Express PRA for *Candidatus* Liberibacter solanacearum

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Initiation for the revision: *Regulation as regulated Non-Quarantine Pest on seed potatoes.*

Express PRA	***Candidatus* Liberibacter solanacearum**
Phytosanitary risk	*Classification no longer applicable since Lso is listed in Decision (EU) 2019/2072 as a regulated non-quarantine pest on seed potatoes.*
Conclusion	The bacterium *Candidatus* Liberibacter solanacearum (Lso) is present in Europe, North and Central America, North Africa and Oceania. However, the distribution of individual haplotypes is geographically limited and related to the distribution of the specific vector. Currently, the vector *Bactericera cockerelli* and the haplotypes **A**, **B** and **F** (Lso**A**, Lso**B**, Lso**F**) that cause damage on plants of the nightshade family (Solanaceae) are known from North and Central America as well as from New Zealand and Australia, but are not established in Europe. The haplotypes C, D and E infect Apiaceae and are established in Europe – like their vectors. Since 2012, Lso is listed in the A1-list of EPPO. *The bacterium is listed in Decision (EU) 2019/2072 as a regulated non-quarantine pest on seed potatoes. The vector B. cockerelli is listed as Union quarantine pest.* Due to appropriate climate conditions, it is assumed that Lso (Lso**A**, Lso**B**, Lso**F**) as well as the vector *Bactericera cockerelli* can establish outdoors in Germany. The establishment in other EU-Member States is possible, too. Due to the high damage potential mainly for potatoes, Lso and the vector *B. cockerelli* pose a significant phytosanitary risk for Germany and other EU Member States. According to current knowledge, Lso infected seeds of Apiaceae bear no or a negligible phytosanitary risk. Based on this risk analysis, it is assumed that the vector *Bactericera cockerelli* and the haplotypes **A**, **B** and **F** of *Candidatus* Liberibacter solanacearum can establish in Germany and other Member States and may cause severe damage on Solanaceae. *Thus, the distribution of all haplotypes of Lso via seed potatoes must be prevented.*

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Taxonomy	Kingdom: Bacteria; Phylum: Proteobacteria; Classification: Alphaproteobacteria; Genus: Liberibacter; Species: *Candidatus* Liberibacter solanacearum Currently, seven haplotypes (genetically slightly different forms) of the bacterium (**A**, **B**, C, D, E, **F** and U) are known. Here, all haplotypes are considered, however, the focus lies on the haplotypes **A**, **B** and **F** that occur on plants of the nightshade family (Solanaceae) and may cause damage to them.
Common name	Zebra chip disease; Abbreviation: Lso (LsoA, LsoB, LsoC, …)
Synonyms	*Candidatus* Liberibacter psyllaurous
Biology	Lso is an obligate parasite on its host plants and vectors. Until today, it is not possible to keep it on growing medium and to propagate it (thus, the name affix *Candidatus*). While feeding on an infected host plant, psyllids (Triozidae) ingest Lso and distribute it via the haemolymph (body fluid of invertebrates that surrounds tissue and organs), amongst others, into the salivary glands. The propagation of Lso takes place within the vector. Only after building a sufficient high bacteria population of within the vector, it becomes infectious (latency period app. 2 weeks). Female psyllids are able to pass on the bacterium to the progeny (HANSEN et al., 2018). While sucking on the phloem of the host plant the infectious vector transmits Lso back to the plant where it distributes. Anew, propagation of Lso takes place within the plant and in the case of sufficiently high concentrations of the bacterium, the typical disease symptoms show (HAAPALAINEN, 2014). Infected potato tubers do not germinate or lead to weak seedlings that die early (MUNYANEZA, 2015). In previous examinations on tomatoes and potatoes, no transmission of the bacterium from infected mother plants to seeds was observed (EPPO, 2017). Several haplotypes may be present in an infected plant (BEN OTHMEN et al., 2018). In the case of different haplotypes in the same distribution area, there were no hints on a genetic exchange between these haplotypes. That indicates a stable biological separation of the haplotypes (NELSON et al., 2011).

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<tr><th>Express PRA</th><th>Candidatus Liberibacter solanacearum</th></tr>
<tr><td></td><td>Lso is very susceptible to heat and does not tolerate temperatures above 32°C. Lso develops slowly at temperatures below 17°C (Munyaneza, 2015).

In experiments, the transmission of Lso to Catharanthus roseus and other herbs via the plant parasite Cuscuta campestris was detected. The plant enters into the phloem of the host plant with suction organs and lives on it.</td></tr>
<tr><td>Is the pest a vector?</td><td>No</td></tr>
<tr><td>Is a vector needed?</td><td>Yes. The vectors are psyllids (Superfamily: Psylloidea; Order: Hemiptera; Family: Triozidae) that feed on the phloem of the plants. They are specialised on certain host plant families.
<table>
<tr><td>Haplotypes</td><td>Vector</td></tr>
<tr><td>A, B</td><td>Bactericera cockerelli

<u>Host plants</u>: relatively polyphagous, present on species out of 20 plant families, mainly on solanaceous plants (Solanales; plants of the nightshade family (Solanaceae) and plants of the morning glory family (Convolvulaceae)), as well as plants of the mint family (Lamiales);

<u>Distribution</u>: Canada, USA, Guatemala, Honduras, Mexico, Western Australia, New Zealand, Norfolk Island (Ouvrard, 2019), El Salvador, Nicaragua (EPPO, 2019b)</td></tr>
<tr><td>C</td><td>Trioza apicalis (carrot psyllid)

<u>Host plants</u>: Apiaceae;

<u>Distribution</u>: Europe (Austria, Czech Republic, Slovakia, Denmark, Finland, Germany, France, UK, Hungary, Italy, Poland, Slovenia, Sweden,, Switzerland, Norway), Ukraine, Belarus, Russia, Mongolia (Ouvrard, 2019)</td></tr>
<tr><td>D, E</td><td>Bactericera trigonica

<u>Host plants</u>: Apiaceae;

<u>Distribution</u>: Europe (Cyprus, Czech Republic, France, Greece, Hungary, Italy, Malta, Portugal, Spain, Canary Islands, Slovakia, Switzerland, Turkey), Asia (Iran, Israel), Africa</td></tr>
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<tr><td></td><td>(Tunisia, Morocco, Egypt, Algeria) (OUVRARD, 2019), Serbia (EPPO, 2019a)</td></tr>
<tr><td>F</td><td>Presumably Bactericera cockerelli (see above), however, the evidence is still pending.</td></tr>
<tr><td>U</td><td>Presumably Trioza urticae (evidence for successful transmission is still pending)

<u>Host plants</u>: Urticaceae;

<u>Distribution</u>: Europe (Germany, Austria, Czech Republic, Denmark, Finland, France, UK, Greece, Hungary, Ireland, Lithuania, Poland, Slovakia, Slovenia, Sweden, Madeira, Norway, Turkey), Belarus, , Tajikistan, Russia, Mongolia, Japan, India, China, Israel, Iran, Afghanistan, Algeria, Lebanon (OUVRARD, 2019)</td></tr>
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Lso was detected in further Triozidae: Bactericera tremblayi, Bactericera nigricornis, Trioza anthrisci. There is no detection of a successful transmission of the bacterium to a host plant via these potential vectors. Bactericera tremblayi is not able to transmit Lso (ANTOLINEZ et al., 2017). Trioza apicalis is not able to transmit LsoC to potatoes (HAAPALAINEN et al., 2018a). It cannot be excluded that further currently unknown vectors that are established in Europe are able to transmit the bacterium.
</td></tr>
<tr><td>Host plants</td><td>The vectors are specialised on individual plant families. Thus, an outdoors transmission of the bacterium to other families is very unlikely (ANTOLINEZ et al., 2017; MUNYANEZA et al., 2016).
<table>
<tr><td>Haplotypes</td><td>Host plants</td></tr>
<tr><td>A, B</td><td>Solanaceae: Solanum tuberosum, S. lycopersicum, Capsicum annuum, Nicotiana sp.</td></tr>
<tr><td>C</td><td>Apiaceae: currently only on carrots (Daucus carota)</td></tr>
<tr><td>D, E</td><td>Apiaceae: carrots (Daucus carota), celery (Apium graveolens), fennel (Foeniculum vulgare), parsnip (Pastinaca sativa), chervil</td></tr>
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<tr><td></td><td>(Anthriscus cerefolium), parsley (Petroselinum crispum)</td></tr>
<tr><td>F</td><td>Previously detected only once on an individual potato tuber in the USA (Swisher Grimm & Garczynski, 2019)</td></tr>
<tr><td>U</td><td>Nettle (Urtica dioica) (Haapalainen et al., 2018b)</td></tr>
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Exemption: It was possible only once, in a laboratory trial to transmit LsoB to a few carrot plants via B. cockerelli. Under outdoors conditions, the risk of such a transmission is negligible (Munyaneza et al., 2016). LsoE was detected in a few symptomatic potatoes in Spain (EPPO, 2017). LsoC was found on asymptomatic potatoes next to carrots in Finland. The infection was not passed on to the next plant generation (Haapalainen et al. 2018a).
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<tr><td>Symptoms</td><td>
<u>On Solanaceae</u>:
Infected potatoes show dark stripes insides that show particularly after frying („zebra chips”). The vascular bundle ring in the tuber discolours to brown and necrotic spots occur (fig. 1b). The potato tubers are smaller but occur more numerously. Often they are distorted and do not germinate or prematurely without dormancy. The seedlings are lean and very weak. Symptoms on plant parts above ground are similar to a phytoplasm-infection: growth disturbances (shortened and thickened internodes, rosetting), chlorosis or purple discolouring of the leaves, leaf rolling, leave wilt and necrosis followed by the dying of the plants (Munyaneza, 2015; Abb. 1a).
The symptoms above ground on Capsicum sp. (fig. 1c) and tomatoes correlate with those on potato plants. The plants may also die. Additionally, infected tomato plants (fig. 1d) may build deformed (strawberry-like) fruits.
Tobacco (Nicotiana tabacum) shows an infection with severe leaf chlorosis, distorted growth and wilting (fig. 1d).
The vector Bactericera cockerelli causes the plant yellowing.
<u>On Apiaceae</u>:
Carrots: leaf roll; yellowish, bronze or purple discoloured leaves; shortened shoots and roots, increased building of
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<tr><td></td><td>lateral roots. These symptoms are similar to an infection with the bacterium Spiroplasma citri or phytoplasms (IPPC, 2017).

Celery: celery infested with Lso builds an unusually large number of shoots and shows twisted stems (IPPC, 2017).</td></tr>
<tr><td>Presence of the host plants in Germany</td><td>Widely distributed.</td></tr>
<tr><td>Presence of the host plants in the Member States</td><td>Widely distributed.</td></tr>
<tr><td>Known infested areas</td><td><table>
<tr><th>Haplotypes</th><th>Distribution</th></tr>
<tr><td>A</td><td>USA, Mexico, Central America, New Zealand (Swisher Grimm & Garcynski, 2019; EPPO, 2017), Canada (Henrickson et al., 2019)</td></tr>
<tr><td>B</td><td>Mexico, Southern USA (EPPO, 2017)</td></tr>
<tr><td>C</td><td>Austria, Estonia, Finland, Norway, Sweden, Great Britain, Germany (Haapalainen et al., 2018a)</td></tr>
<tr><td>D</td><td>Spain, France, Morocco, Greece, Italy, Belgium, Tunisia, Israel</td></tr>
<tr><td>E</td><td>Spain, France, Morocco, Italy, Portugal, Tunisia</td></tr>
<tr><td>F</td><td>Oregon (USA) (Swisher Grimm & Garcynski, 2019)</td></tr>
<tr><td>U</td><td>Finland (Haapalainen et al., 2018b)</td></tr>
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<tr><td>Pathways</td><td><u>Haplotype A, B, F</u>:

Introduction of infected Bactericera cockerelli (all stages, including eggs);

Fruits of Solanaceae (tomatoes, Capsicum spp., eggplant, tamarillo, physalis); green plant parts favour the introduction of B. cockerelli;

Solanaceae for planting (other than seeds) from third countries where the vector and the pest are present;

The import of seed potatoes as well as of plants for planting of Solanaceae including potatoes and tomatoes (except seeds)</td></tr>
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	from the currently known infection areas into the EU is prohibited. <ins>Haplotype C, D, E:</ins> In contrast to a study of BERTOLINI et al. (2014), in a number of additional trials, non-infected plants could be cultivated from infected Apiaceae seeds. A transmission of Lso via seeds of Apiaceae is unlikely and does not occur at all or only rarely (LOISEAU et al., 2017). Possible pathways are plants for planting (other than seeds), infected vectors (*Trioza apicalis, Bactericera trigonica*) and green plant parts (carrot leaves, celery with leaves).
Natural spread	Vectors (see above)
Establishment and distribution to be expected in Germany	In case of the introduction of the infected vector the distribution and rapid establishment of Lso**A**, Lso**B** and Lso**F** is expected.
Establishment and distribution to be expected in the Member States	In case of the introduction of the infected vector the distribution and rapid establishment of Lso**A**, Lso**B** and Lso**F** is expected.
Known damage in infected areas	Starch that is stored in potato tubers is transformed by the bacterium to soluble sugar. It is not possible to use infected potato tubers for processing and they are not suitable for other commercialisation. Infected tubers produce weak seedlings. Thus, they are not usable as seed potatoes. Until 2004, the disease was sporadically of economic importance on potatoes in North America, but has since caused millions of dollars in damage to American potato cultivation.. Complete fields became unusable for the cultivation of potatoes. In 2008, Lso was detected in New Zealand and within only a few years, led to disastrous losses in the potato cultivation (MUNYANEZA, 2015). For a long period, LsoC, LsoD and LsoE were overlooked in Europe. Examinations of old seed stocks of commercial and wild Apiaceae showed that a large amount of seeds carried the bacterium already in the 1970s. Nevertheless, the bacterium was detected for the first time in Finland in 2010. Presumably, the observed damage events on Apiaceae caused by the bacterium mainly are primarily due to more favourable conditions for the respective vector (MONGER & JEFFRIES, 2018).

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Limitation of the endangered area in Germany	Plants of the nightshade family: Vegetable cultivation companies in Baden-Württemberg and North Rhine-Westphalia (south-west part), outdoors crops (potatoes) and greenhouse resp. tunnel crops (tomatoes, peppers). Outdoor crops (potatoes) in Saxony and in southern Saxony-Anhalt. Parts of lower and middle Frankonia (Würzburg, Schweinfurt, Nürnberg). Rhine valley (Karlsruhe to Worms and the southern part of the region Pfalz). Carrots: Carrot cultivation areas: the humid lowland in Schleswig-Holstein, Lower Saxony and North Rhine Westphalia. There is also a risk for the Eastern part of Rhineland Palatinate.
Damage to be expected in endangered area in Germany	Lso may cause severe infections or even epidemics on potatoes and tomatoes, with subsequent economic losses, outdoors and in greenhouses. Damage mainly is a quality loss of the fruits up to loss of marketability . Symptom development and crop loss depend on the number of psyllids.
Damage to be expected in endangered area in the Member States	The estimation of damage expected for Germany can be applied for other Member States that cultivate host plants (see above).
Control and measures	The prevention of the introduction of the vector and of infected plants/plant parts is the most effective measure. Currently, the import into the EU of Solanaceae from areas infected with Lso**A**, Lso**B** and Lso**F** is prohibited so that this risk can be estimated as low. Fruits intended for the processing industry are cooked, heated or treated otherwise so that neither the pest nor its vector can survive. The control of the vectors that normally are located beneath the leaves is difficult (monitoring with yellow sticky traps and massive use of specific insecticides for different stages). The psyllids should be controlled regularly during the migration period. However, though the insecticides may largely protect against a colonization by the psyllids, already a few psyllids may infect the host plant with Lso. No effective means against the bacterium are available.
Detection and diagnosis	The occurrence of the typical „Zebra chip“-symptom can mainly be observed after the frying of the potatoes. The detection of Lso as well as the identification of haplotypes is

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	done by means of a molecular examination via real-time PCR resp. PCR (LI et al., 2009; TERESANI et al., 2014; RAVINDRAN et al., 2011; MUNYANEZA et al., 2009; JAGOUEIX et al., 1996). Several samples with symptomatic plant parts should be taken because the bacterium may be heterogeneously distributed within the plant (FAO, 2017). In case that the bacteria concentration within the plant material is low, the identification of haplotypes might not always be possible. *Bactericera cockerelli* can be identified morphologically. Identification keys are available (OSSIANNILSSON, 1992; CARNEGIE et al., 2017). A diagnostic protocol for Lso is available (FAO, 2017). Currently, a new diagnostic protocol for the detection of Lso is prepared by EPPO. EFSA prepared a "Survey card" that informs on the bacterium and the vector including procedures for detection and diagnosis (EFSA, 2019).
Remarks	
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Fig.1 Symptoms on Solanaceae caused by *Candidatus* Liberibacter solanacearum: a) on a potato plant; b) on potato tubers (left raw, right fried); c) on a pepper plant; d) on a tobacco plant and e) on a tomato plant (Source: Joseph Munyaneza, USDA)