South African Action Plan for Huanglongbing and Asian Citrus Psyllid – preparedness, early detection and rapid response

Compiled by Citrus Research International and HLB Steering Committee

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Content layout

1. Executive summary of Action Plan

The various incursion scenarios and response actions are summarised in Table 1. For more detail, please study sections B to L.

Table 1. Summary of scenarios and response actions

Incursion scenario*	Quarantine zone**	Delimiting survey	Permitted movement of Las and ACP host propagation material from nurseries in a quarantine zone ***
A-1: ACP point incursion	5-km	- Identify suitable inspection / trap sites according to the presence of host plants on likely corridors along which the pest may have spread up to 100km from the detection site - Aim to achieve coverage of 100 km range of survey corridors from detection site, informed by the presence of host material and geographic layout. Three teams of two people to cover this range within a week. - PCR trapped/collected ACP for Las testing	HLB Safe nursery
A-2: ACP range expansion	District	-As per A-1 -General trapping and surveys in districts bordering Q-zone -	HLB Safe nursery
B-1: Las point incursion but no vector activity	400-m	- Survey 1 km, 700-, 400-, 200-, 100- and 50m perimeters (12 sites per perimeter). - PCR trapped/collected ACT and symptomatic host material for Las testing	HLB Safe nursery
B-2: Las point incursion, ACT present and active, but ACP absent	5-km	- Survey 5-, 3-, 1-km, 700-, 400-, 200-, 100- and 50m perimeters (12 sites per perimeter) and four 30-km transects (6 sites per transect), as well as 4 sites immediately adjacent (within 7m) of the find site. - PCR trapped/collected ACT for Las testing	*Regional only*: HLB Safe nursery with <50-m buffer zone *Movement out of region*: HLB Safe nursery with >50-m buffer zone
B-3: Las point incursion, ACP present and active	5-km	- As for B-2	*Regional only*: HLB Safe nursery with < 800-m buffer zone *Movement out of region*: HLB Safe nursery with >800-m buffer zone, **or** >400-m buffer plus vector-free treatment and monitoring in the 400 to 800-m zone
B-4: Las + ACP range expansion	District	- As for B-2	As for B-3

*ACP = Asian Citrus Psyllid; Las = HLB pathogen; ACT = African Citrus Triozid

**Radius of quarantine zone surrounding detection sites

***Movement will ONLY be permitted from nurseries in compliance with the HLB Safe System (*inter alia* trees grown in insect-protected structures, with or without buffer zones surrounding the nursery), but subject to CBS-specific movement restrictions in R.110

2. Background

Huanglongbing (HLB) is a disease associated with three phloem-limited bacteria: '*Candidatus'* Liberibacter asiaticus (Las), *Candidatus* 'Liberibacter' *africanus* (Laf) and *Candidatus* 'Liberibacter' americanus (Lam). HLB is spread by the insect vector, Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and African Citrus Triozid (ACT), *Trioza erytreae* Del Guercio (Hemiptera: Triozidae). HLB associated with Las is the most devastating disease of citrus worldwide and presently occurs in Ethiopia (Saponari et al., 2010) and Kenya (Ajene et al., 2020), and in Mauritius and Reunion. The presence of Asian Citrus Psyllid, ACP, the primary vector of HLB, was first reported in East Africa in Tanzania (2015) and Kenya (2016). Both Asian HLB and ACP occur in countries that are frequent trading partners with southern African countries and traders and travellers pose a threat of inadvertently spreading the disease or the Asian vector.

Citrus industry representatives and governments in a number of citrus producing countries have developed contingency, strategy or action plans to inform the actions required for effective early detection, containment and control strategies [Australia: Pegg et al., 2013); USA-California: CDFA, 2018, Albrecht et al., 2020)]). The California Department of Food and Agriculture (CDFA) plan has been operationally implemented on a large scale following the outbreak and spread of ACP and detection of HLB in California.

This document, modelled to a large extent on the CDFA plan, has been developed as the South African Action Plan for HLB and ACP, specifically to enable early detection, rapid response and guide ensuing actions required to safeguard South Africa from the biosecurity threat posed by HLB and ACP.

In this action plan, early detection and rapid response measures are detailed for HLB, associated with Las and vectored by ACP. ACP is also able to vector African Greening (caused by Laf), which is still absent in some citrus production areas in South Africa. The endemic occurrence of ACT, the triozid vector of African Greening, which is also able to vector Las, further highlights the need for rapid response plans in South Africa.

3. Oversight and coordination (HLB Steering Committee)

The endorsement of the action plan, changes to it and high-level coordination of implementation will reside with the HLB Steering Committee. The composition of the HLB Steering Committee will include senior representation from the Directorates Plant Health (PH) and Inspection Services (IS) of the Department of Agriculture, Land Reform and Rural Development (DALRRD), Department of Environmental Affairs (DEA), industry and the scientific community. Members of the Steering Committee must have executive decision-making authority. Some of the roles of the stakeholder groupings include the following:

DALRRD: The HLB Steering Committee meetings will be convened by DALRRD. DALRRD will be responsible for official functions. Actions are executed by an Executive Officer of DALRRD in terms of

the control measures R.110 of the Agricultural Pests Act, 1983 (Act No. 36 of 1983). DALRRD is responsible for the timeous updates to legislation and regulations, most notably the import conditions for material of HLB or ACP alternative host plants as well as R.110, to ensure legality and timeous enforcement of the measures stipulated in this Action Plan.

DEA: In support of DALRRD, DEA will assist with official functions relevant to environmental settings.

Citrus Research International (CRI): As the citrus industry body responsible for technical matters, CRI will promote industry awareness, preparedness and implementation of the Action Plan. Through its Biosecurity and Citrus Improvement Scheme (CIS) divisions, CRI will be responsible for ongoing revision of the Action Plan to ensure alignment with global best practice. CRI and DALRRD will coordinate surveys and diagnostic testing. Through the CIS, CRI will liaise with Scheme stakeholders and will oversee implementation of the HLB Safe System in citrus nurseries.

Citrus Growers Association (CGA): Will engage commercial citrus growers to support effective implementation of the Action Plan, ensure awareness within the industry and provide funding support.

Agricultural Research Council (ARC): Will provide Post Entry Quarantine and diagnostic services.

South African Citrus Nurserymen's Association (SACNA): As a stakeholder grouping represented on CIS Advisory Committee (CISAC), SACNA will assist with actions (awareness, preparedness, surveillance, containment, eradication and control measures) as it pertains to their members.

South African Nursery Association (SANA): As a stakeholder grouping represented on CISAC, SANA will assist with actions (awareness, preparedness, surveillance, containment, eradication and control measures) as they pertain to their members.

Representatives from provincial working groups: Will support DALRRD and DEA with awareness, surveillance, containment, eradication and control measures.

4. Preparedness

An important task of the HLB Steering Committee is to identify and assess preparedness tasks. This includes ensuring that the necessary legislation is in place, as well as inter-agency and inter-governmental cooperation agreements and a framework for provincial engagement. Other important preparedness tasks involve funding, stockpiling, and emergency registration of remedies.

5. Awareness and training

Public awareness aids prevention and early detection. Awareness material on HLB and its vectors will be produced and distributed to alert and inform the public and all stakeholders. Distribution points for awareness material and target groups must be identified and awareness campaigns run, including follow up surveys. Appropriate awareness material with suitable communication media must be provided at points of entry, with priority being given to higher risk areas or routes. Additional training should be provided to inspectors, extension officers, field workers, nursery personnel and other

relevant stakeholders regarding HLB and its vectors, as well as identification of host species. Support from agricultural extension officers in provincial governments is regarded as a strategic resource, and should be utilised. Awareness campaigns should, where possible, include SADC and other African countries as needed.

6. Surveillance

6.1 Incursion detection surveys

Survey protocols approved by the HLB Steering Committee are appended to the Action Plan as Appendix A. A regular surveillance programme for HLB, African Greening and ACP should be in place for early detection. Early detection surveys should include scouting for the detection of ACP and symptoms of HLB on citrus, sampling of symptomatic citrus plants for testing, and trapping or sampling of ACP and ACT for identification and HLB diagnostic tests. Official and officially endorsed detection surveys should be aimed at high-risk areas, based on epidemiological models and world experience, and surveillance should extend into Africa and surrounding islands. The latter can be conducted through industry projects and should be included in inter-governmental cooperation. Targeted surveillance should include areas with a high demography of relevant Eastern countries, African countries and east-African islands, as well as areas where there is a concentration of trade and travel exchange with such countries.

Surveillance should include all species of *Citrus*, *Fortunella* and *Poncirus trifoliata*, which are hosts for Las and its vectors, as well as highly suitable alternative hosts for Las or ACP (Table 2) at residential properties, commercial and abandoned orchards, nurseries, parks, retail outlets, weekend markets, roadside stalls and natural bush. It is therefore important that scouts and DALRRD inspectors receive the necessary training to identify hosts.

Table 2. Hosts of Las, ACP and ACT

Host		Host status*				
Botanical name	**Common name**	**ACP**	**ACT**	**Las**	**Laf**	**LafCl**
Citrus	All types	I	I	I	I	I
Fortunella	Kumquat	I	I	I	I	
Poncirus trifoliata	Trifoliate	I	I	I	I	
Murraya paniculata (syn. *M. exotica*)	Orange Jasmine, China boxwood, mock orange	I	III-IV	III	III-IV	
Murraya koenigii (syn. *Bergera koenigii*)	Curry leaf	I	I	III-IV	III-IV	
Choisya ternata	Mexican orange	I				
Clausena anisata	Horsewood, Perdepis	V	I	V		I
Zanthoxylum capense (syn. *Fagara capensis*)	Small knobwood, Kleinperdepram	V	II	V		
Calodendrum capense	Cape chestnut, wilde kastaiing, Kaapse kastaiing, Umbhaba	V	IV, III [+]	V		
Vepris lanceolata (syn. *Vepris undulata*)	White iron wood	V	I	V		
Vepris reflexa	Bushveld white-ironwood	V	II [•]	V		
Oricia / Teclea		V		V		

* Group I (highly suitable), Group II (intermediate to low suitability), Group III (not suitable), Group IV (non-host), Group V (potential host)

[+] Poor development on host due to hairy leaves, but adults survive well on the host

• Hard leaves make it a poor host

Surveillance should be extended to ports of entry. High risk ports should be profiled and projects rolled out by DALRRD to search passenger luggage.

Grower based surveillance should be promoted and monitored in the various citrus growing areas in southern Africa. In addition to these more targeted survey programmes, more informal community surveillance that is broad-based and reactive to awareness campaigns and community feedback should be encouraged. Official surveys should follow specific protocols as adopted by the HLB Steering Committee, whilst samples from informal or community surveys should at least provide information on host, address or GPS coordinates, and contact details to allow official follow-up and verification if needed; ideally trees from which samples were drawn should be physically marked. Positive detection in surveys not conducted under the auspices of DALRRD-Inspection Services (IS) will first be validated by means of an official detection survey within 6 calendar days of the unofficial detection; if Las or ACP is confirmed, this survey will be expanded to include a delimiting survey.

Reports of illegal imports should be followed up and plant material traced and diagnosed. Awareness and monitoring (use of internet surveillance software) should be extended to include internet trading of host plants.

6.2 Sample analysis and reporting

Sampling methods and diagnostic protocols approved by the HLB Steering Committee are appended to the Action Plan as Appendix B. The approved diagnostic tests for ACP and ACT vectors should include morphological identification by a specialist approved by the Steering Committee, and PCR diagnostics as appropriate. The infective nature of the insect vectors is determined by means of PCR diagnostics. The approved diagnostic tests for Las in plant material is real-time quantitative PCR detection.

The HLB Steering committee will accept diagnostic reports from any laboratory approved by DALRRD using the approved protocols and procedures. These laboratories must obtain permits allowing them to receive, analyse and dispose of sampled insects or plant material as prescribed. Approved laboratories must provide results within 6 days of sample submission. These laboratories will be responsible to receive, document and analyse samples according to Good-Laboratory-Practice and approved SOPs. Results of the analyses will be reported to the client, and, bearing in mind the mandatory notification of detection of quarantine pests to DALRRD-IS and DALRRD-Plant Health (PH), DALRRD must also receive the detailed report. In all cases where a Las or ACP diagnosis has been requested, the laboratory and/or specialist will be obliged to report the detailed report to the Steering Committee. Strict confidentiality must be maintained until results are suitably verified and publication is approved by the HLB-SC.

7. Rapid response to incursions into pest free areas

7.1 Delimiting surveys

Upon a single positive detection of Las or ACP and confirmation by CRI diagnostics laboratory that has been accredited by DALRRD, institute delimitation surveys within 6 calendar days after confirmation of detection. Delimiting surveys for the vector (ACP/ACT) should be carried out 3-monthly. Delimiting surveys for the pathogen should be repeated at least every 6 months, in order to monitor spread and/or success of eradication/containment measures.

The type of delimiting survey carried out will depend on different detection scenarios as stipulated below. In these scenarios, a point incursion is defined as a single initial detection point whereas range expansion is defined as concurrent multiple detections of the invasive organism within a region:

7.1.1 Scenario A: ACP incursion

- A delimiting survey procedure will be initiated as a matter of urgency (within 7 days) in response to the first reported detection of ACP. Considering that the detection is unlikely to be a point incursion, and the pest may have been present for some time already and more widely dispersed, the point of detection merely provides a starting point for delimitation.

7.1.1.1 Delimiting survey procedure

- Plot the initial find site on Google Earth. With the aim of identifying the boundary of current distribution and likely corridors along which the pest may have spread, plot potential inspection and trap sites on Google Earth.
- The number of inspection (visual, tap- or sweep net sampling) and trap sites will be determined by presence and density of host plants and available resources required to complete the initial delimitation within one week.
- In addition to the detection site itself, and any likely host sites in close proximity, 10 to 20 inspection sites should be identified on the 5- to 10-km perimeter of the area where the find occurred, considering the nature of the original detection site and the availability of host plants in the vicinity.
- Identify suitable inspection / trap sites according to the presence of host plants on likely corridors along which the pest may have spread up to 100-km from the detection site.
- Aim to achieve surveillance coverage of the 100-km range of corridors with even coverage, but as informed by the presence of host material and geographic layout.
- These inspection points should be located optimally to ensure maximum chance of psyllid detection, i.e. in orchards against windbreaks, closest to natural vegetation, prevailing wind side, and facing the direction of the most likely incursion pathway. Transport corridors and abandoned orchards should be included.
- Identify inspection points furthest from the find site and survey back towards the find site.
- Whereas the Google Map plotting of potential inspection sites will be the first step, the final location of the sites will be informed by each inspector selecting the optimal sites when present in the locality.
- One ACP trap should be placed on citrus trees, or other ACP hosts if no citrus is available, at each inspection point.
- Visual inspection and tap sampling for ACP should be conducted on 5 branches per tree on at least three citrus trees or other ACP hosts at each inspection point.
- After trap placement and tap sampling for ACP, approximately two minutes should be spent looking for Las symptoms on the trap tree and trees adjacent to it. Only symptomatic material will be sampled (10 symptomatic leaves per site).
- ACP traps should be checked after one week initially, and then every second week after that.

The above survey procedure represents the initial delimitation response. The findings will inform the nature of the follow-up delimitation. If no ACP are detected at any of the inspection sites, intensify surveys in closer proximity to the detection site and continue surveillance of the original survey sites (inspections and trap monitoring*)* for at least six months at monthly intervals. If any ACP are detected at any of the survey points, the locality will inform a new quarantine zone for which

delimiting surveys (as above) should extend beyond that of the bordering quarantine zones 100-km. If the infestation is wide-spread or deemed to be as a result of range expansion, the quarantine area may be expanded to each magisterial district affected. Once no new range detections are recorded for a period of six months, intensify the surveys inwards to define the boundaries of the area of presence. Depending on laboratory capacity, ACP specimens captured or collected will be tested for the presence of Las.

7.1.2 Scenario B: Las incursion

- A delimiting survey procedure will be initiated as a matter of urgency (within 7 days) in response to the reported detection.

7.1.2.1 Delimiting survey procedure

- On Google Earth, mark out 12 evenly distributed (similar to clock hour markings) potential inspection sites on the perimeters each of the circles with a radius of 5-km, 3-km, 1-km, 700-m, 400-m, 200-m, 100-m and 50-m surrounding the original find site. Also mark out 4 inspection sites immediately adjacent to the original detection site.
- Additionally, four transects radiating from the find site for a distance of up to 30 km should be mapped out. On these transects, at least 6 inspection sites should be marked at approximately 5 km intervals (only where plants potential host plants might be present). These transects should be placed so as to intersect as much host material (especially citrus plantings) as possible, and need not be evenly distributed. Inspection sites can also deviate from the transect lines to incorporate host plantings adjacent to them.
- Whereas the Google Maps plotting of potential inspection sites will be the first step, the final location of optimal sites will be determined by each inspection team when present at the locality.
- Utilise only the survey points that have HLB and vector hosts in the immediate vicinity, or move them to such points if close by.
- One ACP trap should be hung at each inspection site.
- Each inspection site will be surveyed for presence of its vectors by visual and tap sampling methods. Visual inspection and tap sampling for vectors should be conducted on 5 branches per tree on at least three citrus trees (preferably those with new flush) or other hosts at each inspection point.
- Surveys should commence at the final inspection sites on the outermost circle (5 km radius) and move inward.
- This survey plan may be adjusted depending on location (in residential, near natural vegetation, in commercial citrus orchards), area topography, the size of the known outbreak and the availability of hosts in the area. The survey should be planned to incorporate as many inspection sites as can be surveyed by 3 teams of two people within a period of one week.
- As Las will most likely be identified in vectors before host plants, trapped and captured insect vectors at each inspection point will be subjected to Las PCR diagnostics subsequent to positive identification.

- After trap placement and tap sampling for vectors, approximately two minutes should be spent looking for Las symptoms and vector feeding damage on the trap tree (including tops, use ladders if required) and trees adjacent to it. Only symptomatic material will be sampled (10 symptomatic leaves per site).
- Follow-up surveys and actions will be determined by the findings of the initial delimitation survey. Each inspection site with Las-positive samples will then become the central point for a new survey, following the procedures described above.
- In regions officially recognised as vector-free, 30-km transects and 5 km and 3km perimeters will not be utilised and the number of inspection points per perimeter circle will be halved (6 per perimeter). This trapping and surveying will confirm whether vectors are indeed absent. If any vectors are discovered, the survey procedure will default to the plan described above, with increased inspection sites on the perimeters and along 30-km transects.

7.2 Record keeping, pest distribution and area status

DALRRD as the National Plant Protection Organisation (NPPO) will be responsible for data capturing of all surveys, inspections and samples collected and analysed for Las or ACP; this database should include GPS coordinate positions, which should be plotted on a map of Southern Africa. The database should also include information required for trace-back and trace-forward evaluations to determine the origin (source material) of the outbreak and the potential spread that might have occurred already. This database and map should be continuously updated and be available to the HLB Steering Committee, who will track pest distribution and demarcate area status.

7.3 Quarantine and official orders

Upon detection of Las and/or ACP, the area of the detection will be quarantined. The area to be quarantined will depend on detection scenarios as referred to below.

7.3.1 Quarantine period

An area may be removed from quarantine after the pest and/or disease has been declared eradicated, *i.e.* when no ACP has been detected for six maximum life-cycle periods (*i.e.* 12 months), or when no infected ACP or HLB positive host plants were detected for a period of at least 3 years, considering that symptom expression may be delayed for 2 years after infection. Official orders may in such cases be withdrawn or amended.

7.3.2 Scenarios

7.3.2.1 Scenario A: ACP incursion

A-1: ACP point incursion

- Quarantine area: 5-km radius around find site
  - An official order must be issued to the land users in the quarantine area to:
  - depending on the nature of land use (nursery, commercial producer, home garden, etc.), specify quarantine measures to prevent the movement of any host plant material from the area excluding fruit without leaves (but includes propagation material, plant parts and green waste), and
  - order treatment or control of all ACP host plants with approved insecticides, or implement prescribed containment and/or control measures to be applied to all ACP host plants within

800-m from the find site (these measures should be implemented within a prescribed time period not exceeding 2 weeks), and

- issue a public notice specifying the quarantine area and specifying control measures, and
- ensure compliance of land users to containment and control measures as stipulated in the orders will be inspected at least twice a year; this will include confirmation of treatment or control orders and management of any regrowth, record keeping of prescribed treatments, and compliance to movement restrictions, and
- enforce orders must remain in force until the quarantine measures have been officially withdrawn, or amended, or eradication has been declared successful.

- Regional ACP containment: order commercial citrus growers within the region to participate in prescribed area-wide vector monitoring programmes, including vector control using systemic and contact insecticides
- Permitted movement of propagation material from quarantine zone:
  - Only from nurseries in compliance with the HLB-Safe System, but subject to point H below, and subject to CBS-specific movement restrictions in R.110, and subject to treatment, loading and movement protocols specified in the HLB-Safe System.

A-2: ACP range expansion

Transition from point-incursion response to regional management response will be based on the abundance of point-finds, the history of ACP detection in adjacent areas or districts, and expert opinion as endorsed by the HLB Steering Committee.

- Quarantine area: magisterial district or magisterial districts in the broader citrus production region in which a detection site is located, or districts if adjacent district is within 5-km of a detection site.
  - Official order to the land users in the quarantine area: as described for A-1 for highly suitable host species (Table 2); control or treatment of less suitable alternative ACP host plants will not be mandatory.
- Regional ACP containment: official order to commercial citrus growers within the implicated region(s), including regions bordering the expansion frontier, to participate in prescribed area-wide vector monitoring programmes, including vector control using systemic and contact insecticides
- Permitted movement of propagation material from quarantine zone: as described for A-1

7.3.2.2 Scenario B: Las incursion

B-1: Las detected but no vector activity (only pest-free areas as listed in annex)

- Quarantine area: in the absence of vector activity, the quarantine area will be limited to the 400-m inner quarantine area
- Determine the origin by trace-back investigation and address the issue there.
  - An official order must be issued to the land users in the quarantine area to:

- immediately treat and destroy host plants that tested positive for HLB or that are reasonably suspected to be infected/infested according to prescribed guidelines, and
- depending on the nature of land use (nursery, commercial producer, home garden, etc.), specify quarantine measures to prevent the movement of any Las host plant material from the area excluding fruit without leaves (but includes propagation material, plant parts and green waste), and
- order destruction of all Las host plants, or implement prescribed containment and/or control measures to be applied to all highly suitable Las host plants within 400-m from the find site (these measures should be implemented within a prescribed time period not exceeding 2 weeks), and
- issue a public notice specifying the quarantine area and specifying control measures, and
- ensure compliance of land users to containment and control measures as stipulated in the orders will be inspected at least twice a year; this will include confirmation of destruction orders and management of any regrowth, record keeping of prescribed treatments, and compliance to movement restrictions, and
- enforce that orders remain in force until the quarantine procedures have been officially withdrawn, or amended, or eradication has been declared successful, which is when no ACP were detected for at least 6 months or no HLB were detected for at least 3 years.

- Permitted movement of propagation material from quarantine zone:
  - Only from nurseries in compliance with the HLB-Safe System, and subject to point H below, and subject to CBS-specific movement restrictions in R.110.

<u>B-2</u>: Las point incursion, ACT present and active

- Quarantine area: 5-km radius around find site
  - Official order to the land users in the quarantine area: as described for B-1, except that the area in which destruction or treatment of all highly suitable Las host plants is ordered is increased to 800 m surrounding the find site.
- Permitted movement of propagation material from quarantine zone:
  - Only from nurseries in compliance with the HLB-Safe System, and subject to point H below, and subject to CBS-specific movement restrictions in R.110, and only from HLB-Safe Nurseries with a 50-m buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2) and with an 800-m vector-free treatment and monitoring zone surrounding the nursery.
  - HLB-Safe nurseries not complying with the 50-m buffer zone or 800-m vector treatment or monitoring zone, will only be allowed to move material regionally, as specified in point H below.

<u>B-3</u>: Las point incursion, ACP present and active

- Quarantine area: as described for B-2
  - Official order to the land users in the quarantine area: as described for B-2

- Regional ACP containment: official order to commercial citrus growers within the region to participate in prescribed area-wide vector monitoring programmes, including vector control using systemic and contact insecticides
- Permitted movement of propagation material from quarantine zone:
  - Only from nurseries in compliance with the HLB-Safe System, and subject to point H below, and subject to CBS-specific movement restrictions in R.110, and only from HLB-Safe Nurseries with an 800-m buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2) surrounding the nursery.
  - HLB-Safe nurseries not complying with the 800-m buffer zone will only be allowed to move material regionally, as specified in point H below.

B-4: Las + ACP range expansion

Transition from point-incursion response to regional management response will be based on the abundance of point-finds, history of Las and ACP detection, as well as detection of Las-positive ACP/ACT.

- Quarantine area: magisterial district or magisterial districts in the broader citrus production region in which a detection site is located, or districts if adjacent district is within 5-km of a detection site.
  - Official order to the land users in the quarantine area: as described for B-2
- Regional ACP containment: official order to commercial citrus growers within the implicated region(s), including regions bordering the expansion frontier, to participate in prescribed area-wide vector monitoring programmes, including vector control using systemic and contact insecticides.
- Permitted movement of propagation material from quarantine zone: as described for B-3.

8. Permitted movement of plant material from quarantine zones

8.1 **Fruit**: fruit without leaves is **not** subject to a movement restriction.

8.2 **Leaves**: removal of leaves sampled for diagnostic or nutrient analyses may be permitted by means of an official removal permit, subject to the provisions that leaves are cleaned by brushing to remove insects and debris, placed in insect-secure bags sealed in a sturdy container (e.g. cardboard box) for transport to the laboratory, and be destroyed by means of incineration or autoclaving after analyses. A copy of the removal permit must accompany the sample.

8.3 **Propagation material**: movement of propagation material and nursery trees (excluding harvested and treated rootstock seed) will be restricted as specified in R.110 and prior orders issued by DALRRD. At the discretion of DALRRD, movement from HLB or ACP quarantine zones may be permitted subject to R.110 and certain provisions as stated in the cases below (a copy of the removal permit must accompany the sample):

8.3.1 Supply of budwood from quarantine or nucleus block facility

Supply of budwood from these facilities will not be restricted, subject to the provisions below, and in which case the movement restrictions stipulated in R.110 will not be applicable:

- Absence of Las and its vectors in the facility is demonstrated through at least 1 year's up-to-date scouting and insect monitoring records using yellow sticky traps with a minimum trapping density of 1 trap per 25 m^2 in all facility rooms and at least two traps per room. Additionally, one trap will be placed in the entrance section/vestibule of all rooms. Approximately half the traps in each room should be placed at ceiling height, as ACP tend to move upward and towards light. These could be attached to a simple "pulley system" to enable easy access. For example, if a room requires 5 traps, 3 traps will be at plant height level and two traps at ceiling height. Traps are to be monitored every second week and replaced every second month. Following Las incursion in the area, the trapping density must be doubled to 1 trap per 12.5 m^2, with traps monitored every second week, and replaced every second month. The old traps will be sent to the designated Trap Reader at CRI Nelspruit for microscopic evaluation.
- The facility meets the minimum physical and procedural requirements and is officially recognised as a quarantine or nucleus block facility. A record of weekly inspections of the integrity and insect-security of structures and maintenance thereof must be kept. These requirements as well as Las and vector freedom must be confirmed by DALRRD following inspections every 3 months; frequency of inspections will be increased to monthly inspections for facilities in quarantine zones.
- Budwood was produced from disease-free material in approved insect-secure structures for its entire production cycle and processed according to the CIS's Procedural Guide, and inspected and passed compliance by the authorised CIS representative on a bi-annual basis.
- Supply of hardened-off budwood (without leaves) that was treated with a contact insecticide from facilities meeting these provisions will not be restricted from ACP (only) quarantine zones, but the supply of said material from Las quarantine zones will only be permitted subject to the following procedural provisions:
    - Budwood supply to a recipient in Africa will only be permitted after the source plant has been tested as free from Las by means of comprehensive diagnosis, which will include direct PCR testing from the source plant, biological indexing for a period of 6 months, followed by direct PCR testing of the source and bio-index plants; diagnosis must be repeated in case a breach of insect-security occurs during this testing period.
    - Budwood supply to other countries in the world will be allowed if the import conditions of that country can be complied with.
    - Interim release of budwood to a foundation block in southern Africa meeting the provisions stated below will be permitted if the initial direct PCR tested the source plant free from Las. Multiplication plants may be established in said facility, but supply will not be permitted until the source plant has been diagnosed as free from Las.
    - In cases where the facility is surrounded by a 5-km buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2), these diagnostic provisions will not be required.

8.3.2 Supply of budwood from foundation block or budwood multiplication facility

Supply of budwood will not be restricted if the facility meets the provisions below:

- Facility is surrounded by a 5-km buffer zone without plantings of citrus and highly suitable ACP hosts (Table 2). The buffer zone should be monitored on an annual basis to ensure compliance, and

- Facility is not located in a commercial citrus growing region and vector pressure and transmission risk from citrus plantings or highly suitable ACP alternate hosts (Table 2) surrounding the facility is monitored through an ongoing vector monitoring programme at 12 sites on the 5-km perimeter of the buffer zone, and
- Based on official quarterly inspection all applicable structures were inspected and passed compliance, and
- Absence of HLB and its vectors in the facility is demonstrated through at least 1 year's up-to-date scouting and insect monitoring records (7-day monitoring programme) using approved surveillance and trapping methods, and
- Budwood was produced from disease-free material in approved insect-secure structures for its entire production cycle and processed according to the CIS's Procedural Guide.

8.3.3 Supply of citrus trees from CIS-certified citrus nurseries

Movement of nursery trees may be restricted by R.110, but movement from HLB or ACP quarantine zones may be permitted (HLB Safe System compliance auditor or HLBSC risk management subcommittee), dependent on the incursion scenario (point F above) and level of compliance with the provisions below:

a. Nursery is surrounded by a buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2):

i. No buffer zone required for Scenarios A-1, A-2 and B-1.

ii. Scenario B-2: 50-m buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2) and with an 800-m vector-free treatment and monitoring zone in which the Las- and ACP transmission risk from neighbouring citrus plantings (up to a distance of 800 m from the nursery) must be effectively mitigated through registered and recorded vector control measures. Efficacy of control measures would be monitored through an ongoing HLB and vector monitoring programme in those plantings. Treatment thresholds on bearing trees would be similar to threshold of ACT stipulated in CRI production guidelines that is either one or more immature vector specimen on a new growth or one or more adult vector specimen on traps, and

iii. Scenarios B-3 and B-4: 800-m buffer zone without plantings of citrus and hosts listed as highly suitable for Las and ACP (Table 2). In cases where nurseries are not fully compliant with the 800-m buffer zone, a derogation can be considered when Las- and ACP transmission risk from neighbouring citrus plantings or alternative ACP hosts within the 800-m buffer zone is mitigated through vector-free treatment and monitoring measures, as described for Scenario B-2, provided that the nearest citrus plantings is not within 400 m from the nursery.

b. Absence of HLB and its vectors in the nursery is demonstrated through up-to-date scouting and insect monitoring records (7-day monitoring programme) for a least one year using approved surveillance and trapping methods, and

c. The production nursery must be a CIS-certified nursery, and trees were produced in compliance with the HLB Safe System, and the level of compliance specified on the Declaration of Compliance, and

d. Nursery trees were treated with a registered systemic insecticide 30 (minimum) to 90 (maximum) days prior to despatch, and with a contact insecticide within 3 days of despatch but after final topping in preparation for despatch (trees to be despatched must be free from any flush growth), and

e. Nursery trees must be inspected by a DALRRD inspector prior to loading and despatch and must be insecticide treated and topped, free from any flush growth and free from diseases or pests and in compliance with the HLB Safe System, as confirmed through inspection of nursery records prior to issuance of movement permit, and

f. Trees must be loaded in a truck of which the loading bin is enclosed to prevent entry of insect vectors, and trees must be subjected to the prescribed insecticide fumigation treatment in the enclosed loading bin, and

g. Strict adherence to the loading and movement protocol should be ensured by a DALRRD inspector.

A Declaration of Compliance will be issued based on the nursery's level of compliance to the HLB Safe System and the declaration will be signed in acceptance by the nursery. At the discretion of DALRRD, movement of nursery trees may be permitted as follows:

- **Level 1 (Comply with provisions a, b, c, d, e, f, g):** movement from HLB or ACP quarantine zones may be permitted, dependent on incursion scenario (point F above) and as specified under provision a.
- **Level 2 (Comply with b, c, d, e, f, g):** movement from ACP quarantine zones will be allowed, but movement from HLB quarantine zones will be allowed only to the magisterial district or magisterial districts in the broader citrus production region in which the nursery is situated:
  - **Cederberg** (Clanwilliam, Citrusdal, Klawer, Vredendal)
  - **Swartland** (Piketberg, Porterville, Malmesbury, Riebeeck-Kasteel, Riebeeck-Wes)
  - **Boland** (Hex River valley)
  - **Wynland** (Stellenbosch, Paarl, Wellington)
  - **Southern Cape** (Robertson, Bonnievale, Montagu, Swellendam, Buffeljagsrivier, Heidelberg, Riversdale, Herbertsdale, Reenendal)
  - **Gamtoos** (Cambria, Patensie, Hankey, Loerie)
  - **Sundays' River Valley** (Kirkwood, Sunland, Addo)
  - **Katrivier** (Fort Beaufort, Victoria East, Skhutshwana, Ripplemead, Balfour, Adelaide, Cookhouse)
  - **KwaZulu-Natal South** (Ixopo, Richmond)
  - **KwaZulu-Natal Mid** (Nkwaleni, Swart Mfolozi, Weenen)
  - **KwaZulu-Natal North** (Louwsburg, Pongola)
  - **Vaalharts** (Hartswater, Jan Kempdorp, Ganspan)
  - **Onderberg** (Malelane, Schoemansdal, Hectorspruit, Komatipoort)
  - **Mbombela** (Nelspruit, Schoemanskloof, Barberton, White River)

- **Hoedspruit**; **Ohrigstadt + Burgersfort**
- **Letsitele** (Letsitele, Constantia, Trichardtsdal, Tzaneen, Modjadjiskloof)
- **Levubu**
- **Musina-Tshipise**
- **Beitbridge + Weipe + Pontdrift**
- **Groblersdal + Marble Hall**
- **Mookgopong** (incl. Mokopane, Bela-Bela)
- **Brits + Rustenburg**
- **Orange River** (Augrabies, Kakamas, Keimoes, Upington, Groblershoop, Kanoneiland)

8.3.4 Supply of propagation material from non-HLB-Safe nurseries

Movement of citrus trees and plants/trees from other Rutaceae host species will **not** be allowed from retail nurseries or re-sellers of citrus trees *located inside HLB or ACP quarantine zones* that do not comply with the HLB-Safe System.

9. Containment and control measures

Mandatory tree removal in quarantine zones will be applicable to all trees confirmed to be infected by an approved diagnostic test. If suspect trees are not immediately removed while awaiting results from diagnostic tests, containment measures in terms of vector control measures should at least be implemented, even if trees are not in quarantine zones.

9.1 HLB-infected tree removal

- HLB-infected tree removal will remain a mandatory control measure until official notice to the contrary.
- HLB-infected trees should be sprayed with foliar insecticides within 7 days of detection.
- After treatment, the tree should either be physically removed and the remaining stump treated with undiluted glyphosate herbicide, or the tree trunk should be completely girdled by means of deep hatchet cuts that are subsequently treated with undiluted imazapyr herbicide.
- It is critically important that any regrowth from the stump be prevented as these shoots may harbour the pathogen and will attract insect vectors. Re-treatment with herbicide may be required.

9.2. Vector control

- In case of point incursion of Las or ACP (Scenarios A-1, and B-1, B-2, B-3), all host plants in the designated quarantine area will be treated with registered foliar **and** systemic insecticides within 7 days of detection (at registered rates).
- Within 7 days of detection, all host plants in the quarantine area will also be treated with contact foliar insecticides (at registered rates).
- For regional ACP containment under scenarios A-1, A-2, B-3 and B-4, vector control programmes should be structured so that year-round protection against ACP and ACT is maintained.

9.3. Record keeping

Details of control measures applied (action, detail, date, person, follow-up date and action) will be kept for each quarantine zone and follow-up actions will be coordinated by the HLB Steering Committee.

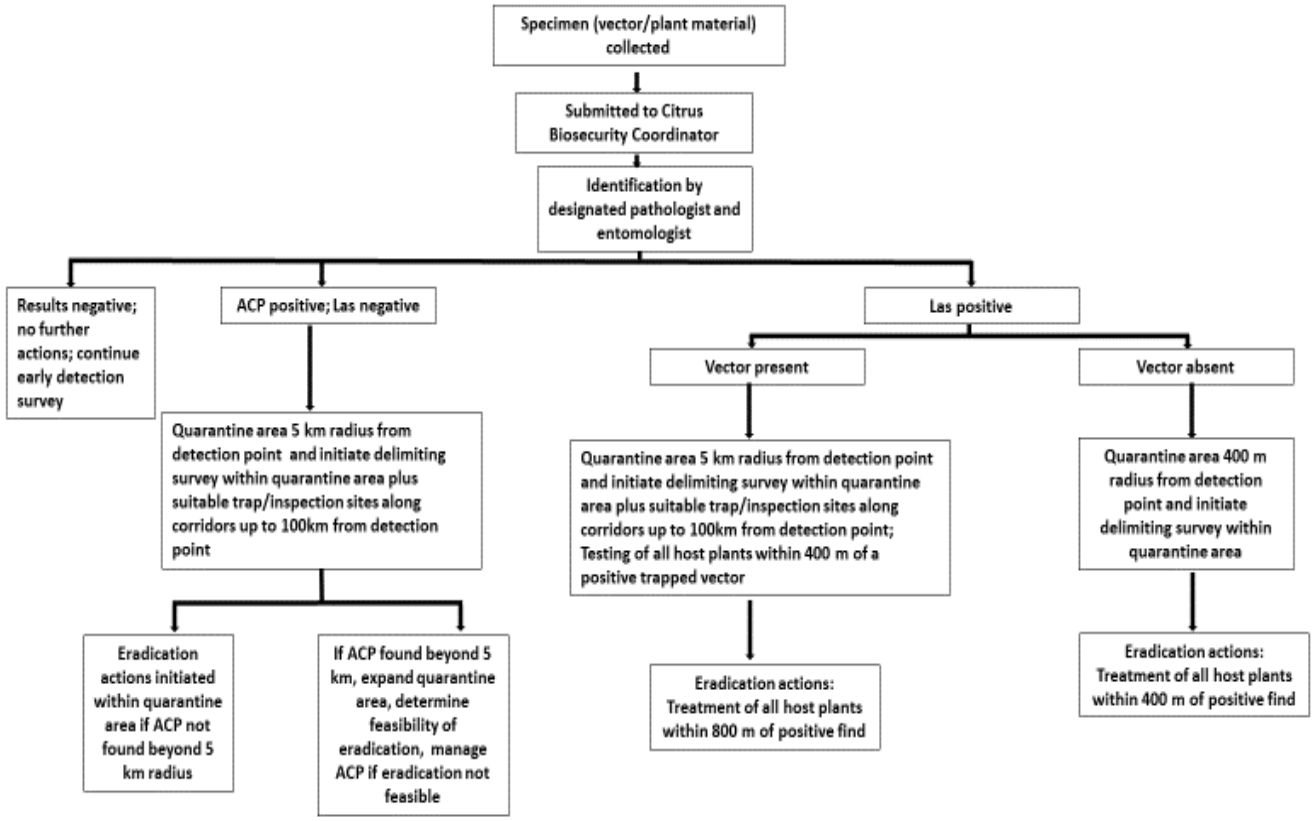
10. Resources

Each stakeholder / agency will be responsible for its own funding during all phases of this Action Plan. Additional emergency funding and funding for stockpiling should be established as part of the preparedness responsibility of the HLB Steering Committee.

11. Communication

The official means of communication would be via email, in which all stakeholders on the HLB Steering Committee will be copied for their attention. Detail of samples and reports and identity of landowners will be treated confidentially by Committee members, and not be divulged by any other role players. Likewise, inspectors, field officers and laboratory personnel must handle said information as strictly confidential. However, information on areas affected by quarantine will not be confidential. The HLB Steering Committee will be responsible for engagement with government, industry and media, and will do so on the basis of reports or media release statements endorsed by the Committee.

12. Sequence of events



Appendix A. Survey protocols for the South African Action Plan for Huanglongbing and Asian Citrus Psyllid – preparedness, early detection and rapid response

I. SURVEY METHODOLOGY (HLB and vectors)

In order to develop, manage and coordinate technology transfer, survey strategies and any possible containment or eradication actions, a Citrus Greening Surveillance and Rapid Response Subcommittee should be established, comprising representatives from all major role players.

A. Detection Surveys and Monitoring

Surveys are conducted by the Directorate Inspection Services (IS), CRI, growers and other interested parties. It is important that growers (representing the biggest surveillance capacity) must be aware of the HLB-ACP risks, vector feeding damage, symptoms of the disease and characteristics of the vector. Extensive surveillance for the detection of symptoms of HLB, sampling of plants for symptomatic diagnostic tests, and sampling of psyllid for asymptomatic diagnostic tests should be in place in strategic and high-risk areas.

Detection surveys must be carried out on *Citrus* (commercial species and varieties), and species of *Murraya* (e.g. orange jasmine), *Bergera* (e.g. curry leaf) and *Clausena* (e.g. wampee), at residential properties, commercial and abandoned orchards, nurseries, parks, retail outlets, weekend markets and roadside stalls.

B. Data Capture

During surveys, data, especially the location and GPS coordinates of the site of detection or occurrence, must be recorded. Data collected during a preliminary investigation should be used to estimate the potential for spread and the anticipated rate of spread, and to identify endangered areas. Information gathered must be recorded on a survey form. A pre-agreed/approved form should be used by all role players.

C. Sampling

- Samples should be collected from the symptomatic areas/branches of the host trees (new flush if available);
- each sample collected should contain green twigs of 15 cm to 20 cm long with approximately 20 leaves, preferably with the petiole still attached and with good recognisable symptoms;
- if a variety of symptoms are present, the preferred samples (in order of preference) would be:
- branches with blotchy mottled leaves;
- branches that contain shoots that are almost entirely yellow;
- branches that have leaves with yellow veins;
- branches with leaves that have either green islands on a yellow background or yellow islands on a green background

- Root samples can be taken for validation
- branches with nutrient deficiencies that have a ‘rabbit’s ear’ appearance (small, upright leaves);
- branches with leaves that show chlorosis and ‘vein-corking’;
- branches with Zn and/or Fe deficiencies that are not related to blight or other known causes;
- the total number of HLB symptomatic plants must be recorded;
- symptoms should, if possible, be photographed before they are removed from plants. The entire plant should also be photographed and the affected area of plant included. Other photographs should include a perspective of where the symptomatic plant is located with respect to other plants;
- trees with HLB-like symptoms are labelled along with the first and last trees of the row to indicate presence of a suspect tree within that row, for sample collection and/or ultimately tree eradication.

i. Sample handling and shipping

- Each sample should be placed in an individual sealable plastic bag (zip-lock);
- leaves should be packed between dry paper towels and the twigs bundled together with a rubber band;
- fruit needs to be wrapped with paper towels and placed in paper bags;
- a completed sample submission form should be included in the bag (or stapled) with each sample;
- samples should be double bagged. When multiple samples are being submitted, multiple samples can be placed in one or more larger sealable bags, however each individual sample needs to be bagged individually and have its own individual completed sample submission form attached;
- samples must be kept as cool as possible (but not frozen) and out of the sunlight;
- if at all possible, the samples should be shipped or hand delivered the same day that they are collected, in order to reach the laboratory within 24 hours;
- measures should be taken to avoid the presence of the insect vector in the sample bag.

ii. Assigned laboratories

Samples resulting from a detection survey have to be confirmed by accredited and assigned plant pest and disease diagnostic laboratories. These laboratories will be responsible to receive, document and analyse samples.

iii. Sanitation

Collectors must exercise extreme care in handling specimens to ensure that hands, tools, and other collection supplies do not become a source of contamination between samples and particularly between individual sites. Special care must be taken with pruning instruments and other items used for sampling. It is therefore important to rather use hands (covered in disposable gloves) to pick and remove leaves to avoid possible cross contamination from cutting tools. A 10% solution of common household bleach (sodium hypochlorite with 1-2% available chlorine) should be used to sterilise cutting equipment to prevent spread of mechanically transmissible pathogens, such as citrus viroids.

iv. Sampling protocol for submission of suspect Asian Citrus Psyllid (ACP) samples

Preservation of psyllids for collection and extraction is an important component of effective diagnostics. If psyllids are forwarded for detection of the HLB pathogens, psyllids must first be correctly identified as ACP. For psyllid identification, the insects must be placed in a leak-proof vial containing 99% ethyl alcohol.

Psyllid samples taken for PCR analysis of HLB should be collected alive and preserved in alcohol immediately. The insects must be placed in a leak-proof vial containing 95% ethyl alcohol. If population allows, at least 3 insects from a location must be submitted and the sample must be labelled. Psyllids that have been dead for more than 2 days (including those on sticky traps) will not yield accurate results when analysed using PCR. Psyllids lose bacterial titre over time and the pathogens cannot be reliably detected after 7 days. Therefore, if used, survey traps need to be checked at very close intervals (every 1 to 3 d), especially in areas where ACP has been detected. Negative PCR results from old traps may therefore not indicate absence of HLB in an area.

D. ACP trapping

The procedure indicated in *Cutting Edge (CE) No. 240. Surveillance of the Asian Citrus Psyllid*

in citrus production areas in Southern Africa Aruna Manrakhan, Peter Stephen, MC Pretorius, Tim Grout and Paul Fourie Citrus Research International (CRI) should be followed.

Indicated below are some extracts from CE 240.

Yellow sticky traps or lime-green ACP traps should be placed along orchard edges on a citrus farm. Traps should not be placed close to indigenous trees to prevent trapping of endemic *Diaphorina* species.

- In large commercial citrus plantings use 6 traps per 100 ha.
- For nurseries, use 6 traps per 10 ha.
- Label traps with nursery/farm name, trap number, date placed and date serviced.
- Place one trap per citrus tree on the outside canopy (Figure 2) and preferably on either the southern or eastern side of the tree. Hang the trap at about 1.5-2 m above ground.
- Clear leaves away around the trap.
- Remove waxy paper covering the yellow sticky card.

Inspect and collect traps at least once a month and replace with new traps. Place collected trap flat onto a transparent plastic film (Cling Wrap) and cover the two sticky sides with the film. Avoid creases in the plastic film for easy identification of insects.

https://insectscience.co.za/wp-content/uploads/2018/03/No-240-ACP-Surveillance-newsletter.pdf

E. Survey Tools for ACP

Survey methodologies can comprise of:

- visual inspections for adults on mature leaves, particularly on the undersides of leaves in between flush cycles;
- visual inspections for eggs, nymphs on flush growth from 5 mm to 50 mm long, particularly in spring, within 14 days of buds opening;
- visual inspections of young flush growth (5 mm to 50 mm long) for honeydew, particularly in spring, within 14 days of buds opening;
- beating foliage, particularly young flush growth, to dislodge adults into a 300 mm-diameter pan containing a shallow amount of mineral oil;
- Sweep netting for vectors, and
- use of yellow sticky traps to trap flying adults can also be used.

F. Survey Tools for HLB

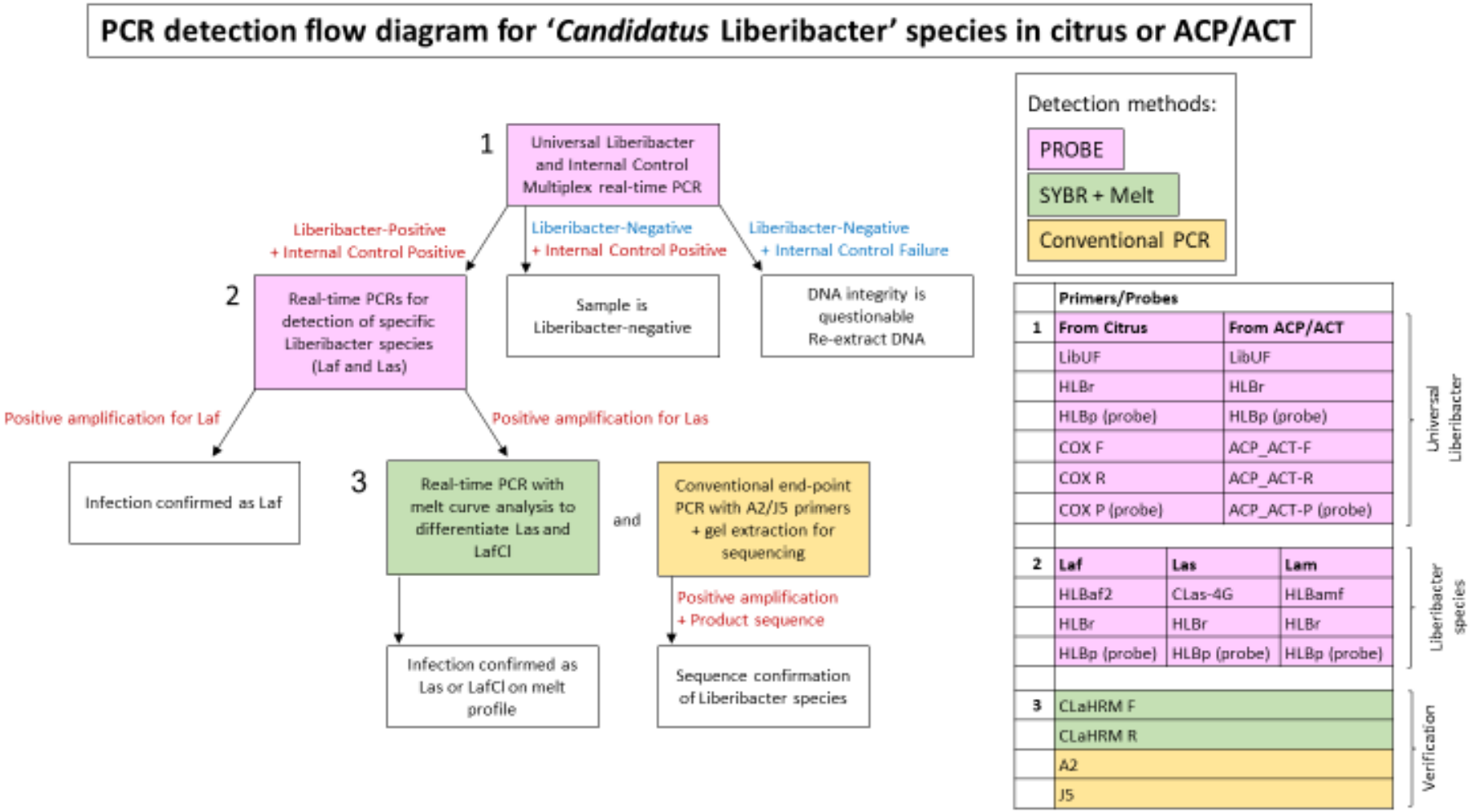
i. Symptomatology

Field recognition of HLB, based on visual symptoms, requires well trained personnel. A simple aid for the identification of suspect HLB symptoms can be used: use a pen to draw two circles symmetric about the leaf mid rib, and compare the leaf colour within the two circles. If one is yellow, and one green, it could be HLB.

Trained surveillance teams should walk orchards, nurseries, parks and gardens by foot, looking for the following HLB symptoms: yellow shoots, mottled leaves, small sometimes misshapen fruits with aborted seeds and darkening of the columella, and evidence of citrus psyllids or their activity.

Only symptomatic leaves with blotchy mottle symptoms, and root samples (for validation only) should be relied on for positive PCR results for '*Ca*. Liberibacter' and these should be specifically collected. Symptoms become evident on younger plants sooner after infection than on mature trees, especially if budwood was infected, so younger plants should be included.

Appendix B. Sampling methods and diagnostic protocols



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