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**Management of Huanglongbing and its Vector,
the Asian Citrus Psyllid, *Diaphorina citri***

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Introduction

Huanglongbing (HLB), a disease apparently caused by the bacterium *Candidatus Liberibacter* spp. and transmitted within the American continent by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, 1908, is currently recognized as the most devastating citrus disease on a global scale (Cortéz et al. 2013).

Management of HLB is complicated and requires a regional or an area-wide strategy for both the pathogen and the vector. Such a strategy includes, among other approaches, use of propagative and planting material free from the bacterium, early detection, removal of infected plants, and psyllid control. Reducing the populations of the pathogen-carrying vectors as much as possible is an important part of integrated pest management and will assist in slowing the spread of the disease (Pacheco et al. 2012).

The technical reasons for implementing and maintaining area-wide control of ACP, especially in a region where HLB is present, are as follows: (1) high capability for the ACP to spread long distances; (2) constant migration of the ACP among citrus orchards or plants; (3) difficulty to totally avoid primary infection caused by migrant infective ACP, even with frequent pesticide applications. Bassanezi et al. (2013) report on the regional removal of the inoculum (diseased plants) and ACP as a result of HLB regional management which in turn leads to: (1) delaying the beginning of the epidemic almost a year; (2) considerably reduction of incidence (by 90%) and progress rate (by 75%) of HLB; (3) decreased need for frequent pesticide usage to control the psyllid and (4) reduction in HLB management costs, due to fewer and more effective pesticide applications. These results are attributed to the reduction of sources of inoculum and ACP in the region which reduces the infective population of ACP (by 90%) from nearby groves and decreases psyllid populations that migrate to managed groves (76 to 96%). Consequently, primary infection decreases more effectively.

Scope

This document describes the components involved in the implementation and operation of a program for area-wide management of HLB and its vector, ACP. This document is intended to generate discussion on how citrus-producing countries can approach management of the disease and its vector.

Definitions

Definitions of phytosanitary terms used in the present document can be found in NAPPO RSPM 5 and in ISPM 5.

1. Producing clean citrus propagative material

1.1 Background

The centre of origin and diversification of citrus is continental Southeast Asia (Indochina) and Australasia (Pfeil and Crisp 2008). Citrus originally moved to new areas as seed, allowing seed-borne citrus diseases to be spread. When citrus began to be moved as budwood or rooted plant material, graft-transmissible diseases of citrus started to spread

around the world. The devastating effects of some of these graft-transmissible diseases of citrus, such as Tristeza (*Citrus tristeza virus*), encouraged the development of technologies to diagnose the pests responsible for the diseases and eliminate them from plant material. These technologies are now in place in many citrus-producing countries and regions and they are a key tool for the management of citrus health. The use of clean propagative material or nursery stock is one of the important means of preventing the spread of HLB and other graft-transmissible diseases (Krueger and Navarro 2007). This, together with surveys to detect and remove infected trees and area-wide reduction of the vector population, forms the basis of regional management programs for HLB and other citrus diseases. The measures described here are intended to result in the production of nursery stock free of all graft transmissible diseases.

1.2 Importation and quarantine

Citrus propagative material is highly regulated because of the high risk of moving diseases into citrus-producing areas in which they do not occur, introducing more severe strains or generally increasing incidence of already present diseases. National plant protection organizations (NPPOs) and regulatory agencies in individual countries use phytosanitary regulations to minimize the risk of introducing pests and diseases through the movement of plant material. The risks associated with large-scale commercial importation of plants for planting are unacceptably high in most instances and it is therefore generally prohibited.

However, because of the benefit of introducing new material such as nursery stock or budwood, regulatory agencies may allow entry of material under an approved series of protocols designed to safeguard the crop and minimize the risk of introducing exotic pests and pathogens. Such protocols generally apply to the introduction of limited amounts of material, which is released only after verification of an acceptable phytosanitary status and/or a post-entry quarantine period. In some countries without post-entry quarantine stations, propagative material not known to be free from disease is prohibited from entering the country.

There are two basic approaches to citrus quarantine. The classical approach involves propagation of imported material through quarantine facilities to contain it, followed by observation and indexing for the presence of pathogens. Infected material is destroyed or subjected to a therapeutic procedure. This approach has been used for many years with success in areas with low disease pressure or threat.

The tissue culture approach involves culturing imported budwood *in vitro*, recovering plants by shoot-tip micrografting (STG) *in vitro*, and testing for the presence of pathogens by indexing and by direct laboratory testing. The material is released from quarantine only when no pathogens are present.

The tissue culture approach is more conservative than the classical approach as therapy is applied under all circumstances, which should reduce the threat from unknown or unreported diseases. In some cases, this approach has been incorporated into regulations that previously gave the quarantine facility at the point of entry discretion as to which approach was used.

It is a requirement in many citrus-producing areas that citrus propagative material comes

from clean sources. However, in some areas there are no legal restrictions on the movement of citrus propagative material within a country or state. This lack of restriction can result in the spread of pests and diseases that may cause severe problems for the citrus industry. In such situations, all material moved within the same country, state, or region is advised to be sanitized for pathogen elimination and included in a certification program. Where no certification program exists, obtaining propagative material from a reliable source of pathogen-tested material outside the country (either a gene bank or a certification program) carries less risk than obtaining material from within the country.

1.3 Producing clean plants and verifying the disease-free status of plant material

Whereas arthropod pests are screened out through processes described under Section 1.4, protocols for producing clean plants and verifying the disease-free status usually involve therapies to rid the plant of pathogens and various methods of testing for pathogens. These protocols are generally carried out under controlled conditions and may be conducted before moving plant material, following the introduction of new plant material, or after a post-entry quarantine period.

Current pathogen-elimination techniques rely mainly on thermotherapy or STG (see NAPPO treatment protocols (TP) for thermotherapy (TP 01: 2015) and STG (TP 02: 2015) for details). STG is recommended and has become the most common technique because it is effective in eliminating all citrus pathogens.

Detection of graft-transmissible pathogens is a fundamental component of a program for the safe introduction of new citrus varieties or the sanitation of existing varieties. Pathogen detection is based primarily upon biological indexing with citrus indicator plants, supplemented by laboratory tests. The specific tests required will be determined by various factors but are ultimately approved by regulatory authorities. Examples of approved methods of testing are provided in RSPM 16: 2013.

Laboratory tests for graft-transmissible pathogens include serological tests, such as enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA), and immunospecific electron microscopy (ISEM); nucleic acid based tests, such as sequential polyacrylamide gel electrophoresis (sPAGE), hybridization, and polymerase chain reaction (conventional and real-time PCR); culture of pathogens on media; and microscopy.

Laboratory tests may offer advantages over biological indexing: they are more rapid, enable the processing of large numbers of samples in a short time, and require fewer human and physical resources so are generally less expensive. A disadvantage is their need for a well-equipped laboratory with specialized and often expensive equipment, as well as training for the technicians carrying out the tests. Quality control measures, such as calibration, optimization, selection of adequate controls, and a high level of expertise in all personnel involved, ensure these tests are run with a high level of accuracy and confidence. However, if a disease of unknown aetiology is present, it may be possible to detect it with an indicator plant but not with a laboratory test (Navarro et al. 1984).

Hence, greenhouse and laboratory tests complement but do not replace each other. For a complete index, if a biological test is available for a pathogen a laboratory test may be used as an adjunct to but should not entirely replace the biological test. In many cases,

laboratory tests are not accepted by the regulatory authorities, and the “index of record” is the biological index. In contrast, for re-testing a large number of pathogen-tested germplasm accessions, laboratory tests are often the only feasible method of testing.

1.4 Facilities

Quarantine and sanitation programs for citrus have some very specific requirements for facilities (Gumpf 1999). Ideally, the facilities should be located in an area with a climate suitable for growing citrus, but not adjacent to areas of commercial citrus production. This is not always practical. It is therefore important that quarantine facilities are designed, constructed, and operated in a manner that minimizes the risk of pathogens escaping from the quarantine material held within them. Measures should also be taken to minimize other phytosanitary risks, such as contamination of other plants in the facility, access by insects and pests, and the spread of fungal pathogens within the facilities. Facilities must also be designed to maintain environmental conditions suitable for indexing. In some cases, regulatory agencies will have specific requirements for facilities design (USDA-APHIS-PPQ 2010).

Access to the facilities should be prohibited to all except those who are authorized and trained in proper phytosanitary conduct. The facilities should be kept locked at all times, behind a fence with a locked gate.

Facilities generally belong to federal or state agencies involved in plant protection, but these agencies may have an agreement with an outside entity such as a research institution to conduct the actual introduction or sanitation program. In any case, the facilities themselves as well as processes and records must be accessible and available for inspection by the phytosanitary authorities.

Facilities should include greenhouses, screenhouses, and a laboratory.

1.4.1 Greenhouses

The greenhouses are used for the production of indicator plants and rootstocks and for biological indexing. They must have at least two chambers, one maintained at a cool temperature and another at a higher temperature, for the detection of graft-transmissible pathogens. The rooms should have independent environmental controls; the environmental control system is the most critical component of the greenhouse as it is vital for maintaining the required temperatures. A third room of intermediate temperature is preferred, and if available should be used for the production of indicator plants and for propagation. Greenhouses should be constructed so that they protect the plants from insects, and for this purpose they should have double doors with vestibules and positive pressure airflow and/or air curtains. Air intakes and exhausts should also be protected (i.e. screened) for insect exclusion.

1.4.2 Screenhouses

The screenhouses are used for protected maintenance of plants. They may be constructed of metal (preferred) or from wooden frames screened with nylon or stainless steel insect-proof materials. In the past, the standard construction utilized “aphid-proof” screens, but current practice is to use “thrips-proof” screens. The roof of the screenhouse should be at least three metres from the ground (preferably four to five metres). Screenhouses should

have a vestibule with double doors and positive pressure airflow and/or air curtains.

1.4.3 Laboratory

The laboratory is used for laboratory testing and therapy. Separate areas within the laboratory for therapy and tissue culture are highly desirable. The design and construction of the laboratory depends on the extent of its use. If limited types of laboratory tests are carried out (e.g. ELISA, sPAGE), a small laboratory may be sufficient. If a larger range of laboratory tests are carried out or a large number of samples is handled, a larger laboratory will be needed. The types of tests carried out will determine the equipment needed.

1.4.5 Plant materials

Most plant resources maintained under protection in a germplasm bank, foundation block, and increase block are maintained as potted trees growing in a sterilized substrate. This has some obvious advantages for disease prevention, fertilization, frost protection, the ability to manipulate and move the trees, and so forth. Growing trees in pots also allows for an easier change in the number of trees maintained per variety or genotype. However, maintaining trees in soil is the only way to provide the large quantities of budwood that some programs supply. Trees maintained under screens or other protective structures, whether in pots or in the ground, are generally not suitable as sources of fruit for accurate characterization and evaluation of fruit quality. Observation of any fruits produced helps to ascertain the trueness to type of genotypes and to detect possible misidentified accessions and possible chimeras. These are very important considerations when releasing budwood for certification.

1.5 Germplasm bank

A germplasm bank, also known as a gene bank, is a collection of the widest range possible of genotypes, which are maintained regardless of their commercial use or potential. A germplasm bank supports scientific research while also supplying specific genotypes to industry via a certification or clean plant system. Maintenance of a germplasm bank is a resource-intensive activity that does not generate revenue; therefore, these banks are usually maintained by a governmental or an academic institution and consequently are subject to competition for resources from other programs and projects. Some germplasm banks have a program for long-term preservation under cryogenic or tissue culture conditions. These programs are very important for long-term preservation of valuable genetic material and diversity, but they are an uncommon activity restricted to a few leading banks.

A germplasm bank, especially one that supports a certification or clean plant system, should be maintained in structures to protect against pests and diseases. When sufficient resources are available, a field-planted collection is an option to serve in characterization and evaluation and as a source of seeds and tissue for research. In some cases, resources are insufficient for protective structures, and the only trees present in a germplasm bank may be field-planted. This is not desirable because trees in the field are vulnerable to pests, diseases, and abiotic stresses, such as high temperatures and moisture stress. The current standard advises protection of germplasm bank material inside a structure.

Many germplasm banks established in the twentieth century and earlier include genotypes of unknown phytosanitary status. Now, only sanitized genotypes are maintained, and the addition of new genotypes to a germplasm bank or release of genotypes from the bank to

a certification or clean plant system requires a sanitation program be in place. Sanitation of propagative material can occur before the material is added to the germplasm bank, while the material is within the bank, or before the material is released to a certification or clean plant system. The latter option is mostly associated with accessions acquired prior to the adoption of current phytosanitary standards; current practice is to have clean material in the germplasm bank.

Documentation of material maintained in a germplasm bank is crucial. At a minimum, origin data and unique identifiers for genotype and individual trees as well as management information (e.g. propagation, location) are necessary. As much additional characterization and evaluation data as possible should be obtained and recorded.

1.6 Foundation block

The basis for all further propagation in a certification or clean plant system is the foundation block. The scope of a foundation block is narrower than that of a germplasm bank. A foundation block directly supports an industry so is concerned with commercial varieties and varieties with commercial potential. Varieties not used in commercial production should be maintained in a germplasm bank at a high phytosanitary status (meeting the standards for the foundation block).

Material maintained in a foundation block must meet a high phytosanitary standard; usually this equates to the absence of all known graft-transmissible pathogens, a status met after passing through an introduction or a sanitation program. Foundation block plantings are periodically re-tested for pathogens as required by regulations. The most important diseases from a re-testing standpoint are endemic diseases that are naturally spread by their vectors. Material in a foundation block that meets the phytosanitary standard can be maintained indefinitely, although practical matters (e.g. tree size) may limit its life.

Depending upon regulations and circumstances, foundation blocks may be maintained by governmental or academic institutions and/or by private nurseries. Foundation blocks in the past have included both protected foundation blocks of initial (mother) material and field-planted or protected foundation blocks that can be located at nurseries. However, the current practice is to protect all blocks of the program and field-planted foundation blocks are being phased out. A field-planting of trees propagated from foundation material is sometimes established by the certification or clean plant system or by researchers or industry representatives in order to observe, evaluate, and document production characteristics. Vegetative material from field-grown trees should not be used for propagation.

Documentation associated with a foundation block includes some of the same information as that used to manage a germplasm bank, such as origin data, phytosanitary data, and management data. Generally, each foundation block tree is supplied with a unique identifier that allows tracing of all buds distributed to the industry to an individual tree in the foundation block. This is important if a disease or an abnormality is observed in trees propagated from buds originating directly or indirectly from the foundation block. The presumed source tree can be checked to determine whether it is the source of the problem or whether the problem originated after the buds left the foundation block facility. Unique identifiers are generally issued and maintained by regulatory agencies.

1.7 Increase blocks

Because of the expense associated with maintaining and re-testing foundation block trees, typically only two to six trees from each variety are maintained. This number is not enough to supply budwood directly to propagate nursery trees. Therefore, material from the foundation block is normally used to establish budwood increase (multiplication) blocks, allowing rapid and efficient multiplication of buds. In some cases, increase blocks can be propagated from other approved, tested source trees.

As with germplasm banks and foundation blocks, increase blocks should be protected. Increase blocks generally have a defined useful lifetime, parameters of which are determined by the regulatory authorities. The lifetime can sometimes be extended after re-testing for specific pathogens. The phytosanitary status of increase block trees must be equivalent to germplasm bank and foundation block trees. As with the germplasm bank and foundation block, the increase block is authorized and inspected by the regulatory authorities.

The final step in a certification or clean plant system is the production of certified nursery trees from either foundation block or increase block material. Production may be protected or in the field: the selection of method will depend upon the phytosanitary conditions in the area, which, in turn, determine contamination risks. The actual requirements for certification are mandated by the regulatory agency and vary from location to location. The preference is to produce and maintain the trees intended for sale under protection, but this is not always feasible.

1.8 Seed source trees

The current practice is to maintain seed source trees in the field because of the difficulties of fruit and seed production using the currently available technologies of protective structures. Seed source trees are tested for a relatively small number of pathogens, and the standards for testing are not consistent across governments or certification programs. Reports of seed transmission of citrus pathogens are sporadic and inconsistent, and most major graft-transmissible citrus pathogens, including the bacterium causing HLB, have not been reported to be seed-transmissible [although *Citrus leaf blotch virus* (CLBV) has been]. The preferred practice is to plant seed source trees that meet all relevant clean plant or certification criteria, protect them against vectors, and re-test them periodically for vector-transmitted pathogens endemic to the area. Seed production plantings are also subject to authorization and inspection by regulatory personnel.

Completely protected production of citrus seed would be preferable; however, this is currently not practical because protective structures would have to be much larger to accommodate seed source trees than to accommodate smaller bud source trees. In addition, flowering and fruiting are impeded or inconsistent under screen. Tissue cultured rootstocks are produced and used by a few commercial nurseries but are not routinely used at this time. Adoption of tissue cultured rootstocks and indicators for certification programs will undoubtedly increase in the future.

1.9 Nurseries

1.9.1 Wholesale nurseries

Wholesale nurseries producing citrus nursery stock should follow all regulations concerning citrus as well as all general nursery regulations. Wholesale nurseries must be authorized by regulatory agencies and accessible for inspection. Origin and sale destination of stock should be recorded. This information should be available to phytosanitary regulators upon request if an event requiring investigation occurs. The actual format for documentation will be determined by nurseries and regulatory personnel depending upon local conditions.

Production of citrus stock for sale should be carried out in protective structures. A pest control program including monitoring, control actions, and appropriate documentation should be in place. Citrus trees should be protected until the time they leave the wholesale nursery. Exposure to unprotected conditions should be minimized during loading of the trees for transport off-site. Wholesale nursery facilities are subject to inspection. Trees should never be returned to a nursery once they have left it. Trees should be treated for insects and mites that are vectors of disease with a systemic insecticide before transport off-site.

1.9.2 Retail nurseries

Retail nurseries should obtain plants for sale to the general public from wholesale nurseries. Citrus trees for sale in at-risk areas (in which HLB or its vector is present) should be maintained in a protective structure. Trees should be monitored and treated for vectors, especially in areas in which HLB or its vector is present and in unprotected situations.

Regulations may limit the maximum time citrus trees can stay in retail nurseries and the number of trees allowed there at any one time, especially if protective structures are not available. Alternate hosts of HLB and its vector should be subject to the same standards as citrus if they are present at the retail nursery, as once they leave the retail nursery they may come into proximity with citrus. Retail nurseries should record sources of citrus trees (i.e. plants from which buds and seed originated). These records would be available to regulators in the event infection is detected in trees purchased from a retail nursery.

2. Regional management for the Asian citrus psyllid

2.1 Background

The regional management of ACP requires a multi-faceted approach that encompasses social, economic, operational, epidemiological, and biological aspects. Bassanezi et al (2013) showed that in Brazil, regional (area-wide) management of ACP was much more effective at significantly reducing populations than management on an orchard-by-orchard basis.

The most effective management of ACP usually occurs when a group of growers in a defined region or area have agreed to work in a coordinated manner. The nomenclature used to designate this concept varies. The most commonly utilized terms are "area-wide management" and "regional management", although other variants also occur. Area-wide management is considered to be the "management of the total pest population within a delimited area" (Hendrichs et al. 2007). Similarly, there are variations in the nomenclature specifying the area or region of a coordinated ACP control program. For instance, in the 24

citrus-producing states of Mexico, there are defined citrus-producing areas known as ARCOs (areas for regional control) where coordinated actions take place in order to reduce ACP populations and decrease the risk of HLB introduction and spread among and within areas through monitoring and chemical and biological control activities. ARCOs coordinate control measures over the entire area based on insect numbers observed during monitoring. The strategy ARCOs use also considers rotation of pesticide groups in order to slow development of resistance in the vector (SENASICA 2014). In the United States, areas similar to ARCOs are called citrus health management areas (CHMAs) in Florida (Rogers et al. n.d.) and Psyllid Management Areas (PMA's) in California (Zaninovich 2015). These areas and designations have been devised specifically to coordinate efforts; however, coordinated efforts can also be implemented in existing designated areas (such as Pest Management Districts), if appropriate.

2.2 Components of regional management

2.2.1 Organization

A HLB working group should be created in each state or region, composed of representatives from relevant agencies, institutions, and organizations to lead the management efforts in the region [e.g. regulatory agencies, local government, the citrus chain and citrus industry (growers, packing and exporter associations, processing associations), certified nursery associations, and citrus research institutions] (SENASICA 2014). The working group assists in compliance with regulations related to HLB and especially in the implementation of area-wide management (Rogers et al. 2014, Rogers et al. n.d.).

A HLB technical group can provide recommendations for management and other technical issues, such as the number, size, and location of regional management areas; prioritization of areas prone to HLB endemic outbreaks; periods of total regional pesticide application; action threshold to control ACP; and rotation of pesticides. The technical group can also participate in training of stakeholders, growers, and other technical staff belonging to state, federal, or extension organizations and growers. Regarding the composition of a technical group, in Mexico, for example, the technical groups that assist the ARCOs are made up of technical staff from the NPPO and local government, as well as the ARCO coordinator, the HLB campaign coordinator, a technician representing the citrus production chain from the state, a technician from the Growers' State Committee, and researchers from citrus research institutes in the region.

HLB is not only a technical problem, but also must be considered from the economic, social, environmental, and commercial points of view and, as such, regional management needs the involvement of all stakeholders. The joining of forces increases the possibility of achieving the goal and reducing individual costs. The roles of some of the authorities and personnel involved in the implementation and operation of ACP regional management are discussed below.

Role of the phytosanitary authority

The phytosanitary (and regulatory) authorities at both the state and the federal level lead the creation of working and technical groups and foster implementation of regional management areas. They also develop protocols; describe strategies, management components, and responsibilities; and follow up on regional control activities (e.g. weekly

monitoring, scheduled applications, and control of focus areas).

Role of researchers

With the objective of making regional management more effective, all its components must be gradually optimized. The importance of each factor – social, economic, operational, epidemiological, and biological – must be taken into account in the management strategy. It is up to the identified subject matter experts to correctly identify all the factors, establish priorities for research, and conduct the research appropriately so that results are quickly applicable. Biological and epidemiological aspects need to be quantified in an experimental form (not only in case studies). These aspects include: (1) the effect of the wind (intensity and direction) and the flight of the vector on its spread; (2) the effect of systemic and contact insecticides applied in the control of the vector to reduce the probability of transmission of the bacteria from infected plants; (3) the importance of phenological states of buds on the biology and population dynamics of the vector and the transmission of HLB; and (4) the influence of the environment and root stock on the intensity and seasonality of species outbreaks and varieties.

Related to the social aspects of regional management, the cause of any lack of cooperation must be identified. This is particularly important for neighbouring growers in the implementation of control actions, because without cooperation among the growers – the principal beneficiaries of the process – regional management will not be effective.

Mamani (2013) noted the main reason for lack of collaboration is a lack of confidence among the producers to participate in a coordinated fashion. Sociologists, psychologists, and anthropologists may be brought in to resolve these issues using participatory extension methods appropriate to the context.

Role of extension personnel

The role of extension personnel is to provide information to stakeholders and work with them to implement the regional management program; develop and implement training workshops focusing on the identification, control, and suppression of HLB and its vector; and provide outreach to the community. The extension service and other available resources should be brought together to enhance current public outreach efforts on pest and disease prevention and management, as well as to educate on the importance of protecting agricultural resources.

Outreach is also necessary to encourage home gardeners to manage HLB and other citrus pests in their gardens, and to help promote a better understanding of how their decisions may affect commercial producers. It is important that home gardeners understand the need to purchase citrus plants only from state-certified nurseries. They must be aware that they should not accept citrus plants from other sources or give citrus plants from their gardens to others.

Role of growers

Growers should undertake regional management in consultation with technical experts to evaluate collected data. In Brazil, a cooperative alert system functions such that growers post weekly observations of sticky traps and outbreaks on a website that publishes information every two weeks on infestation levels and recommendations for applications to hot spots. Growers must be convinced of the importance of participating in order to achieve a reduction in the population of ACP with coordinated application of insecticides and

entomopathogens.

One of the major issues in convincing growers to participate in regional management working groups and programs is their desire to see immediate results from actions taken. This is not possible when dealing with diseases with characteristics such as those of HLB [long incubation time, seasonal and unclear symptoms (especially for early infection), difficulties in controlling the vector, etc.].

Some experiences in Florida have shown that the removal of infected plants is more effective at the beginning of the epidemic at the regional or grove level. Once the infection is widespread, growers are reluctant to remove large numbers of trees and have adopted a management strategy, including improving nutrition, thermotherapy, psyllid control, improved root health, among others.

2.2.2 Social participation in the program

The social participation, under the perspective of plant health and the ARCO's program is developed through organized activities that facilitate the appropriation and implementation of the program strategies and objectives holistically, as well as the generation and gathering of first-hand information to evaluate and monitor the impacts for different strategies in specific sites.

Regional control of ACP implies strengthening communities to coordinate actions among growers, the OASV and other key interested parties in citrus production. For regional control to be successful, it is important to encourage growers to participate in actions, through the social organization and unity, that allow to create a working network for the ARCOs' objectives.

It is crucial that extension workers raise community and growers awareness of the problem and its objectives. Therefore, growers need to understand that their participation is a necessary but not imposed obligation. They should also understand that their active participation in surveillance, monitoring and pest control will bring direct benefits to their standard of living, making them responsible for the protection of their patrimony and national citrus production

For this to be successful, the staff working as extension personnel should have field, organization and communication experience as well as technical knowledge of the disease, its vector and the ARCO's program in order to design strategic, technical planning and participatory methodologies, strategies to encourage social participation, and social and community organization. Their input is also important in gathering and analysing data on social characteristics of citrus production areas, to help decide on the establishment of ARCOs and the operational campaign against HLB.

2.2.3 Operational activities

Program coordination

The area-wide management program coordination should be led by a phytosanitary authority (state, regional or national) or by industry stakeholders tightly engaged with research and extension personnel. Developing regional or area-wide management areas such as CHMAs and ARCOs will be a critical first step in developing a plan for regional or

area-wide management, followed by appointing coordinators for each of these management areas. The need to establish a coordinating body for each management area is very important to ensure continuity of the program.

Training

Every effort should be made to provide training workshops for growers and technicians, focusing on disease symptomatology, recognition of symptoms, insect identification, authorized insecticides for management of ACP, biological control organisms, and the organization, components, and operation of ACP and HLB area-wide management bodies (e.g. ARCOs and CHMAs). There should be efforts to conduct sessions for the trainers and to hold coordination meetings among researchers, extension personnel, and growers.

Communication and outreach

One of the most important aspects of a management program is building an outreach and communication strategy that focuses on developing training materials on the management of HLB and its vector so as to inform producers, growers, nursery owners and workers, and the public. The use of social media and websites is crucial to delivering information on HLB and its vector to the public widely and quickly. Extension publications together with trade journals and the scientific literature are important stakeholder information and training tools. Early detection through public and stakeholder education will ultimately serve to manage ACP and HLB.

The use of websites maintained by the working groups will assist in data and information management and dissemination. For example, treatment schedules as well as recommended pesticides may be published on a dedicated webpage.

2.2.4 Prioritization of areas for control

Ideally, regional areas for the control of ACP should be established in the entire citrus-producing area of the country, and grant autonomy as well as economic and operational responsibility to the growers. This is a highly complex undertaking that should be seen as a goal for the medium and long term.

The phytosanitary authority should prioritise the creation of regions for area-wide management of ACP and HLB where conditions are conducive to HLB outbreaks. The following biological and epidemiological criteria should be taken into account when assessing such conditions: (1) host abundance; (2) host susceptibility; (3) number of and distance between sites (as sources of infection); (4) inoculum load; and (5) direction of the predominant wind. These criteria will also form the basis for the size, shape, and surface area of the control area as well as the number of participating growers and other factors.

As an example, Mora-Aguilera et al. (2013) proposed a methodology to determine the number, size, and location of ARCOs for the regional control of ACP in the 24 citrus-producing states in Mexico. Known as “@RCOs HLB v1.0”, it defines regional areas for control based on the principles of risk prevention and protection (endemic characteristics). This methodology was used to simulate real low-risk epidemic scenarios in Brazil (São Paulo), the United States (Florida), and Mexico (Yucatán and Colima) in a mathematical model.

As well as the criteria listed above, in practice the number and location of regional areas for control are also determined by availability of infrastructure and human and economic resources in the citrus-producing states, and the desired level of suppression of risk of the disease (the goal should be 80–90% suppression - Mora-Aguilera et al. (2013)). This latter consideration is a dynamic element which helps prioritise regional areas for control of ACP by the level of municipal risk. By applying epidemiological approaches, rational criteria based on the principles of prevention and protection are used to establish regional areas for control of ACP in Mexico.

2.2.5 Monitoring of the vector

Trapping

Green or yellow traps are used to measure ACP populations on a regional scale and according to host species to: (1) evaluate the impact of total regional chemical spray applications and biological releases; (2) determine regional application times; (3) avoid unnecessary applications; and (4) identify insect outbreaks by orchard (sources of infestation). Data should be collected weekly and delivered to a centralized point for processing. Information systems should hold data that enable the use of analysis for decision-making at different levels (e.g. state, orchard).

In Mexico, a monitoring system for *D. citri* based on trapping (called SIMDIA) has been designed and implemented (see <http://www.siafeson.com/simdiatecnicos/>). The system provides information on psyllid infestation levels at the national, state, ARCO, orchard and trap levels, allowing for timely decision-making at these different levels. For example, scheduled regional pesticide applications can be brought forward or cancelled, or specific sites where the insect population is on the increase can be targeted.

Tapping

Tap sampling (“tapping”) is an efficient method for monitoring moderate to high ACP populations. For example, in the USA, the recommendation is to conduct routine monitoring by applying 100 taps per block of any reasonable size, taken in groups of 10 blocks per location in 10 different locations, with five taps on the periphery and five in the interior of the block. This protocol provides population numbers with approximately 25% precision down to about one vector per 10 taps. Inspection of 10 young shoots per location, if available, to determine infestation percentage and flush density is also recommended. Very low populations are better detected using sticky cards or vacuum sampling methods.

Tap sampling was adapted in Florida by the Citrus Health Response Program (CHRP) with the goal of monitoring 6,000 “multi-blocks” every three weeks. Fifty tap samples are taken, 10 in each of the four cardinal extremes of the block and 10 in the center. This information is uploaded in a timely manner to the CHMA management areas website where it is available to the multi-block owner and whoever else he or she designates. The data are also mapped and made available to members of participating CHMA and others to chart surveillance progress and population densities of ACP in groves.

Visual Observation

APHIS PPQ has also been making visual observations of nymphal densities per flush year round since 2010 in order to monitor the impact of biological control efforts against ACP in south Texas.

Detection of HLB outbreaks and determination of infestation level

To detect presence of the bacterium, symptoms are looked for in plants and insects are collected for laboratory diagnosis. Scouting for HLB is worthwhile if the incidence is low enough to justify removal of symptomatic trees. Incidence of more than 3–4% per year is often deemed too high by growers to be economically justifiable. HLB inspection for detection and removal purposes needs to be undertaken frequently: at least four times a year. Scouts must be trained to recognize symptomatic trees and have the equipment to inspect tall trees at sufficient height.

If the objective is to evaluate the effectiveness of management programs or provide information on positive HLB detection, it may be useful to devote time to estimating HLB incidence. One simple method is to note the number of symptomatic trees among the 50 or 100 in some or all of the blocks being monitored for ACP.

It should be recognised that inconclusive or suspect samples (ie.those with any measureable detection by qPCR , that exceed Ct thresholds) are likely infected with Liberibacter but simply do not meet the standards for regulatory purposes. Such cases should be submitted to ongoing surveillance and to active management of the vector.

2.2.6 Rational use of insecticides

For applications both in the entire region and in individual outbreak sites found through monitoring, priority must be given to insecticides registered by the competent authority for specific usage against ACP. The ARCOs' protocol used in Mexico (<http://www.senasica.gob.mx/?doc=9364>) emphasizes the rotation of toxicological groups to manage insect resistance and to avoid emergence of secondary pests, such as the citrus blackfly (*Aleurocanthus woglumi* Ashby, 1915).

Chemical control of ACP will still provide benefits in terms of increased yield even if incidence of HLB approaches 100%. However, under those conditions, a threshold approach during the growing season may be more cost-effective than monthly sprays. The choice of insecticide will depend on any other target pest present at the time of the application spraying; however, rotating the mode of action and class of pesticide is recommended. Back-to-back application sprays of the same class (mode of action) of insecticide are not recommended. The choice of chemical depends on several parameters, including what has already been used during the season in order not to exceed the maximum allowable amount per season; the pre-harvest interval of the insecticide, because dormant sprays are made during the harvest season; and costs deemed reasonable by the grower.

Coordinated insecticide application

When growers are organized into regional or area-wide management areas (such as ARCOs or CHMAs) insecticide applications can be coordinated efficiently. A coordinator established in each management area can communicate with the growers on the timing of applications, using ACP population levels as the critical factor in determining when these should be. All growers in the area would be urged to spray their orchards within a two-week time frame. Applications can be done by air or, in most cases, on the ground.

Pest outbreaks generally occur when growers do not react quickly during a vegetative flush cycle or when orchards are in close proximity to residential zones with abundant citrus trees. In these situations, targeted spraying of low volume or reduced volume can be carried out.

Many growers have adopted the orchard border treatment program, specifically between major flush cycles. Orchard border treatment prevents incursion of ACP into the orchard. Its success depends on a good monitoring program to detect adults before a new insect outbreak.

ACP control programs are typically part of a multi-pest control approach. Although psyllid-specific sprays are used, most of the sprays are tank mixes that target important pests present in the orchard at the time of application. This multi-pest control strategy avoids the risk of secondary pest outbreaks. When the production goal is fresh fruit for the market, growers should exercise extreme caution in the use of broad-spectrum insecticides, which poses the risk of other pests flaring up (e.g. scales, mites) or chemical residues remaining on the fruit.

Trees should not be treated during their flushing cycle with broad-spectrum chemicals because these will harm natural enemies of citrus pests. Growers should monitor both adult and immature psyllid populations during the flushing cycle. If adult psyllid numbers have been increasing over a three-week period, a treatment should be applied only if there is no leaf or flower flush.

2.2.7 Biological control

Biological control – making use of predators, parasitoids, and entomopathogenic fungi – is a complementary technology to insecticide use for ACP management and is considered to be a tool that is ecological, environmentally innocuous, versatile, and effective (Chien et al. 1989, Étienne et al. 2001, Mellín-Rosas et al. 2011, Alvarado-Martínez et al. 2012, Ramírez-Balboa et al. 2012, Arredondo et al. 2013). For some zones and sites within an area-wide management strategy, it may be difficult to apply chemical sprays; for example, in abandoned orchards or in orchards that do not use chemical sprays, as in the case of organic producers. In the urban environment where homeowners have citrus trees, chemical treatments are expensive and not always feasible or acceptable. In these cases, the use, conservation, mass production, and release of entomophagous insects is particularly helpful. Biological control is not a short-term solution but a long-term one to help control psyllid populations.

2.2.7.1 Biological control with arthropods

Lacewings and debris carriers are favoured predators for ACP because of their high feeding capacity and availability on the market. *Olla v-nigrum* (Coleoptera: Coccinellidae) is also a favoured predator (Pacheco-Rueda and Lomelí-Flores 2012). Predators of the genera *Chrysoperla* and *Ceraeochrysa* have the potential to be used as regulators of *D. citri* nymph populations (Cortez-Mondaca et al. 2011, Pacheco-Rueda and Lomelí-Flores 2012). It is important to take the biological characteristics, predominance in the field and habits of each species into account in order to ensure their effect on *D. citri*.

Among the parasitoids, *Tamarixia radiata* has important advantages – such as its high degree of parasitism on *D. citri* nymphs and its excellent ability for searching for and feeding on the nymphs of its host – that make it an ideal candidate for use as a biological control agent in abandoned orchards or in orchards with little management (Aubert 1987, Étienne et al. 2001, Skelley and Hoy 2004). This parasitoid has been imported and released within control programs on the islands of Réunion and Taiwan (Étienne and Aubert 1980, Chien 1995), as well as in Florida, California, Louisiana, Puerto Rico, and Texas, United States (Hoy and Nguyen 2001). Although there are no records of any official introduction, *T. radiata* is present naturally in Mexico (Sánchez et al. 2015), and augmentative releases have been implemented in various parts of the country.

In Florida, residential plantings are problematic owing to inconsistent spray patterns or no spraying at all, but their negative impact on the psyllid population is small compared to that of abandoned and unmanaged orchards (Hall D., pers. comm.). Texas and California share a concern for ACP spreading from urban neighbourhoods, where many varieties of citrus trees are found, to nearby commercial citrus-producing zones. In most cases, ACP move from residential to commercial citrus and not vice versa, because of the abundance of type and quantity of host material (Setamou, M., pers. comm.). If left unmanaged, populations of the psyllid in residential areas can work against the effectiveness of regional or area-wide management programs such as ARCOs. Control of the psyllid in urban environments must rely heavily on biological control; it is the most practical and acceptable method, as there are too many challenges to funding implementation of chemical treatment.

Release of parasitoids

Releases of *T. radiata* should be made year round on citrus that is flushing and infested with nymphs. The number of parasitoids to be released should be based on the population of ACP nymphs, and take into account the capacity of the production facility (insectary). For example, in Texas, 100–500 parasitoids per site have been released, and in Mexico, 400 parasitoids per hectare have been released in abandoned orchards and 100 parasitoids per 100 linear metres in urban areas. Releases are targeted to parks and residential neighbourhoods as well as areas with host material within one mile of orchards. Parasitism rates have been reported to be much higher within a five mile radius than in other parts of south Texas. An alternative method is that used by APHIS PPQ in both Texas and California where field insectary cages are used to cover large citrus trees in the urban environment to produce and release *in situ* large volumes of parasitoids (Daniel Flores, pers. comm.).

Release of parasitoids in unmanaged orchards

In unmanaged orchards, releases of *T. radiata* may be done at any time of the year as long as eggs or any nymphal instar of the ACP are present in the orchard and temperatures are between 20 and 35°C (Sánchez-González et al. 2015). The parasitoids should be released at the rate of 400 insects per hectare (Sánchez-González et al. 2011b). This rate may lead to a reduction of up to 92.6% of the population of third to fifth instar nymphs of *D. citri* after five months of weekly releases (Sánchez-González et al. 2011a). Studies on the dissemination of *T. radiata* show that the parasitoid disperses in groups, using the wind to assist its dissemination; wind direction and velocity should therefore be taken into account before releasing the parasitoids (Sandoval-Jiménez et al. 2013).

Release of parasitoids in urban areas

There is a growing concern in the citrus industry that ACP is spreading from abandoned

orchards and urban areas to commercially viable orchards, rendering important the management of ACP in these areas.

The rate of release in urban areas should be 100 parasitoids per 50–100 linear metres, depending on the extent of the infestation; or in other words, if more than 20 nymphs per bud per tree are observed, then 100 individuals should be released every 50 metres (CNRCB 2011).

Just as in the case of unmanaged orchards, releases in urban areas may be done at any time of the year as long as eggs or any nymphal instar of the ACP are present in the orchard and temperatures are between 20 and 35°C. The effectiveness of *T. radiata* released in urban areas in parasitizing *D. citri* nymphs can reach up to 71% (Moreno-Carrillo et al. 2012).

2.2.7.2 Biological control by entomopathogenic fungi

For commercial citrus orchards that base their control methods primarily on the use of chemical insecticide applications to keep ACP populations low, entomopathogenic fungi may be considered as a friendlier alternative. Following the persistence time of chemical products, these entomopathogens may be used in a rotation when humidity and temperature conditions are favourable. Research and laboratory testing should be done to determine which species and strains of entomopathogenic fungi are appropriate for different citrus-producing regions that have the psyllid. The choice of strain to be used will depend on the results of validation tests (Sánchez et al. 2015).

In Mexico, the strategy in the ARCOs includes the use of strains of species *Isaria javanica* (prior *I. fumosorosea*) (candidate races CNRCB-CHE 303, 305 y 307, prior Pf15, Pf17 y Pf21) and *Metarhizium anisopliae* (CHE-CNRCB 224, prior Ma59) (Mellín-Rosas et al. 2009, Ayala et al. 2015). These strains are kept in the Entomopathogenic Fungi Collection of the Plant Health General Directorate of SENASICA.

Area-wide management programs in Texas and Florida have shown that ACP can be effectively controlled by relying on insecticide sprays in the dormant winter season and before major flush cycles (David Hall, pers. comm., David Bartels, pers. comm., Wright 2015, Chow et al. 2013). However, populations of ACP in Florida are becoming less susceptible to some insecticides (David Hall, pers. comm., Stelinski 2013), and the use of entomopathogenic fungi has less adverse effects than insecticides on human health and the environment (Chow et al. 2013). ACP in the United States is susceptible to several entomopathogenic fungi. *Isaria fumosorosea* is showing positive results in south Texas; in one study, 94% of ACP adults and nymphs were killed within four days of infection (Chow et al. 2013).

Application of entomopathogenic fungi

In commercial orchards that meet the conditions for temperature (22–28°C) and relative humidity (>80%) (Zimmermann 2008), applications of entomopathogenic fungi may be made in the entire area. In Mexico, these environmental conditions are expected from November to December and from January to February; however, it will depend on where they will be applied and to prior evaluation of their effectiveness in the field.

In general terms, applications of entomopathogenic fungi to control ACP are made at a concentration of 1×10^7 conidia/ml. The amount of fungi to be applied per hectare will depend on the water required to cover the area. If a residual population of the psyllid is detected after application, the fungi must be applied again 10 days after the first application, but only in areas where the psyllid is present and that meet the environmental conditions. Applications should be made in the evening hours (after 16.00 hours) as the fungus will survive better under the conditions of temperature and relative humidity at that time. The equipment used for the application of the entomopathogenic fungi should allow for all the foliage of a psyllid-infested tree to be sprayed in order to increase the probability of infection, while guaranteeing the dose of 1×10^7 conidia/ml. The equipment should be free from residues of fungicides, insecticides, fertilizers and herbicides. Preparations of fungi should be applied on the same day they are made.

3 Additional tools for management of Huanglongbing and its vector

The following information is adapted from the Executive Summary of the Technical Working Group report, Area wide control of Asian citrus psyllid (*Diaphorina citri*) (USDA 2009).

3.2 Cultural management

The following tactics are recommended as appropriate in the development of area-wide control programs:

- Area-wide removal of symptomatic trees to reduce inoculum
- Destruction of abandoned orchards.
- Encouragement of urban dwellers to replace ACP host plants with non-host plants in their gardens or, failing that, to control the psyllids. Caution should be exercised when choosing a new plant to avoid attracting other significant pests.
- Management of untreated citrus and other hosts such as orange jasmine (*Murraya paniculata*) and box orange (*Severinia buxifolia*) through mass release of the parasitoid *T. radiata* (especially in urban areas) .
- Flush management such that reproduction by ACP is limited to twice per year. This would greatly reduce psyllid populations.
- Planting of new citrus blocks so as to reduce the relative length of edges with regard to the enclosed area.
- Adjust production practices such as nutrition and watering, for better care of the root system

3.3 Outreach, education, coordination, and extension

- Extension specialists should disseminate the appropriate information through established mechanisms in each state or area.
- Appropriate information should be disseminated to the general public and stakeholders: agricultural news media, home gardeners (backyard trees), tribal governments, packers and shippers, migrant farm labourers, people who might move fruit or plants from one place to another, farmers' market personnel, floral market personnel, and ethnic grocery stores personnel.
- The provision of information to all commercial growers, packers, urban growers, and so forth on the importance and timing of the area-wide control program will be essential.

- Recruiting urban area residents and stakeholders in the area-wide program to report any psyllids will provide information to extension personnel and regulatory officials on the presence of ACP in new areas,
- Involve urban area residents and stakeholders, especially near commercial groves, to encourage participation in biological control programs.

4. Regulatory recommendations

Another option to insecticide application and biological control is to consider regulatory action, for example to eliminate sources of inoculum, which poses risks not only for HLB but also for other high-risk citrus pests. To strengthen regional management, NPPOs may consider establishing regulations related to:

- Detection and identification methodology for HLB and ACP
- Certification of disease-free propagative material
- Implementation of area-wide management
- Removal of plants contaminated with HLB
- Movement of HLB-free propagative material
- Movement of fruit free from plant material
- Restriction of movement of unprocessed fruit from areas with *D. citri* into uninfested areas
- Establishment of quarantine areas
- Training and outreach campaigns

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