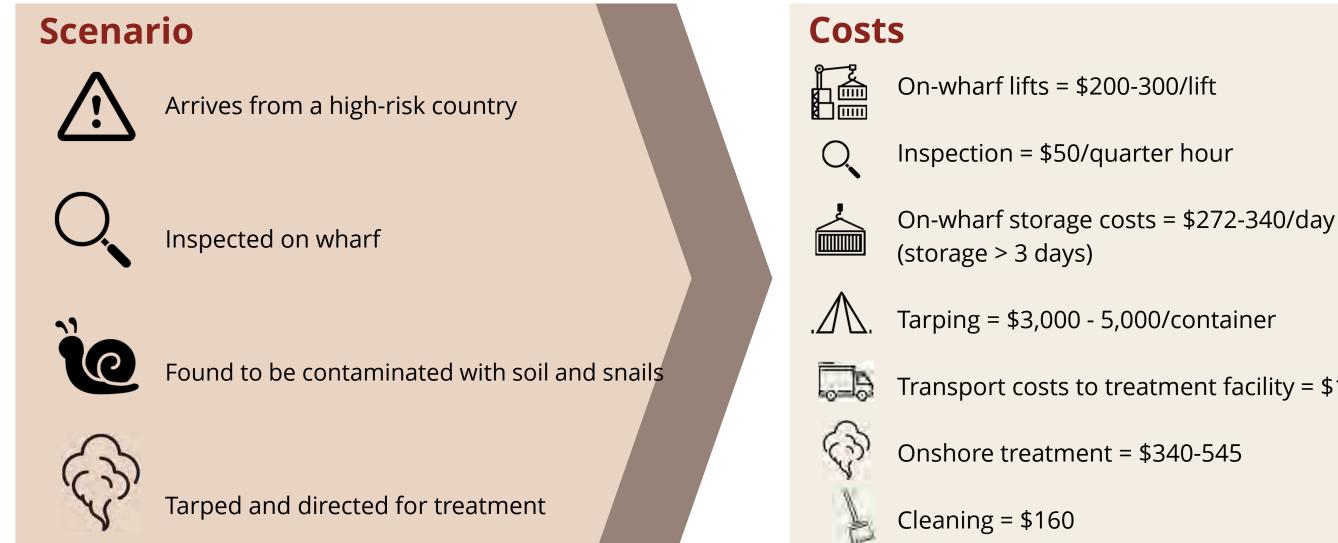
### Exotic invasive ants \$8.5 billion



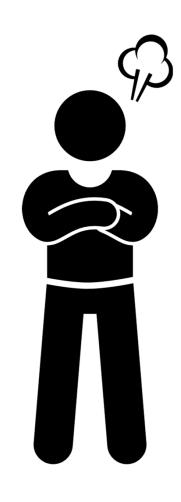
Asian Honey bees \$0.7 billion

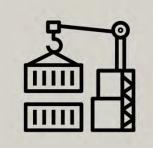


### **Inspection and treatment costs**



Transport costs to treatment facility = \$125/hour





## **CONTAINER LOGISTICS: KEY**

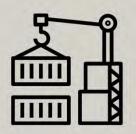


### Container depot

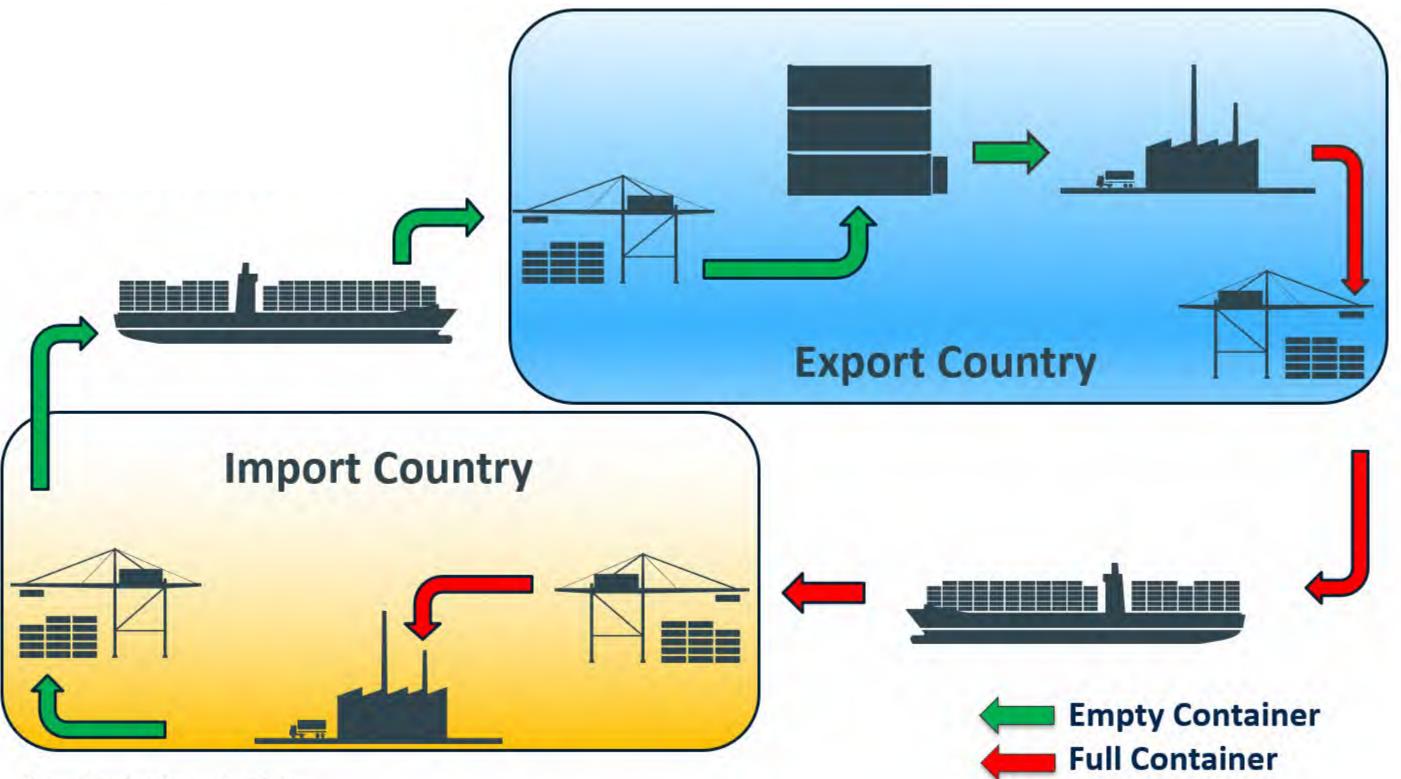
Pack or unpack point (warehouse) Container terminal



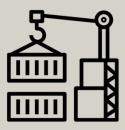
### Container vessel



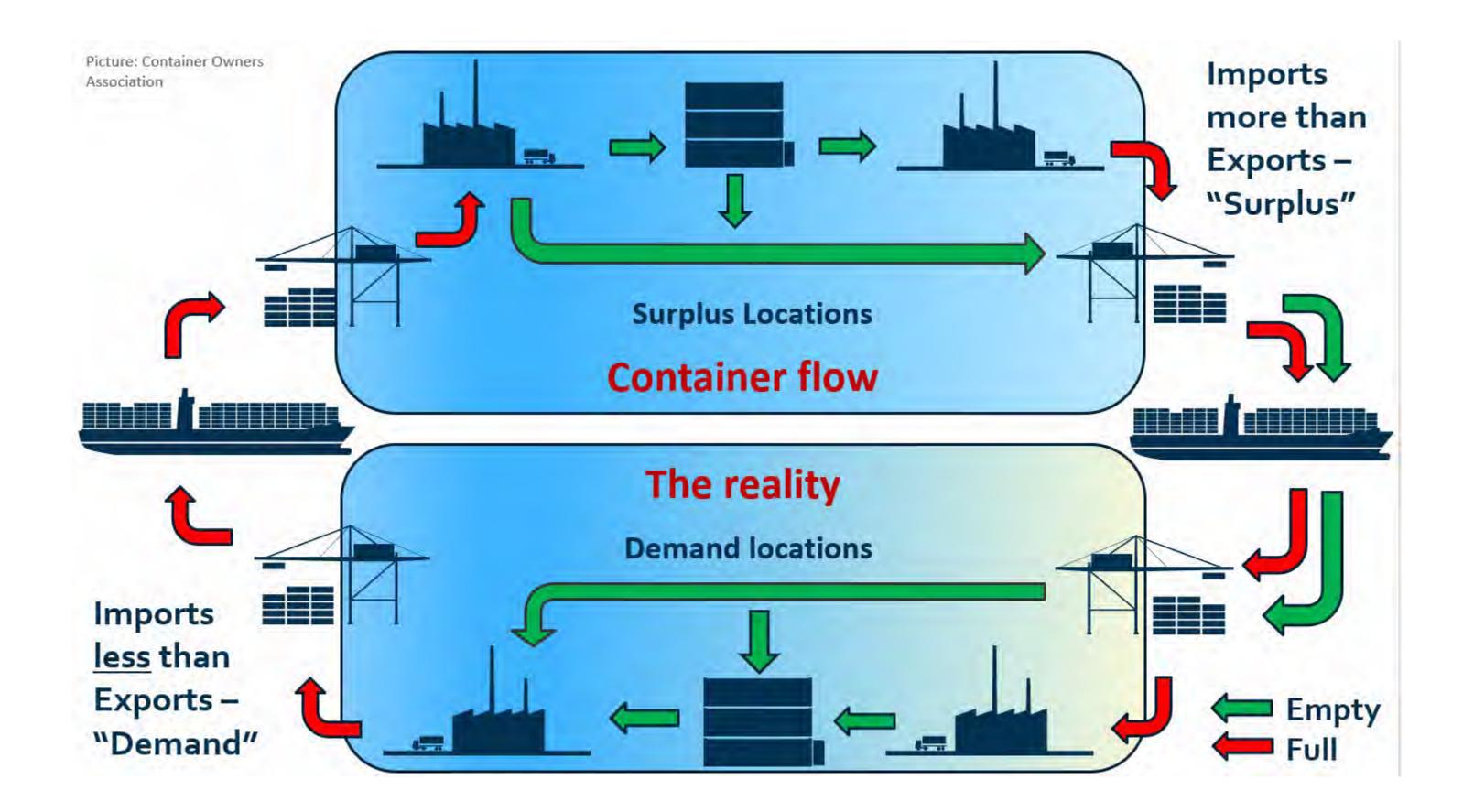
## CONTAINER LOGISTICS: WHAT MOST PEOPLE THINK



Picture: Container Owners Association

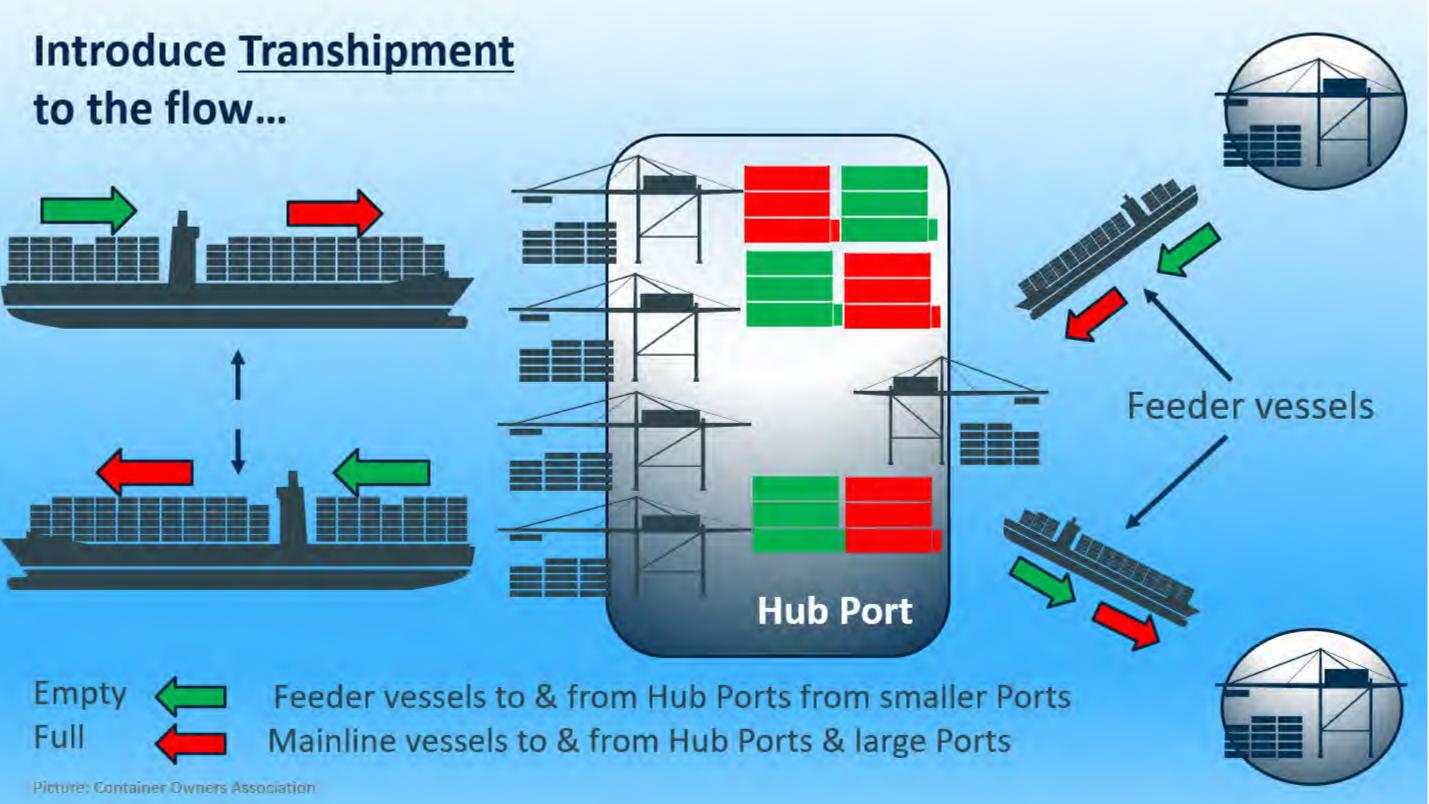


## **CONTAINER LOGISTICS: THE REALITY**

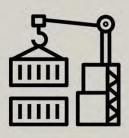




## **Introduce Transhipment** to the flow...

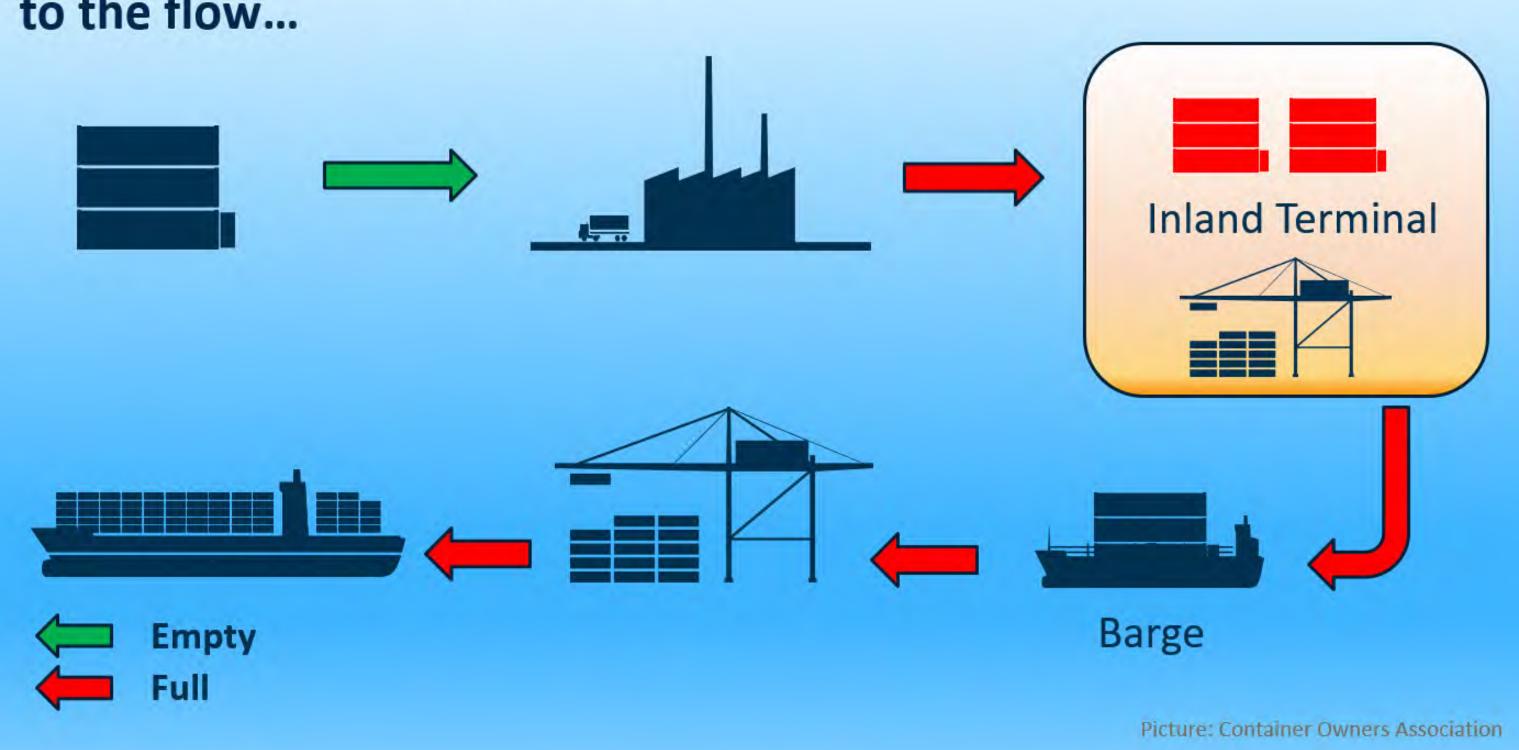






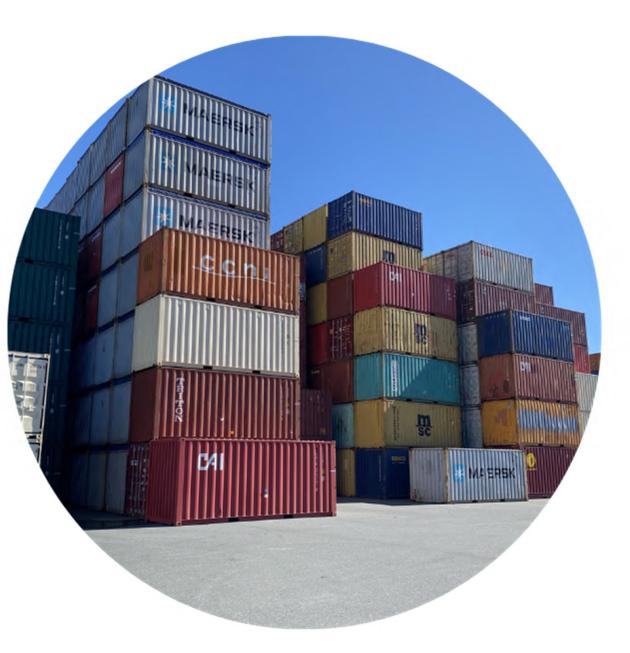
## **CONTAINER LOGISTICS: INLAND MOVEMENTS**

## **Introduce Inland Movements** to the flow...











Risks include (external and internal):

- Containers placed on grassy areas or soil are more likely to be contaminated by insects, snails and plant parts, including seeds.
- Entry of pests through container doors left open.
- Soil entering the container on the feet of persons, lacksquareor on the wheels of handling equipment.
- Cross contamination from cargoes.





Risks include (mainly external):

- Contamination due to positioning of containers on mud
- Lights that attract insects
- Cross contamination between containers
- Contamination from natural habitat
- Pest habitats or resident pest populations





Risks include (mainly external):

- Cross contamination between containers
- Cross contamination from ship holds



A moth infestation discovered by US Customs and Border Protection on a cargo ship in Jan, 2013 (Source: US CBP)



Risks include:

- Contamination from infested goods
- Contamination from packaging

Khapra beetle is an example of this









Innovative technologies

**Onshore industry** arrangements

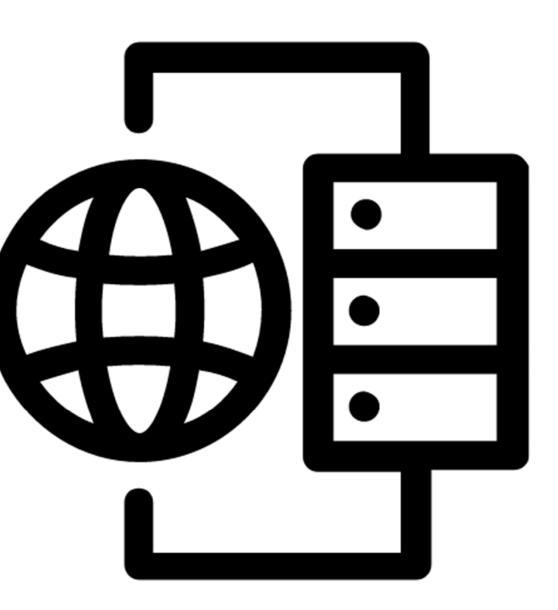
**Offshore supply** chain assurance

#### **Education and** awareness

#### **Offshore quality** systems



- Data and intelligence sharing •
- What is working  $\bullet$
- Opportunities for joint management of systems
- Offshore assurance schemes
- Innovative technologies lacksquare



# **RESOURCES**



agriculture.gov.au/import/arrival/pests
 Australian Biosecurity

#### **7 TIPS FOR KEEPING CONTAINERS CLEAN**

Help us keep hitchhiker pests out of Australia by keeping sea containers clean. Avoid border delays and unexpected costs by following these tips.



**Inspect:** Visually inspect containers for pests like khapra beetle and contaminants such as grain residue or soil. Pay attention to the bottom rails, forklift pockets, twist lock fittings, internal surfaces, container tops and the underside and cross members, if safe to do so.



**Clean containers:** Vacuum, sweep or pressure water wash containers before packing goods to remove contaminants and pests. Avoid scraping containers across the ground when moving.



**Clean storage areas:** Keep storage areas clean. Store containers away from pest habitats or resident pest populations. Store containers on paved/sealed storage and handling areas where possible. Use traps and other pest control methods to keep storage areas pest free.



**Pack clean goods:** Only pack containers with clean goods. Store goods in a clean, enclosed area prior to packing. Keep the container doors closed prior to and after packing goods. Use clean handling equipment when packing goods.



Keep away from lights: Don't keep containers under bright lights as they attract insects that can infest goods and the container.



Use higher grade containers: Use higher grade containers e.g. containers with no cracks in the floorboards, to reduce the risk of sheltering pests.



Avoid contaminated areas: Avoid driving through areas that can contaminate the container e.g. waste water, manure and other animal faeces.













Investigations for confirmation of country freedom from certain quarantine significant pests in Sri Lanka



Thushara Wickramaarachchi, PhD Additional Director/IPPC Official Contact Point **National Plant Quarantine Service** Katunayake, Sri Lanka



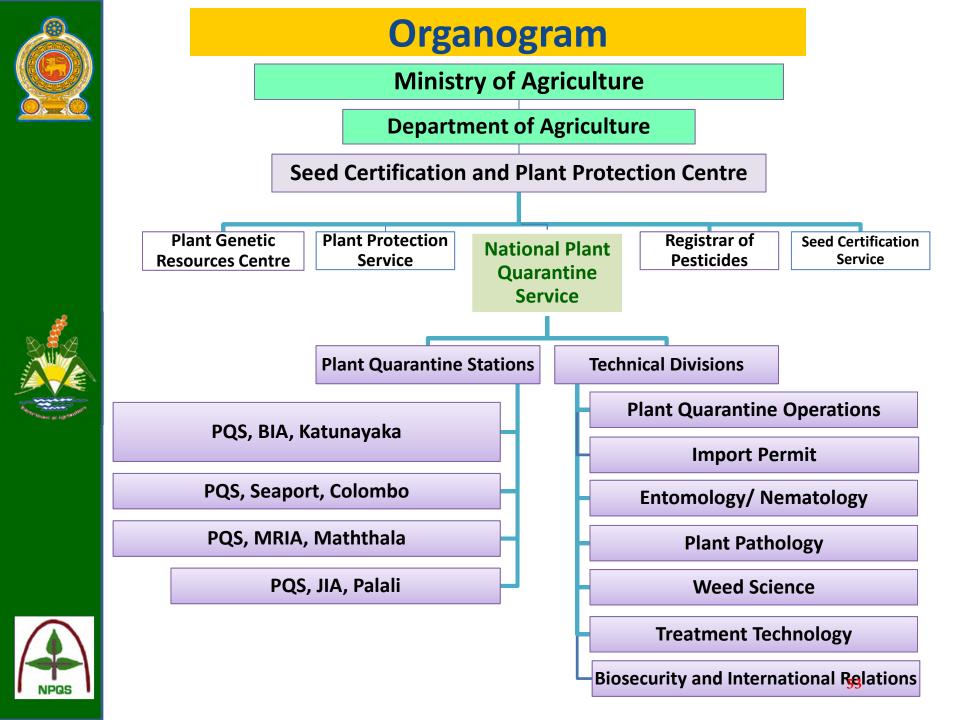
T: 0094 11 2252028 F: 0094 112253709 M: 0713044144 E: npqs@doa.gov.lk, wartwa@gmail.com 51

# National Plant Quarantine Service (NPQS) Sri Lanka

ATOMAS ANTIME









#### Vision

 Facilitate the International movement of healthy plants and plant products for the development of national agriculture and related industries

# **Objectives**

- Prevention of introduction, establishment and spread of dangerous alien pests within the country
- Involvement in domestic pest control programmes
- Development of treatment technologies to eradicate pests of quarantine importance
- Promotion of export of healthy plants and plant products





#### Mandate

Responsibility of enforcing and implementation of Plant Protection Act No.35 of 1999 and Regulations made there under in relation to plant quarantine activities. It also conducts research and development activities in plant quarantine aspects







### Recent concerns in trade of plants and plant products to EU from Sri Lanka

- EU banned the imports fresh curry leaves from third countries due the citrus greening disease as per Commission Implementing Directive 2014/78/EU of 17 June 2014
- EU restricted importation of *Xylella fastidiosa* host plants from third countries until country freedom is assured as per Commission Implementing Regulation (EU) 2020/1201 of 14 August 2020





### Investigating the presence of *Candidatus* Liberibacter *asiaticus* CLas) in *Murraya koenigii*







## Significance of Citrus Greening (CG) = **Huanglongbing (HLB)**

- The most serious citrus diseases in the world
- Caused by non-culturable fastidious phloem limited bacterium know as Candidatus Liberibacter asiaticus (CLas)
- Symptoms: Yellow shoots, blotchy mottling and chlorosis, reduced foliage and tip dieback of citrus plants, eventually declining and die
- CG is widespread in Asia, Africa, and the Saudi Arabian Peninsula
- DNA hybridization studies revealed the presence of CLas in Sri Lanka (Nakashima et al, 1998)











# Transmission of CG

- It can be transmitted by
  - Asian Citrus Psyllid-ACP (Diaphorina citri)
  - African Citrus Psyllid (*Trioza erytreae*)
  - Grafting
  - Dodder
  - Possibly by seed
- ACP is harmful both as the insect vector of the CG and as a significant citrus pest in its own
- Identification of ACP
  - 45°C feeding angle of adults
  - Full of eggs and nymphs on new flushes
  - Lot of excreted wax like substances
  - Notched leaves
- Alternate hosts of ACP
  - Murraya koenigii
  - Murraya paniculata
  - Calamondins
  - Orange jasmine (Chinese box) Murrya exotica
- Curry leaves are exported to EU as spice crop







#### Hosts of ACP



#### Curry leaves (Murraya koenigii)



Calamondins



#### Murraya paniculata



Orange jasmine (Chinese box) Murrya exotica 60







# **Transmission of CG**

- Due to the serious and destructive nature of citrus greening disease, EU has restricted the movement of plants that are hosts of CG and ACP
- Host plants for both CG and ACP are considered regulated articles in USA
- In 2015, EU banned importing of fresh curry leaves from third countries due the citrus greening disease
- Plants that are host of ACP but not host of CG be accepted in USA a compliance agreement





#### Investigation of presence of CLas in Murraya koenigii

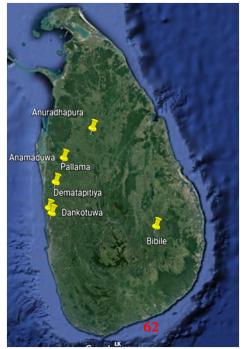
#### **Objectives**

- To investigate the presence of *Candidatus* liberibacter *asiaticus* (CLas) in *Citrus* spp and *Murraya Koenigii* from selected areas in Sri Lanka
- To confirm of the absence of Clas) in Murraya koenigii



#### Methodology

- 66 samples of citrus (35) and curry leaves (31) were collected from 6 locations of major citrus growing area
- DNA extracted from each sample were tested by PCR using specific primers which amplified the 1160 bp fragment of 16S rRNA gene of CLas {EPPO Diagnostic Protocols -PM 7/121 (1) }







#### Investigation of presence of CLas in Murraya koenigii

#### ...Methodology

- Viruliferous ACP were introduced into health citrus and curry leaves (10 from each) plants in a insect proof cage
- After I month of inoculation, DNA from each plant were tested for presence of CLas



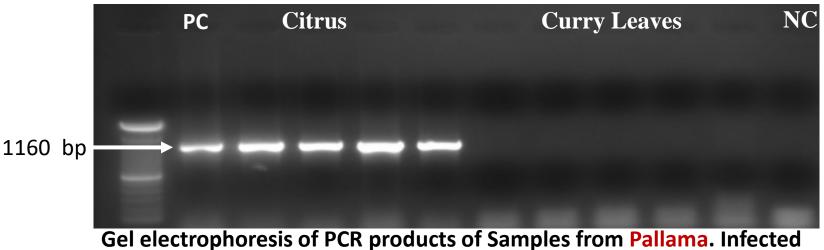




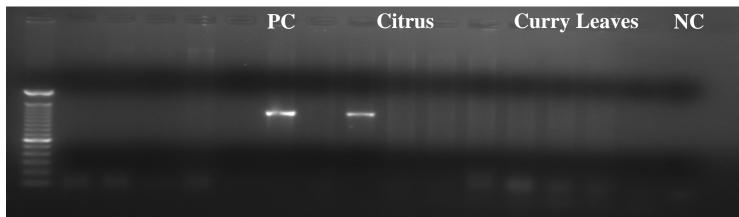
Healthy citrus and *Murraya* plants were inoculated with viruliferous CLas in a insect proof cage



 Out of 35 samples of citrus, 15 were found infected with CLas where as all 31 samples of *Murraya* found uninfected with CLas



samples showed amplification of 1160 bp fragment









Summery of the PCR results of artificially innoculated citrus and *Murraya* plants using viruliferous CLas

| Gel image<br>number | Plant numbers  | Results  | Gel image<br>number | Plant number   | Results  |
|---------------------|----------------|----------|---------------------|----------------|----------|
| 2.1                 | M 01           | Negative | 2.12                | C 01           | Negativ  |
| 2.2                 | M 02           | Negative | 2.13                | C 02           | Negativ  |
| 2.3                 | M 03           | Negative | 2.14                | C 03           | Negativ  |
| 2.4                 | M 04           | Negative | 2.15                | C 04           | Negativ  |
| 2.5                 | M 05           | Negative | 2.16                | C 05           | Negative |
| 2.6                 | M 06           | Negative | 2.17                | C 06           | Negative |
| 2.7                 | M 07           | Negative | 2.18                | C 07           | Negative |
| 2.8                 | M 08           | Negative | 2.19                | C 08           | Negative |
| 2.9                 | M 09           | Negative | 2.20                | C 09           | Positive |
| 2.10                | M 10           | Negative | 2.21                | C 10           | Negative |
| 2.11                | M 11 (control) | Negative | 2.22                | C 11 (control) | Negative |

 Only one citrus plant artificially inoculated was found positive for CLas where as no single plant of *Murraya* found positive



It was generally found that ACP was shown more attraction towards *Murraya* plants



- Beloti *et al*, 2018 could not observe the successful transmission of of CLas to *Murraya* by ACP and it was treated as immune to CLas
- Curry leaves plants are proved to be more attractive to the ACP than citrus plant (Beloti et al, 2017)
- Curry leaves is not a host of CLas, but is good host for the ACP insect vector (Teck *et al*, 2011 and Westbrook *et al*, 2011)
- The presence of CLas in leaf samples of citrus were identified by amplification of 1160 bp fragment of 16S rRNA (Athukorala *et al*, 2020)





### Conclusion

- CLas is in association with citrus plants in Sri Lanka.
  Thus country is not free from CLas.
- Murraya is not a host of CLas though it is a good host of ACP. Thus, Murraya is treated as immune to CLas
- There is a potential to export fresh curry leaves due to the absence of CLas in curry leaves under a special compliance agreement with EU





# Confirmation of country freedom from *Xylella fastidiosa* in Sri Lanka







# Significance of Xylella fastidiosa (Xf)

- Xylella fastidiosa is a Union quarantine pest that is known to occur in the EU. It is regulated in the EU as a harmful organism under Plant Health Regulation (EU) 2016/2031
- Xf lives in the xylem vessels of plants. The xylem vessels can become blocked by biofilms of the bacteria exudates leading to leaf scorch diseases characterized by the desiccation of leaves and dieback
- Infections may be asymptomatic but can produce a range of disease symptoms from minor leaf scorch through to extensive die-back and plant death
- Has a wide range of potential intraspecific hosts including over 220 species which can be infected naturally
- Target host species more likely to be infected with several subspecies of *Xylella fastidiosa*
  - Prunus dulcis, Prunus avium, Polygala myrtifolia, Spartium junceum, Nerium oleander, Rhamnus alaternus and Rosmarinus officinalis





# **Vectors of Xylella fastidiosa**

- natural spread of the bacterium is exclusively through xylem-feeding insect, Philaenus spumarius
- The most likely pathway for introduction of Xf is the importation of plants for planting and / or infected insects (vectors) originating from areas where the pest is present
- Following detection of Xf outbreaks in Europe, the EU has implemented several risk reduction options to combat this plant disease and prevent its entry and spread
- Xf is quarantine listing pest in EU thus all hosts are prohibited to import into EU
- All nonEuropean Cicadellidae (leafhoppers) that are known to be vectors of Xf such as Carneocephala fulgida, Draeculacephala minerva and Graphocephala atropunctata are also regulated in EU





#### Hosts of Xf



Plum leaf scald



**Cherry leaf scorch** 



Polygala leaf tip burn



**Coffee leaf scorch** 



Pierce's disease of grape



Almond leaf scorch



**Citrus Variegated Chlorosis** 





#### **Confirmation of country freedom from** *Xylella fastidiosa* in Sri Lanka Objectives

- To investigate the presence of Xf in Sri Lanka
- To study the presence of Xf in potential host plants in Sri Lanka

#### Methodology

- Site Inspection of places of production for *Xylella fastidi*osa was conducted in accordance with the guidelines of PM 3/82 (2)
- Sixty seven fresh leaf samples of 23 species were collected from Colombo, Gampaha, Kandy, Nuwara Eliya, Kurunegala, Puttlalam and Anuradhapura districts in Sri Lanka
- Samples were subjected to PCR with forward RST31 5' GCGTTAATTTTCGAA / reverse RST33 GTGATTCGATTGC-3' (Amplicon size- 733 bp.) primers which amplify the 733 bp fragment of *rpoD* gene (Appendix 4 of EPPO Protocol for Xylella fastidiosa)



Positive samples of test DNA of *Xylella fastidiosa* was procured from the French collection of Plant Associated Bacteria, CIRM-CFBP





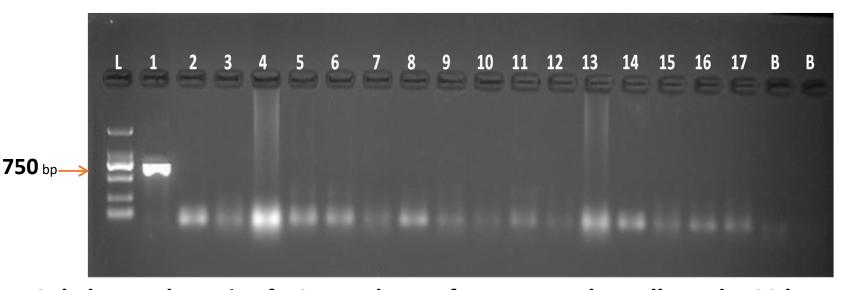
#### Photographs of some samples collected for the study



Cinnamomum zeylanicum, Garcinia morella, Elettaria cardamomum, Coffea spp., Citrus reticulate, Piper nigrum, Averrhoa carambola, Muntingia calabura, Rosa spp., Persea Americana, Citrus spp., Senna auriculate, Coffea spp.



- The desired band with the size of 733bp, was not observed from any of the samples studied
- The positive control of *Xylella fastidiosa* produced the band with the size 733bp as expected







Gel electrophoresis of PCR products of some samples collected. 733 bp amplified in positive control (L:2000D DNA Marker, 1:Positive control (*Xf,733bp*), 2&16:NPQS 282, 3&13:NPQS 327, 4&10:NPQS 350, 5&14:NPQS 270, 6&11:NPQS 284, 7&15:NPQS 265, 8&17: NPQS 342, 9&12:NPQS 262, B:Negative controls)



- This conventional PCR is suitable for the detection and identification of Xf and the test is based on Minsavage *et al.* (1994).
- This primer pair can specifically amplify the 733bp fragment of *rpoD* gene (Appendix 4 of EPPO Protocol for Xylella fastidiosa)





### Conclusion

- Xylella fastidiosa is not known to present in Sri Lanka on the basis of inspection, sampling and molecular testing carried out by NPPO, Sri Lanka using a test listed in Annex IV of Regulation (EU) 2020/1201
- However, the investigations will be continued periodically to confirm the freedom of Xf
- Work in progress with DG-SANTE to obtain a conditional permission for resume the trade of Xf host plants to EU











# Thank you