

# EPPO Datasheet: '*Candidatus Liberibacter solanacearum*'

Last updated: 2020-04-22

Only Solanaceae haplotypes of '*Candidatus Liberibacter solanacearum*' are included in the EPPO A1 List.

## IDENTITY

**Preferred name:** '*Candidatus Liberibacter solanacearum*'

**Authority:** Liefiting, Perez-Egusquiza & Clover

**Taxonomic position:** Bacteria: Proteobacteria: Alphaproteobacteria: Rhizobiales: Phyllobacteriaceae

**Other scientific names:** *Liberibacter psyllaourous* Hansen, Trumble, Stouthamer & Paine, *Liberibacter solanacearum* Liefiting, Perez-Egusquiza & Clover

**Common names:** zebra chip disease

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**EPPO Categorization:** A1 list

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**EU Categorization:** RNQP (Annex IV)

**EPPO Code:** LIBEPS



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## Notes on taxonomy and nomenclature

This bacterium was first described from solanaceous plants and psyllids, almost simultaneously in New Zealand and the USA. The name '*Candidatus Liberibacter psyllaourous* (Hansen *et al.*, 2008) was initially proposed, but '*Candidatus Liberibacter solanacearum*' (Liefiting *et al.*, 2009c) was finally retained as the validly published name. Until now, '*Ca. L. solanacearum*' has not been cultivated in axenic medium to allow the Koch's postulates to be verified, hence its '*Candidatus*' status. The bacterium is genetically diverse and ten haplotypes of '*Ca. L. solanacearum*' have been described (Nelson *et al.*, 2011, 2013; Teresani *et al.*, 2014; Swisher Grimm and Garczynski, 2019; Haapalainen *et al.*, 2018b; Mauck *et al.*, 2019; Haapalainen *et al.*, 2019; Contreras-Rendón *et al.*, 2019). These haplotypes also differ in their host ranges, psyllid vectors and geographical distributions. In particular, four haplotypes (A, B, F and G) are associated with diseases of potatoes and other solanaceous plants, whereas four others (C, D, E and H-European) are associated with diseases of carrots and other apiaceous crops. Haplotype H European was also described in plants of the family Polygonaceae. Further described haplotypes, H North America and U, were not associated with a disease and were described in plants of the families Convolvulaceae and Urticaceae, respectively. Haplotype H North American (reported in Mexico) is different from haplotype H European (reported in Northern Europe). In the EPPO region, several haplotypes have been detected in apiaceous crops, as well as in several psyllid species, but the solanaceous haplotypes of '*Ca. L. solanacearum*' have never been detected. Therefore, it is important to note that only Solanaceae haplotypes of '*Ca. L. solanacearum*' are included in the EPPO A1 List.

## HOSTS

'*Candidatus Liberibacter solanacearum*' is known to primarily infect solanaceous species, including potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), tomatillo (*Physalis peruviana*), tamarillo (*Solanum betaceum*), tobacco (*Nicotiana tabacum*), and several weeds in the family Solanaceae (Hansen *et al.*, 2008; Liefiting *et al.*, 2008a,b, 2009a,c; Abad *et al.*, 2009; Crosslin & Munyaneza, 2009; Lin *et al.*, 2009; Munyaneza *et al.*, 2009a,b,c; Secor *et al.*, 2009; Wen *et al.*, 2009; Brown *et al.*, 2010; Crosslin *et al.*, 2010; Munyaneza, 2010, 2012; Rehman *et al.*, 2010; Sengoda *et al.*, 2010). This *Liberibacter* species is transmitted to solanaceous species by the potato/tomato psyllid, *Bactericera cockerelli*.

This bacterium also infects apiaceous species including carrot (*Daucus carota*), celery (*Apium graveolens*), celeriac (*A. graveolens rapaceum*), parsnip (*Pastinaca sativa*), parsley (*Petroselinum crispum*), fennel (*Anthriscus cerefolium*

), chervil (*Anthriscus cerefolium*) and several weeds in the family Apiaceae. (Munyaneza *et al.*, 2010a; Teresani *et al.*, 2014; Monger & Jeffries, 2017; Hajri *et al.*, 2017). In the Northern part of Europe, the bacterium is transmitted to apiaceous crops by *Trioza apicalis*, while in the Southern part of Europe and the Mediterranean Basin, it is vectored by *Bactericera trigonica* (Nissinen *et al.*, 2014; Antolínez *et al.*, 2017a).

Different reports suggest that the host range of ‘*Ca. L. solanacearum*’ is larger than the Solanaceae and Apiaceae families. In a laboratory study, two plants belonging to the Convolvulaceae family, sweet potato (*Ipomoea batatas*) and field bindweed (*Convolvulus arvensis*), were tested positive after transmission trial with *B. cockerelli* (Torres *et al.*, 2015). In 2018, ‘*Ca. L. solanacearum*’ was reported in stinging nettle (*Urtica dioica*) and the psyllid *Trioza urticae* in Finland (Haapalainen *et al.*, 2018b). Recently, ‘*Ca. L. solanacearum*’ was detected on plants belonging to Polygonaceae family (*Fallopia convolvulus* and *Persicaria lapathifolia*) (Haapalainen *et al.*, 2019).

**Host list:** *Aegopodium podagraria*, *Anthriscus cerefolium*, *Anthriscus sylvestris*, *Apium graveolens* var. *rapaceum*, *Apium graveolens*, *Capsicum annuum*, *Capsicum frutescens*, *Chenopodium album*, *Datura stramonium*, *Daucus aureus*, *Daucus carota*, *Fallopia convolvulus*, *Foeniculum vulgare*, *Galium* sp., *Heracleum sphondylium*, *Lycium barbarum*, *Lycium carolineanum*, *Nicotiana tabacum*, *Pastinaca sativa*, *Persicaria lapathifolia*, *Petroselinum crispum*, *Physalis ixocarpa*, *Physalis peruviana*, *Physalis virginiana*, *Solanum americanum*, *Solanum betaceum*, *Solanum dulcamara*, *Solanum elaeagnifolium*, *Solanum lycopersicum*, *Solanum melongena*, *Solanum pseudocapsicum*, *Solanum tuberosum*, *Solanum umbelliferum*, *Urtica dioica*

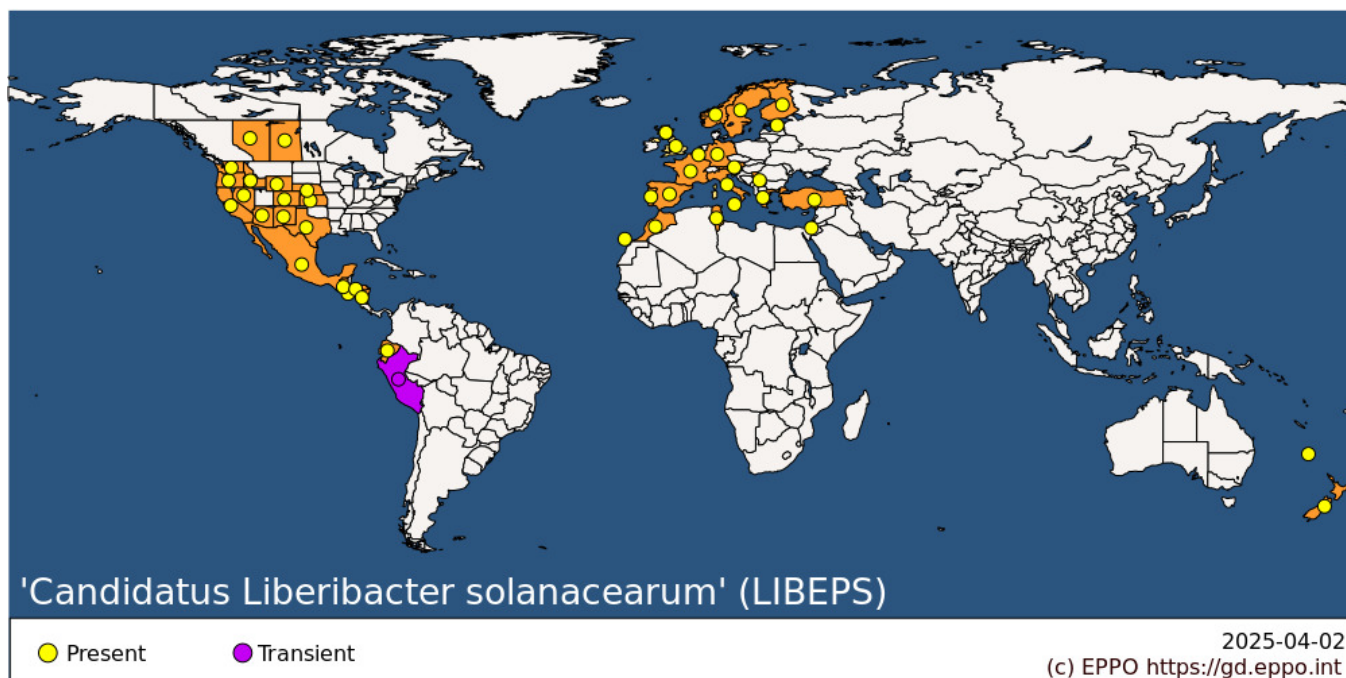
## GEOGRAPHICAL DISTRIBUTION

‘*Ca. L. solanacearum*’ haplotypes A and B have been found from Nicaragua through Central America, USA and Canada. Haplotype A was introduced in New Zealand by infected psyllids (Gill, 2006). It is also reported in Norfolk Island (Australian external territory).

Haplotype C has been found in Northern Europe. Haplotypes D and E occur in Southern Europe and the Mediterranean region. Haplotype U has been described in stinging nettle in Finland.

Recently described haplotypes H-European and H-North American were reported in Finland in Apiaceae and Polygonaceae family plants and in USA in Convolvulaceae family plants respectively (Haapalainen *et al.*, 2019; Contreras-Rendón *et al.*, 2019).

However, the distribution of ‘*Candidatus Liberibacter solanacearum*’ could be wider than that which has been reported. While testing collections of apiaceous seeds, Monger and Jeffries (2017) detected the bacterium in seeds coming from countries that had not reported it: Czech Republic, Denmark, Egypt, Japan, Lebanon, the Netherlands and Syria. This study also reported that some apiaceous seeds from 1973 contained the bacterium suggesting that ‘*Ca. L. solanacearum*’ has been present in Europe for a long time. Likewise, the study of Mauck *et al.* (2019) allowed the detection of the bacteria in a plant (*Solanum umbelliferum*) collected in 1970 in California, USA.



**EPPO Region:** Austria, Belgium, Estonia, Finland, France (mainland), Germany, Greece (mainland), Israel, Italy (mainland, Sicilia), Morocco, Norway, Portugal (mainland), Serbia, Spain (mainland, Islas Canarias), Sweden, Tunisia, Türkiye, United Kingdom (Scotland)

**Africa:** Morocco, Tunisia

**Asia:** Israel

**North America:** Canada (Alberta, Saskatchewan), Mexico, United States of America (Arizona, California, Colorado, Idaho, Kansas, Nebraska, Nevada, New Mexico, Oregon, Texas, Washington, Wyoming)

**Central America and Caribbean:** El Salvador, Guatemala, Honduras, Nicaragua

**South America:** Ecuador, Peru

**Oceania:** New Zealand, Norfolk Island

## BIOLOGY

'*Ca. L. solanacearum*' is a phloem-limited, Gram-negative, unculturable bacterium that is transmitted from infected to healthy plants by psyllid insect vectors (Liefing *et al.*, 2009c). It may be spread experimentally by grafting (Crosslin & Munyaneza, 2009; Secor *et al.*, 2009).

'*Ca. L. solanacearum*' haplotypes A and B are transmitted horizontally by *Bactericera cockerelli* from infected solanaceous plant to healthy solanaceous plants (Secor *et al.*, 2009). Within apiaceous plants, haplotype C is transmitted by *Trioza apicalis* while haplotypes D and E are vectored by *Bactericera trigonica* (Nissinen *et al.*, 2014; Antolínez *et al.*, 2017a). Although *B. tremblayi* (onion/leek psyllid) and *B. nigricornis* may acquire '*Ca. L. solanacearum*' by feeding on plants of Apiaceae in Spain (Teresani *et al.*, 2015), *B. tremblayi* failed to transmit the bacterium to carrot and is unlikely to be a vector in this crop (Antolinez *et al.*, 2017a). Transmission by *B. nigricornis* is still being studied. Haplotype U has been detected in *Trioza urticae* and stinging nettle (Haapalainen *et al.*, 2018b). This Liberibacter species has also been shown to be transmitted vertically (transovarially) in *B. cockerelli* (Hansen *et al.*, 2008). No information is currently available on vertical transmission for *T. apicalis* and *B. trigonica*.

Even if Pitman *et al.* (2011) demonstrated the transmission of the bacterium by infected seed tubers, this mode of transmission seems negligible (Munyaneza, 2012). Although a limited number of experiments have been conducted on Liberibacter transmission, it appears that '*Ca. L. solanacearum*' is not transmitted through true seed produced by infected plants for solanaceous plants (Munyaneza, 2012). For apiaceous plants, several seed transmission experiments in carrot and other Apiaceae were conducted, but the results of Bertolini *et al.* (2014) supporting seed transmission could not be confirmed in more recent experiments (e.g. Loiseau *et al.*, 2017a,b; Oishi *et al.*, 2017; Mawassi *et al.*, 2018; Haapalainen *et al.*, 2018b; Carminati *et al.*, 2019; Denton *et al.*, 2019).

Although the differences in severity of the different ‘*Ca. L. solanacearum*’ haplotypes have been studied in a limited number of experiments, they have shown that symptoms and consequences of B were more severe than those of A on tomatoes and, to a lesser extent, on potatoes (Mendoza-Herrera *et al.*, 2018; Harrison *et al.*, 2018). Studies on the genetic diversity of ‘*Ca. L. solanacearum*’ strains suggested that haplotypes hosted by the same plant family did not necessarily cluster together (Nelson *et al.*, 2013; Hajri *et al.*, 2017; Haapalainen *et al.*, 2019; Contreras-Rendón *et al.*, 2019). The discovery of ‘*Ca. L. solanacearum*’-infected potato plants and tubers in Finland (haplotype C) and Spain (haplotype E) might suggest that all haplotypes could infect potato, but that transmission is limited between the different plant families because of the lack of a vector that is able to feed efficiently on plants of both families (Apiaceae and Solanaceae) and then transmit the bacterium to potato. Similarly, it was observed that *B. cockerelli* could not efficiently transmit haplotype B to carrot plants (Munyanzeza *et al.*, 2016). In Finland, both volunteer and cultivated potato plants growing at the edge of an infected carrot field were found to be infected with haplotype C; however, potato plants and tubers were asymptomatic (Haapalainen *et al.*, 2018a, 2018b). In Spain, haplotype E infections, most likely vectored by *B. trigonica* (Antolinez *et al.*, 2017b), were detected in symptomatic potato tubers in Castilla y Leon (Palomo *et al.*, 2014) and Cantabria (EPPO, 2017). However, *B. trigonica* was not able to transmit ‘*Ca. L. solanacearum*’ from potato to potato (Antolinez *et al.*, 2017b). In any case, more studies are needed to better understand the meaning of the genetic diversity among the different strains. A global consensus is also needed on the nomenclature of the genetic variants of the bacterium.

Effects of environmental conditions on ‘*Ca. L. solanacearum*’ are not well known. However, temperature has a significant effect on the development of this bacterium. Compared to citrus greening *Liberibacter* species, ‘*Ca. L. solanacearum*’ appears to be heat sensitive as it does not tolerate temperatures above 32°C (Munyanzeza *et al.*, 2012a).

Generally, the biology of ‘*Ca. L. solanacearum*’ depends on the life cycle of its vectors. It has been shown that ‘*Ca. L. solanacearum*’ could alter its vector physiology, creating an environment that is potentially more favorable to the development of the bacterium and thus, its transmission (Molki *et al.*, 2019). Details concerning *B. cockerelli* are available in the corresponding EPPO data sheet (EPPO, 2013). *T. apicalis*, which vectors haplotype C, is a univoltine species and adults overwinter on coniferous trees (Hodkinson, 2009). *B. trigonica*, vector on apiaceous crops in Southern Europe and the Mediterranean region, has 2 to 3 generations per year and adults overwinter on evergreen shrubs (Hodkinson, 2009).

## DETECTION AND IDENTIFICATION

### Symptoms

The characteristic above-ground plant symptoms of ‘*Ca. L. solanacearum*’ infection in potato, tomato and other solanaceous species resemble those caused by phytoplasmas and include stunting, erectness of new foliage, chlorosis and purpling of foliage with basal cupping of leaves, upward rolling of leaves throughout the plant, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous, small, misshapen, and poor quality fruits (Munyanzeza *et al.*, 2007a,b; Liefting *et al.*, 2009a; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Munyanzeza, 2010, 2012). In potato, the below-ground symptoms include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and crisps or chips processed from affected tubers show very dark blotches, stripes, or streaks, rendering them commercially unacceptable (Munyanzeza *et al.*, 2007a,b, 2008; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Miles *et al.*, 2010; Munyanzeza, 2012; Munyanzeza & Henne, 2012). The symptoms in potato tubers have led to the disease being named ‘zebra chip’ (Munyanzeza *et al.*, 2007a,b; Munyanzeza, 2012).

Symptoms in carrots infected with ‘*Ca. L. solanacearum*’ include leaf curling, yellowish, bronze and purplish discoloration of leaves, stunting of the carrot shoots and roots, and proliferation of secondary roots (Munyanzeza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b). Collectively, these symptoms resemble those caused by leafhopper-transmitted phytoplasmas and *S. citri* in carrots (Font *et al.*, 1999; Lee *et al.*, 2006; Cebrián *et al.*, 2010; Munyanzeza *et al.*, 2011b). Similar symptoms of vegetative disorders are observed on other apiaceous crops but the infected plants can also be asymptomatic. More details concerning symptoms are available in the EPPO diagnostic protocol of the bacterium (EPPO, 2020a).

## Morphology

Similarly to other Liberibacters, scanning electron microscopy images of '*Ca. L. solanacearum*' in sieve tubes of infected plants revealed that this bacterium has a rod-shaped morphology (Liefting *et al.*, 2009a; Secor *et al.*, 2009). The bacterium is about 0.2  $\mu$ m wide and 4  $\mu$ m long (Liefting *et al.*, 2009a).

## Detection and inspection methods

Detection methods for '*Ca. L. solanacearum*' have been developed and include conventional and quantitative real-time polymerase chain reaction (PCR) (Hansen *et al.*, 2008; Crosslin & Munyaneza, 2009; Li *et al.*, 2009; Liefting *et al.*, 2009a; Lin *et al.*, 2009; Wen *et al.*, 2009; Crosslin *et al.*, 2011; Munyaneza, 2012; Ravindran *et al.*, 2011; Teresani *et al.*, 2014). Uneven distribution and variation in the Liberibacter titre in different parts of infected plants has been observed, making detection of this bacterium by PCR sometimes inconsistent (Crosslin & Munyaneza, 2009; Li *et al.*, 2009). Visual symptom inspections in some infected plants such as potato tubers can be very reliable (see symptoms above). A detailed EPPO diagnostic protocol for the bacterium is available (EPPO, 2020a). Mixed infections of '*Ca. L. solanacearum*' and phytoplasmas have been reported in potato (Liefting *et al.*, 2009b; Munyaneza, unpublished data) and carrot (Munyaneza *et al.*, 2011b). Furthermore, mixed infections of Liberibacter, phytoplasmas, and *S. citri* have been detected in carrots in the Mediterranean region (Alfaro-Fernández *et al.*, 2012a).

## PATHWAYS FOR MOVEMENT

'*Ca. L. solanacearum*' can be moved by its vectors or via its host plants, in particular by plants for planting. During international trade, infected planting material could carry the disease, or possibly also infective vectors (most likely as eggs). Seed potatoes infected with '*Ca. L. solanacearum*' generally do not germinate but, in rare cases, may produce infected plants (Henne *et al.*, 2010; Pitman *et al.*, 2011). However, these seed-borne infected plants are often weak and short-lived and do not significantly contribute to the disease spread (Munyaneza, 2012). Seeds of solanaceous plants do not transmit the bacterium (Munyaneza, 2012). And, to date, there is insufficient evidence that transmission by carrot seeds occurs. Indeed, at least five studies, following one report which stated the contrary, found that carrot seeds do not transmit '*Ca. L. solanacearum*' (Bertolini *et al.*, 2014; Loiseau *et al.*, 2017a,b; Oishi *et al.*, 2017; Mawassi *et al.*, 2018; Haapalainen *et al.*, 2018b; Carminati *et al.*, 2019). Most importantly, psyllid vectors have to be present to further spread the bacterium in the field.

## PEST SIGNIFICANCE

### Economic impact

The complex bacterium/vectors has caused serious damage to the potato and tomato industries in the Americas and New Zealand (Munyaneza *et al.*, 2007a,b, 2008; Liefting *et al.*, 2009a; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Rehman *et al.*, 2010; Guenthner *et al.*, 2012), as well as to the carrot industry in Europe (Munyaneza, 2010; Munyaneza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b).

'*Ca. L. solanacearum*' was first identified in 2008 (Hansen *et al.*, 2008; Liefting *et al.*, 2008a,b) and shown to be associated with zebra chip disease of potato. This disease had been observed since the 1990s with increasing impacts and was linked to *B. cockerelli* for the first time in 2007 (Munyaneza *et al.*, 2007a,b). First reported in Mexico in the 1990s, zebra chip was documented as causing serious economic damage in parts of Southern Texas in 2004-2005. In the case of potato, plant growth is negatively affected; crisps or chips made from zebra chip-infected tubers show dark stripes that become markedly more visible upon frying, and hence are commercially unacceptable. Whole crops might be rejected because of high levels of the disease, occasionally leading to abandonment of entire potato fields. Potatoes for the fresh market are severely affected by zebra chip (Munyaneza *et al.*, 2011a). Infected tubers usually do not sprout and if they do, produce hair sprouts or weak plants (Henne *et al.*, 2010; Pitman *et al.*, 2011). This Liberibacter species also severely affects other important solanaceous crops, including tomato, pepper, eggplant, tamarillo (Liefting *et al.*, 2009a; Munyaneza *et al.*, 2009b,c; Brown *et al.*, 2010), and tobacco (Aguilar *et al.*, 2013). In 2013, based on the fact that in Texas and New Zealand, the annual loss caused by zebra chip was respectively

estimated at 33 million USD and 50 million NZD, Soliman *et al.* estimated that the annual direct economic impacts of an infestation of solanaceous crops by ‘*Ca. L. solanacearum*’ in Europe would reach 222 million EUR. The detection of the bacterium in a country can also have consequences on the export market. In New Zealand, export gains for red capsicums were reduced by 5.22 million NZD in 2008, and the tomato industry lost nearly 3 million NZD (Teulon *et al.*, 2009).

In Europe, first reports mentioned that damage to carrots could reach up to 100% crop loss (Munyaneza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b). However, in the framework of the POnTE project, among seven answers from different European countries, a survey revealed that only Finland indicated to experience notable damage due to ‘*Ca. L. solanacearum*’. In Spain, one of the first countries reporting the bacterium, indicated that they were not experiencing any damage due to the bacterium (De Winter, 2019). At least, the impact on carrot production and apiaceous production seems to vary considerably between the European regions.

## Control

At present, applications of insecticides targeted against the potato and carrot psyllids or the use of netting are the only means to effectively manage diseases associated with ‘*Ca. L. solanacearum*’ (Munyaneza, 2012; De Winter, 2019). See the datasheet for *B. cockerelli* for more details. No plant resistance to the disease has yet been identified (Munyaneza *et al.*, 2011a).

## Phytosanitary risk

‘*Ca. L. solanacearum*’ and its insect vector *B. cockerelli* have been found to be serious and economically important pests of potatoes, tomatoes, and other solanaceous crops in Western and Central USA, Mexico, Central America and New Zealand. They would result in similar damage if introduced into the EPPO region. Quarantine considerations have emerged in some regions where ‘*Ca. L. solanacearum*’ has been documented. Some countries are now requiring specific testing for ‘*Ca. L. solanacearum*’ prior to allowing import of potatoes (Crosslin *et al.*, 2010; Munyaneza, 2012). Furthermore, Australia has put in place additional quarantine requirements for the importation of fresh tomato and pepper from New Zealand after 2006, where growers need to ensure that crops for export have been produced in areas free of *B. cockerelli* or the exported produce must be free of the psyllid.

‘*Ca. L. solanacearum*’ has been documented in carrots and other apiaceous crops in different European countries and in the Mediterranean Basin. Due to these reports and to the paper of Bertolini *et al.* (2015) which suggested that seed transmission might occur for carrot, even if their results could not be reproduced since then, some countries have put in place quarantine requirements for the importation of apiaceous seeds.

Different studies on the ability of transmission of ‘*Ca. Liberibacter solanacearum*’ revealed that the risk of transmission by *B. cockerelli* from potato to carrot and by *B. trigonica* from carrot to potato is negligible (Munyaneza *et al.*, 2016; Antolínez *et al.*, 2017b). These observations suggest that the main pathway of introducing ‘*Ca. L. solanacearum*’ into solanaceous species would be the introduction of infective *B. cockerelli* into the EPPO region.

## PHYTOSANITARY MEASURES

EPPO recommends that vegetative material for propagation and produce (such as fruits) of Solanaceae should come from areas free of *B. cockerelli* and ‘*Ca. L. solanacearum*’. Seed and ware potatoes should come from areas free of zebra chip. Alternatively, high grade seed potato may be imported under post-entry quarantine, and ware potatoes may be imported only for industrial processing purposes. Countries have also been recommended to establish a national regulatory control system for *B. cockerelli* and ‘*Ca. L. solanacearum*’ (in particular for haplotypes A and B) to protect their potato crops and ensure a prompt and effective official action in the event of an outbreak (EPPO, 2020b).

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