ANSES Opinion
Request No 2016-SA-0235

The Director General

Maisons-Alfort, 25 April 2019

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on a "pest risk analysis for huanglongbing disease in the European Union"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 25 April 2019 shall prevail.

On 31 October 2016, ANSES received a formal request from the Directorate General for Food (DGAL) to undertake the following expert appraisal: a pest risk analysis for huanglongbing disease in the European Union.

1. BACKGROUND AND PURPOSE OF THE REQUEST

1.1. Background[1]

Huanglongbing (HLB, or citrus greening or yellow dragon disease) is currently one of the greatest threats to the health of citrus crops. Therefore, the introduction and spread of the various species of *Candidatus* Liberibacter spp. (CL) – three of which (*Candidatus* Liberibacter africanus, americanus and asiaticus) are the causal agents of this disease – are prohibited by plant health regulations in the European Union (Annex I, Part A, Section 1 of Directive 2000/29/EC, June 2014 version).

This disease affects a large part of South-east Asia as well as America and Africa. It is absent from Europe. As for the overseas territories of France, it has been found in the West Indies and on Reunion Island.

[1] Please note that the text in the section below (1.1 Background) is an exact repetition of the text of the request.

In the summer of 2015, the disease was suspected in a citrus orchard in the Algarve region of Portugal. Nonetheless, following additional tests for the detection of this bacterium, combined with an intensive monitoring and sampling programme, the region was declared free of '*Candidatus* Liberibacter asiaticus' by Portugal's national plant protection organisation (NPPO) (EPPO, Rsf-1602).

Furthermore, in the same year, the disease was first reported in North Africa, specifically in Egypt, a country that exports citrus fruit to Europe (Tolba and Soliman, 2015)[2].

1.2. Purpose of the request

In order to protect citrus-producing European Mediterranean countries from possible economic impacts arising from these bacteria, a risk assessment was requested for the entire European Union, with the aim of preventing both the introduction of this pest into Europe and its spread if introduction should occur, since one of its vectors, *Trioza erytreae*, is present in Portugal and Spain.

ANSES was thus asked to undertake a pest risk analysis (PRA) for *Candidatus* Liberibacter spp. for the entire European Union.

2. ORGANISATION OF THE EXPERT APPRAISAL

2.1. Procedure: means implemented and organisation

ANSES entrusted examination of this request to the Working Group (WG) on "HLB", reporting to the Expert Committee (CES) on "Biological risks for plant health".

The methodological and scientific aspects of this group's work were regularly submitted to the CES on "Biological risks for plant health". They were presented to the CES for discussion on 08/11/2016, 17/01/2017, 14/03/2017, 08/06/2017, 12/09/2017, 07/11/2017, 23/01/2018, 20/03/2018, 15/05/2018, 04/07/2018, 18/09/2018, 13/11/2018 and 22/01/2019.

The Agency issued an interim report in response to this request on 23 October 2018.

The report produced by the WG takes account of the observations and additional information provided by the CES members. Its conclusions were unanimously adopted by the CES.

This work was therefore conducted by a group of experts with complementary skills.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

2.2. Prevention of risks of conflicts of interest

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the Agency's website (www.anses.fr).

[2] It should be noted that between the submission of the formal request to ANSES and the conduct of the pest risk analysis (PRA), Egypt was considered as free of HLB by the NPPO.

3. ANALYSIS AND CONCLUSIONS OF THE CES AND WG

3.1. Categorisation

The '*Candidatus* Liberibacter asiaticus' (CLas), '*Candidatus* Liberibacter africanus' (CLaf) and '*Candidatus* Liberibacter americanus' (CLam) bacteria, responsible for HLB disease, are regulated pests (Annex I, Part A, Section 1 of Directive 2000/29/EC). They are carried and transmitted to cultivated citrus by two insect vectors, *Diaphorina citri* and *Trioza erytreae* psyllids, which are also regulated organisms (Annex I, Part A of Directive 2000/29/EC).

The pest risk analysis (PRA) area is the European Union (EU). It includes the Azores and Madeira but not the Canary Islands. None of the three bacteria have been found in the EU. The same is true for *Diaphorina citri*. However, *Trioza erytreae* has been reported in Spain and Portugal, including Madeira.

The disease can also be transmitted via the grafting of infected budwood[3] or from infected rootstocks. Moreover, *Candidatus* Liberibacter spp. (CL spp.) bacteria have been transmitted by several species of parasitic plants of the *Cuscuta* genus in experimental conditions.

Most of the host plants of CL spp. bacteria belong to the Rutaceae family, which includes the *Citrus* genus, whose species and hybrids are intensively cultivated in countries in the south of the EU. In their current area of distribution, the three bacterial species have negative impacts on yields (premature fruit drop), harvest quality (decline in the quality of fruit juices) and trees (degraded physiology, premature death). The symptoms of chlorosis in *Citrus* induced by CL spp. can be misleading and resemble abiotic stress such as nutrient deficiency.

The most threatened parts of the PRA area cover all citrus-producing regions (southern Portugal, southern and eastern Spain, Corsica, southern Italy, Sicily, Greece, Croatia, Cyprus and Malta).

3.2. Conclusion of the pest risk analysis

Entry

The eight pathways considered relevant took into account the entry of HLB from imports of infected plant material or via insect vectors.

The four pathways of HLB via infected plants for planting (plants, budwood, seeds) or plant parts for consumption (foliage and fruit) are:

- Plants and budwood of *Citrus* spp. and plants of *Murraya paniculata* (pathway 1: "P1");
- Seeds of *Citrus* spp. and *Murraya* spp. (pathway 2: "P2");
- Cut flowers/cut branches/foliage of *M. paniculata* and *Citrus hystrix* (pathway 3: "P3");

[3] Budwood consists of a shoot free from foliage, bearing buds suitable for grafting.

- Fruit of *Citrus* spp. (pathway 4: “P4”).

The four pathways of entry via contaminated or non-contaminated insect vectors are:

- Plants of *Citrus* spp. (pathway 5: “P5”);
- Plants of *M. paniculata* (pathway 6: “P6”);
- Fresh leaves for consumption of *Murraya koenigii* and *Citrus hystrix* (pathway 7: “P7”);
- Fruit of *Citrus* spp. (hitch-hiker behaviour) (pathway 8: “P8”).

The pathway via plants and seeds of *Cuscuta* spp. (an obligate parasitic plant) was excluded for several reasons, in particular because no intentional import of plants and seeds is likely. However, the entry of this species could occur through the contamination of other imported seeds.

There are already phytosanitary measures for imports to the EU of fruit, seeds and plants for planting of several Rutaceae species. The pest risk analysis undertaken by the WG did not take into account the implementation of these specific plant health measures for these pathways. Nonetheless, it is important to keep in mind that phytosanitary measures have an inevitable impact on some of the parameters taken into consideration in the analysis, such as volumes of imports, which are assumed to be non-existent when plant parts are prohibited as imports.

The highest probability of entry for CL spp. in the PRA area concerns the pathway of plants or budwood from *Citrus* spp. and *Murraya paniculata* (“P1” pathway). In the absence of regulations as mentioned above, if these plants or budwood come from infected regions, the probability of association at origin will be high, as will the probability of the bacteria surviving during transport. In the absence of control via existing quarantine procedures, the probability of the bacteria entering the PRA area without being detected will be high. This association at origin and this survival are linked to a high probability of the disease being transferred to orchards via the planting of contaminated ‘plants for planting’, itself accentuated by the presence of the vector psyllid, *T. erytreae*, in the threatened area. This series of successful events for the entry of HLB (association at origin, survival, transfer) also involves other pathways, namely the plants of *Citrus* and plants of *Murraya* pathways (“P5” and “P6” pathways respectively) as host-commodities of psyllids contaminated by HLB. Thus, the risk of entry into the PRA area of the psyllid vector that is not yet present (*D. citri*) was deemed likely with a medium level of uncertainty, with the corresponding pathways most at risk being those of imported plants (“P5” – plants of *Citrus* – and “P6” – plants of *Murraya paniculata* – pathways).

Nevertheless, it is important to note that, despite the regulatory prohibition of imports, intended to guarantee the reduction of risk at entry, there have been reports in the PRA area of orchards planted with *Citrus* and of nurseries selling young *Murraya* plants, all coming from contaminated countries. These illegal imports constitute a pathway of entry for high-risk material.

The analysis of the other pathways showed that entry of the bacteria and/or insect vectors is unlikely due to a lack of association at origin, a low level of survival during transport or the unlikelihood of transfer, despite the existence of frequent large import flows as is the case with citrus fruit. There are uncertainties relating to the precise quantification of flows of certain plant products, especially foliage of *Murraya paniculata* and *Citrus hystrix* for example, as well as certain aspects of biology such as the survival of insects during transport.

The overall probability of entry of the bacteria responsible for HLB, considering all of the pathways, was deemed likely with a low level of uncertainty.

Establishment

The main ecological factors that influence the potential for establishment of HLB are host plants, climate and vectors.

Citrus plants, hosts to CL spp. bacteria, are widely cultivated in Mediterranean regions in the PRA area. The regions where the climatic conditions in the PRA area are suitable for the establishment of the bacteria responsible for HLB, since they are similar to those in its current area of distribution, are temperate and warm-climate regions (Csa and Csb climates according to the Köppen-Geiger classification). Moreover, the bacteria responsible for HLB have become well established in new areas, outside of the disease's area of origin, and show adaptation traits. Lastly, the non-specific symptoms of the disease and the long latent period (from three months to two years) before symptom expression facilitate not only its entry but also its establishment (before any eradication measures are taken) and spread.

The vectors are key species for the completion of the HLB life cycle, especially during the transmission and spread stages. The climatic conditions in the area of potential establishment are also favourable for *D. citri* and *T. erytreae*, which are the insect vectors of the bacteria responsible for HLB. *T. erytreae* has become well established outside of its area of origin, for example recently in the Iberian Peninsula (in northern Portugal and in Galicia, in the north-west of Spain). Based on the available observations, *D. citri* also has a high capacity for establishment in new areas. These factors, combined with the presence of host plants and the absence of natural competitors and enemies, are suitable to the establishment of the vectors.

The overall probability of establishment of the bacteria responsible for HLB and the vectors of HLB was therefore deemed high with low uncertainty.

Spread

The rate of spread of HLB was deemed high with a low level of uncertainty.
Natural spread is likely via *T. erytreae*, which is already present in the PRA area, and may be accentuated if *D. citri*, which is constantly expanding its range (in East Africa in particular), is introduced. Indeed, the rate of spread of the insect vectors was considered high with a low level of uncertainty, as the presence of host plants and the climatic conditions in the potential area of establishment are favourable for these two insect vectors.

Moreover, human activities represent a major means for the long-distance spread of HLB (by contaminated plants for planting or budwood) or the insects themselves (via the transport of host-commodities infested with the insect vectors).

However, it is more difficult to determine i) the time needed for HLB to reach its maximum extent in the PRA area, and ii) the proportion of the area of potential establishment invaded by HLB after five years, considering the diversity of the climatic zones, citrus planting densities which vary depending on the region within the PRA area, and the latent period for the expression of the disease.

Economic importance

In the current area of distribution of HLB and its vectors, the control methods implemented have not been able to eradicate the disease or maintain its impact below an acceptable economic threshold. In light of these observations, the potential agro-socioeconomic impacts in the threatened PRA area (citrus-producing countries) are, in short: a large drop in yield, a decline in fruit quality and, in the medium term, tree death, loss of income and a decline in employment. HLB may accentuate the negative impact of another disease (such as citrus canker) and sharply accelerate tree dieback.

Overall conclusion of the pest risk analysis

Three species of, *Candidatus* Liberibacter bacteria (CL spp.) (*Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus and *Candidatus* Liberibacter americanus') are responsible for HLB disease.

The main host plants of CL spp. bacteria are citrus, which are intensively cultivated in countries in the south of the EU. All citrus-producing regions (southern Portugal, southern and eastern Spain, Corsica, southern Italy, Sicily, Greece, Croatia, Cyprus, Malta) are therefore threatened.

CL spp. bacteria are transported and transmitted to cultivated citrus fruit by contaminated plant material (including infected budwood and rootstocks) and by *Diaphorina citri* and *Trioza erytreae* psyllids.

The overall probability of entry of HLB (combination of the eight pathways of entry) was deemed likely with a low level of uncertainty. Plants of *Citrus* and *Murraya* for planting are the pathways at the greatest risk since they enable the entry of the bacteria themselves and of contaminated vectors, when these have come from contaminated countries with no effective enforcement of the regulations.

The overall probability of establishment in the PRA area was deemed high (with low uncertainty), considering i) the favourable climate, ii) the presence of citrus, iii) the adaptive potential of the bacteria, and iv) the insect vectors' capacity for establishment outside of their area of origin.

The rate of spread of CL spp. bacteria was deemed high (with a low level of uncertainty), in light of the transmission of the disease by *D. citri* and *T. erytreae* and the role played by human activities in spread via the transport of contaminated host-commodities.

The potential economic impacts affecting production both quantitatively and qualitatively, as well as orchard integrity, are deemed all the greater given that all of the citrus-producing regions in the PRA area will be threatened.

The identified risk is high and is therefore deemed unacceptable, making it necessary to pursue pest risk management.

3.3. Conclusion on pest risk management

Regulations

Since the current legislation has not prevented illegal imports, the strengthening of controls (host-commodities, passengers) in line with these regulations, as well as special requirements for host commodities allowed to enter the PRA area, would help avoid imports of plants (plants, budwood, etc.) potentially carrying HLB and its vectors.

The ban on the transfer of fresh plant material from areas infested with *T. erytreae* to pest-free areas is a measure to be maintained with the aim of preventing the insect from being introduced into pest-free parts of the PRA area. The same is true for compliance with the special requirements with regard to fruit (free from leaves and peduncles for fruit from third countries and specific requirements for fruit from the EU that may bear leaves and peduncles).

High uncertainty will still remain for areas declared free of HLB and its vectors, especially in citrus-producing countries without strong capacities for analysis and expert assessment by national plant protection organisations (NPPOs). The employees of these services should benefit from ongoing awareness-raising and training. It should also be noted that since the disease can have an impact on trade, official reports of its presence or that of its vectors may be delayed. The contracting parties to the IPPC[4] are normally expected to report the presence of the disease or its vectors as soon as this has been confirmed.

Proposed measures to limit the spread of the vectors

Monitoring measures

Measures for monitoring CL spp. and its vectors inside non-infested countries in the PRA area (primarily around the Mediterranean) and in the Azores archipelago are recommended, following the example of those already implemented for *T. erytreae* in Spain and Portugal, which are citrus-producing countries infested with this vector. Outside of the area currently infested, sentinel surveys of private gardens are often used to continuously screen for new outbreaks and detect them as early as possible.

Beyond the PRA area, for the *T. erytreae* species, which is present in the Canary Islands but absent from the other countries around the Mediterranean, a special system could be implemented (if it does not already exist) in order to monitor the plant health status of these countries and safeguard exchanges between the EU and its trading partners. A network for observations as well as data centralisation could thus be considered, as is the case for other pests (crickets) in Africa.

For *D. citri,* reported in the Arabian Peninsula, special monitoring could be proposed (if it has not already been implemented by the relevant NPPOs) in the eastern part of the Mediterranean basin, in order to precisely determine the plant health status of this region following possible introductions, whether occurring naturally or through human activities.

Information and awareness-raising initiatives aimed at nurseries and passengers

Plants in the Rutaceae family, and more broadly certain host species for the disease and its

[4] IPPC: International Plant Protection Convention

vectors, are sold on the Internet or between private individuals at markets. It would be desirable to conduct a survey in nurseries involved in the online sale of species potentially serving as hosts of the disease and its psyllid vectors in order to inform them of the risk. The impact of even more drastic measures (total ban on sales) could be assessed.

Raising passengers' awareness of the risks of HLB introduction and informing them of the regulations in force remain measures to be taken.

Lastly, official trade in plant material inside the PRA area (in particular between Corsica, Spain and other areas producing guaranteed disease-free material) should be better characterised. Indeed, data relating to movements of host-commodities in the intra-European zone are relatively imprecise as to the characterisation of certain plant species, in this case of ornamental plants potentially serving as hosts for vectors.

Recommendations to reduce uncertainty

Some recommendations may contribute to reducing uncertainty identified throughout the analysis, in relation to the lack of knowledge on certain points.

A lack of information still remains with regard to the host plant *Murraya paniculata*, especially in terms of the entry of the disease via plants for planting coming from areas contaminated by the disease but escaping control upon entry into a country in the PRA area, such as a European country that imports but does not produce citrus fruit.

A total ban on imports of plants for planting and fresh foliage of species in the *Murraya* genus from third countries (whether free of HLB or not) would be an additional control measure whose impact on international trade is not known.

A lack of knowledge was also identified with regard to the presence, in the PRA area, of primarily wild plants serving as potential hosts for the disease and its vectors. Regular observation of these plants could be recommended, especially for the Rutaceae family. Awareness-raising aimed at amateur associations or botanist clubs conducting surveys or making observations (participatory science) could also be considered, with the centralisation of data.

Few fundamental studies have been undertaken on potential host shifts of *Candidatus* Liberibacter (CL) spp. In the PRA area, experimental studies dealing with the transmission of CL spp. by *Cuscuta* suggest that *Cuscuta* may play the role of vectors and cause *Citrus* to become infected with CL spp.

Additional studies on the transmission potential of HLB via insects other than the known vectors, that have nonetheless been identified as carrying the bacteria, for example the very common mealybug species *Ferrisia virgata*, are recommended. Furthermore, little information has been collected regarding the presence of host plants of *T. erytreae* in geographical areas located between the current areas where the pest is present and pest-free areas (in Spain, for example).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

ANSES endorses the conclusions of the CES on "Biological risks for plant health" and the Working Group on "HLB".

ANSES reiterates that varying lengths of time have been observed between the reporting of insect vectors and the description of damage due to HLB. Historically, the bacteria responsible for HLB have been detected long after the vector became established in a given region, as was the case in Florida, Cuba, Mexico (seven years), Martinique (two years) and Brazil (62 years), for example. Moreover, observations made regarding HLB in its current area of distribution show that for the moment, no measures are capable of effectively reducing the incidence of HLB once the disease has become established. This leads the Agency to underline the importance of the risk management actions to be taken in the presence of vectors, before HLB is introduced.

Preventing the entry of HLB and its insect vectors, especially *Diaphorina citri,* still absent from the pest risk analysis area (PRA – the European Union for this Opinion), requires strict compliance, both with the regulations in force in terms of the ban on the plants for planting pathways and with special requirements for other pathways including plants for consumption. ANSES also recommends undertaking awareness-raising initiatives to inform passengers of the risks related to the introduction of plant material from the Rutaceae family for ornamental, production or consumption purposes, all the more so because this material may not meet the plant health requirements defined by the regulations and may cross borders without any control.

Of the management options available to limit the establishment of the disease, ANSES recommends strengthening regional monitoring plans for HLB and psyllids (including both *Trioza erytreae*, which is present in the PRA area, and *Diaphorina citri*, which is still absent), especially in citrus-producing regions of the PRA area around the Mediterranean. Emergency plans for the monitoring, eradication and containment[5] of HLB and psyllids should be deployed in the threatened area. This would enable asymptomatic host plants and isolated vector outbreaks to be detected more rapidly. The resulting elimination of contaminated plants and vectors would increase the effectiveness of the HLB control strategy. ANSES stresses the importance of analysing samples collected from asymptomatic materials in regions where one of the vectors is present. Regarding spread, the recommended management options involve the restriction of movements of plant material, in the PRA area, between areas contaminated by psyllids (and later by HLB, if the disease is established) and non-contaminated areas. ANSES also recommends undertaking awareness-raising initiatives to inform nurseries of the risks related to Rutaceae sold on the Internet or between non-professionals at markets.

Lastly, in light of the fact that there is a lack of effective control measures to reduce the incidence of HLB once the disease has become established, ANSES supports recommendations for research with the aim of developing such measures. The avenue of research that seems the most promising is the continuation of efforts to select plant species or plant varieties with at least partial resistance to HLB. Regarding insect vectors, based on successful experiences in various countries (reduction in populations of psyllid vectors, and even non-detection of *T. erytreae* populations on Réunion, after introducing specific parasitoid Hymenoptera), further research is

[5] Also known as contingency plans

recommended to study the relevance and feasibility of implementing a biological control strategy by acclimation in the PRA area, as has been planned in Spain.

Dr Roger Genet

KEYWORDS

HLB, huanglongbing, analyse de risque phytosanitaire (ARP), Union européenne, région méditerrannéenne, agrumes, *Citrus* spp., *Murraya paniculata*, Rutaceae, *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus, psylle vecteur, *Trioza erytreae*, *Diaphorina citri*.

HLB, huanglongbing, pest risk analysis (PRA), European Union, Mediterranean area, citrus, *Citrus* spp., *Murraya paniculata*, Rutaceae, *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus, psyllid vector, *Trioza erytreae*, *Diaphorina citri.*