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# Prevalence of '*Candidatus* Liberibacter solanacearum' Haplotypes in Potato Tubers and Psyllid Vectors in Idaho From 2012 to 2018

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## Abstract

'*Candidatus* Liberibacter solanacearum' (Lso) is an uncultured, phloem-associated bacterium causing a severe tuber disease in potato called zebra chip (ZC). Seven haplotypes of Lso have been described in different hosts, with haplotypes A and B found associated with infections in potato and tomato. In the field, Lso is transmitted by the potato psyllid (*Bactericera cockerelli*), and between 2011 and 2015, a significant change in Lso haplotype prevalence was previously reported in Idaho: from exclusively A haplotype found in tested psyllids in 2012 to mainly B haplotype found in collected psyllids in 2015. However, prevalence of Lso haplotypes in Idaho was not analyzed in potato tubers exhibiting symptoms of ZC. To fill in this knowledge gap, prevalence of Lso haplotypes was investigated in potato tubers harvested in southern Idaho between 2012 and 2018, and it was found to change from exclusively A haplotype in the 2012 season to an almost equal A and B haplotype distribution during the 2016 season. During the same period, haplotype distribution of Lso in psyllid vectors collected using yellow sticky traps also changed, but in psyllids, the shift from A haplotype of Lso to B haplotype was complete, with no A haplotype detected in 2016 to 2018. The changes in the haplotype prevalence of the Lso circulating in potato fields in southern Idaho may be, among other factors, responsible for a decrease in the ZC incidence in Idaho potato fields between an outbreak of the disease in 2012 and a very low level of ZC afterward.

*Keywords*: disease development and spread, epidemiology, prokaryotes, vegetables

Zebra chip (ZC) disease is a bacterial infection of potato plants that triggers a variety of symptoms ranging from scorching and leaf rolling to stunting and ultimately, death (Munyaneza 2012, 2015). In addition to severe tuber yield losses in infested fields, ZC affects tuber quality, because infected tubers develop discolored necrotic lesions around or within the vascular system in the flesh. These lesions are dramatically aggravated by the frying processes, turning into dark streaks, altering flavor quality, and rendering the affected tubers unmarketable for the fried potato-derived product industry (Liefting et al. 2009; Munyaneza et al. 2007; Wen et al. 2009).

First detected in Mexico in 1994, ZC disease spread over Central America and northward into the United States, where it was first recorded in Texas fields in 2000 (Secor and Rivera-Varas 2004); it was later detected in the Pacific Northwest area in 2011. It resulted in an outbreak in potato fields of the Columbia Basin in Oregon and Washington, and the same year, tubers infected with '*Candidatus* Liberibacter solanacearum' (Lso) were also reported in Idaho (Crosslin et al. 2012a, b; Rondon 2012). ZC has also been found in New Zealand, where it had major economic impacts on potato production (Liefting et al. 2008, 2009; Pitman et al. 2011). Consequently, during the past 10 years, ZC has been established as a major threat to the potato tuber industry in the United States, impacting production and triggering efforts to control the disease.

ZC disease has been associated with Lso, an uncultured, phloem-restricted Alphaproteobacteria (Hansen et al. 2008; Liefting et al. 2009; Secor et al. 2009). Lso has been found to naturally infect a range of cultivated solanaceous species, like *Solanum lycopersicon* (tomato), *Capsicum annuum* (pepper), and *Solanum melongena* (eggplant), as well as perennial solanaceaous weeds, including *Solanum dulcamara* (bittersweet nightshade) and *Lycium barbarum* (wolfberry) (Munyaneza et al. 2009a, 2013, 2014; Thinakaran et al. 2017; Wen et al. 2009). The bacterium Lso is present on different crops in Europe and North Africa, where it is responsible for a range of vegetative disorders in apiaceous species, mainly *Daucus carota* (carrots) and *Apium graveolens* (celery) (Alfaro-Fernández et al. 2017; Hajri et al. 2017; Holeva et al. 2017; Nelson et al. 2013; Tahzima et al. 2017; Teresani et al. 2014). So far, seven haplotypes of Lso have been described, designated A, B, C, D, E, F, and U (Haapalainen et al. 2018; Liefting et al. 2008; Nelson et al. 2011, 2013; Teresani et al. 2014; Wen et al. 2009). Haplotypes A (LsoA) and B (LsoB) are associated with ZC and similar diseases in solanaceous plants, including potato, in the Americas and New Zealand, whereas haplotypes C, D, E, and U are found in Europe and the Mediterranean regions, infecting apiaceous species (LsoC, LsoD, and LsoE) or Urticaceae (LsoU)-related species. Haplotype F was described in a single potato tuber grown in the Pacific Northwest, but no other data are available on its prevalence and distribution (Swisher Grimm and Garczynski 2018). It was shown that LsoB caused more severe symptoms in Washington- and Texas-grown potato plants (Harrison et al. 2019; Swisher Grimm et al. 2018). LsoA and LsoB exhibit different geographical distributions, with both haplotypes present in North America and Central America, whereas only LsoA has been found in New Zealand so far.

The wide geographical and interspecies dispersal of Lso in Solanaceae hosts is facilitated through transmission from infected plants to healthy ones by its vector, the potato/tomato psyllid *Bactericera cockerelli* Šulc (*Hemiptera: Triozidae*). Endemic to Central America and the western United States, the psyllid can complete its lifecycle

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on a wide range of solanaceous plants and carries Lso at all life stages (Munyaneza 2012). Psyllid population is thus an important factor in ZC disease outbreaks, and most of the efforts to control ZC have been focused on managing the insect in potato fields during the growing season, incurring large costs of insecticide applications for growers. To aid in managing ZC in potato fields, monitoring programs have been set up in potato-growing areas over the United States, including southern Idaho (Wenninger et al. 2017), which represents around 31% of the potato production in the United States (U.S. Department of Agriculture-Economic Research Service 2016). The program in southern Idaho, set up since the 2012 season, has been very useful to track psyllid population and their Lso infection status in potato fields. Monitoring has also allowed for tracking of psyllid population composition, because four haplotypes of the psyllids have been described and named based on their geographical associations in the United States: Western, Northwestern, Central, and Southwestern (Swisher et al. 2012, 2013a, 2014). Although all four can be found in Idaho, the Western haplotype is predominant in potato fields. No significant differences in Lso transmission have been found among haplotypes so far (Mustafa et al. 2015). Since the start of the monitoring in 2012, Lso was found persisting in psyllids found in the fields at a low rate (<3% of the monitored psyllids), with the exception of the 2012 season, where it reached 26% (Dahan et al. 2017; Wenninger et al. 2017).

To complement the data already available for Lso presence in Idaho potato fields, the purpose of this study was to assess the presence of the bacterium in tubers grown in southern Idaho that were exhibiting symptoms of ZC disease along with haplotyping positive samples to characterize the population of Lso present in plants. We also report here the results of Lso testing on psyllids from the 2016 to 2018 seasons, with tuber testing covering the period from 2012 to 2018.

## Materials and Methods

**Psyllid monitoring.** Monitoring of psyllids in the fields was performed as described in Wenninger et al. (2017). Briefly, yellow sticky cards were deployed in selected potato fields located in the main potato-growing areas of southern Idaho and replaced every week from planting to vine killing during 2016, 2017, and 2018. Caught psyllids were recovered individually from each sticky card and stored at −20°C in 70% (vol/vol) ethanol for additional analysis.

**Tuber sampling.** Overall, 272 tubers were collected or submitted from industry personnel and tested based on visual symptoms spotted after harvest and during storage. The symptoms included typical signs of ZC, such as brown discolorations of the fresh tuber flesh within the vascular system, and less clear signs, such as various discolorations of the tuber flesh. All tubers from 2012 to 2018 were grown in potato fields and/or stored in southern Idaho and selected for suspected ZC symptoms. In 2016 and 2017, some of the tested tubers (74 and 1, respectively) were submitted directly for Lso testing from a potato processing and storage facility in southern Idaho (provided by Justin Ruhl, J.R. Simplot, Nampa, ID).

**DNA extraction.** For psyllids, the procedure followed was the one described in the work by Dahan et al. (2017) with no modification. For tubers collected in 2012, DNA extraction performed on skin pieces followed the protocol of Dellaporta et al. (1983). For tubers grown during the 2013 to 2018 seasons, stolon insertion site was collected on the tubers to be tested whenever possible along with one "eye" and/or skin pieces when possible, and they were extracted using a hexadecyltrimethylammoniumbromide (CTAB) extraction method as follows. Samples were frozen in liquid nitrogen and then ground using precooled mortars and pestles. Powder was recovered and transferred to a 1.5-ml microtube, and 600 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 0.2% 2-mercaptoethanol, 20 mM EDTA, and 100 mM Tris-HCl, pH 8.0) was added to resuspend ground tissue. Suspensions were thoroughly vortexed and incubated for 15 min at 65°C with shaking. Three hundred microliters of a 24:1 (vol/vol) chloroform:isoamyl alcohol mixture was dispensed to each tube, and the samples were vortexed for a few seconds to ensure complete mixing of aqueous and organic phases. They were then centrifuged for 10 min at 16,000 × *g* at 4°C, and the aqueous phase was transferred to a new tube containing 50 µl of 3 M sodium acetate, pH 5.6. After gentle mixing by inversion, tubes were incubated 1 h to overnight at −20°C. DNA was pelleted by centrifugation at 25,000 × *g* for 15 min at 4°C. Pellets were washed with 1 ml 70% ethanol, centrifuged at 12,000 × *g* for 5 min at 4°C, and air dried after decanting the supernatant. DNA pellets were resuspended in 50 µl of dideionized $H_2O$ facilitated by heating at 55°C for 10 min.

**Lso testing and haplotyping.** Lso testing was carried out using the primer pairs OA2/OI2c and OMB_F/OMB_R (Crosslin et al. 2011) and the CAPS_F/CAPS_R primer pairs (Dahan et al. 2017). For psyllid testing, PCR were performed as described in Dahan et al. (2017). For tuber testing, resuspended DNA was diluted 1:10 in water before use, and 1 µl of the diluted material was used in PCR. The nested PCR using the pair CAPS_F/CAPS_R2 was performed on the PCR product from the CAPS_F/CAPS_R amplification diluted at 1:100, which was carried out as described in Dahan et al. (2017). As a control of amplification for tuber samples, the internal transcribed spacer region of nuclear ribosomal DNA of *Solanum tuberosum* was amplified (primers ITSLeu1, 5′-GTC CAC TGA ACC TTA TCA TTT AG-3′ and ITS4, 5′-TCC TCC GCT TAT TGA TAT GC-3′) (Bohs and Olmstead 2001) for the 2016 and subsequent years samples. Lso haplotyping was carried out on positive samples (tuber or psyllid) based on the results of the CAPS_F/CAPS_R or CAPS_F/CAPS_R2 nested PCR and using the cleaved amplified polymorphic sequence (CAPS) technique as described in Dahan et al. (2017). Briefly, 5 or 10 µl of PCR products were digested by the Bsl1 restriction enzyme, and results were analyzed on an ethidium bromide-stained 2% agarose gel. Lso-positive psyllids and some of the tuber samples proved to be difficult to reamplify after the first round of testing, precluding their haplotyping.

## Results

**Psyllid population in south Idaho potato fields for the 2016, 2017, and 2018 seasons.** The three covered seasons were contrasting in terms of numbers of psyllids collected (Table 1). During the 2016 season, 6,519 psyllids were collected on sticky cards and tested for Lso, 91 of which were found to be positive for the bacterium (Table 1). In the two subsequent years, the number of collected psyllids dropped to 883 in 2017 and 490 in 2018, with 5 and 3 Lso-positive psyllids caught, respectively. The Lso incidence was low

**Table 1.** Numbers of total and 'Liberibacter solanacearum' (Lso)-positive psyllids collected on sticky cards in Idaho potato fields during each month of the 2016 to 2018 growing seasons

| | Collecting month[z] | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | May | | June | | July | | August | | September | | October | | Total | | Lso haplotypes | |
| Year | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *c* | *d* |
| 2016 | 3 | 2 | 177 | – | 794 | 85 | 3,652 | 4 | 1,836 | – | 54 | – | 6,519 | 91 | 36 | B |
| 2017 | 8 | – | 24 | 1 | 188 | 4 | 163 | – | 445 | – | 54 | – | 882 | 5 | 4 | B |
| 2018 | 1 | – | 43 | 1 | 133 | 1 | 146 | – | 164 | 1 | 3 | – | 490 | 3 | 3 | B |

[z] No collection date could be assigned to three Lso-negative psyllids. *a*, number of collected psyllids; *b*, number of Lso-positive psyllids; *c*, number of Lso-positive samples haplotyped; *d*, Lso haplotype found. Dashes indicate no psyllids.

overall, with 1.4, 0.6, and 0.6% of the psyllids testing positive in 2016, 2017, and 2018, respectively. Interestingly, in 2016, most of the positive psyllids were found in July. Of the 794 psyllids collected and tested during that month in 2016, 85 were positive, which represents 10.7%. It is noteworthy that, although 3,652 psyllids were retrieved in August 2016, only 4 psyllids were found carrying the pathogen (0.1%). Likewise, in 2017, four psyllids of the five carrying Lso were also caught in July. In 2018, the three Lso-positive psyllids were distributed between June, July, and September catches (Table 1). In terms of the Lso haplotype, over the three considered seasons, all of the individual psyllids testing positive for the bacterium that could be haplotyped were found to carry LsoB (Table 1).

Geographically, in 2016 and 2018, most of the psyllids were collected in the Treasure Valley of southwestern Idaho: 5,712 of 6,519 and 373 of 490, respectively. In each case, Lso-positive psyllids were mostly collected in the Treasure Valley as well (Table 2) (Wenninger et al. 2017). In 2017, an opposite trend was observed, with 64% of the psyllids collected in the Magic Valley of southcentral Idaho as well as three of the five Lso-positive psyllids (Table 2) (Wenninger et al. 2017).

**Tuber testing between 2012 and 2018.** At the end of the 2012 potato-growing season and during storage, 176 tubers from potato plants grown in Idaho were tested for the presence of Lso (Table 3). In total, 48 tubers were found positive: 41 during the first round of screening in 2012 using primer pairs OA2/OI2C and OMBF/OMBR and 7 more detected as Lso positive during the retroactive screening using the nested PCR with the CAPS primers developed for haplotyping and conducted in 2017. Only 16 samples detected as Lso positive during the first screening were also found positive in the second one, meaning that 25 of the samples found in the first round of Lso testing could not be haplotyped. The 23 Lso-positive samples that were haplotyped were all found to be LsoA.

Over the 2013 to 2015 seasons, eight symptomatic tubers collected in southern Idaho were tested for Lso (Table 3). Very few tubers exhibiting visible ZC symptoms were identified and tested during this period, but a few tubers each year were found positive for Lso. Haplotyping of Lso in positive tuber samples from 2013 and 2014 seasons revealed the presence of LsoB only, whereas LsoA was found again in two tubers of the three that tested positive in 2015.

In 2016, 79 tubers from potato plants grown in Idaho were tested for Lso infection based on presence of ZC-like symptoms (Table 3). Among them, 44 were found to be positive for the pathogen. Both haplotypes of the bacteria, LsoA and LsoB, could be detected: 22 samples carried LsoA, whereas 18 samples were infected with LsoB. Two samples had a mixed infection, with both haplotypes LsoA and LsoB present in the same tuber. In 2017, three symptomatic tubers were tested, and the two that turned out positive for the bacterium were found to carry LsoB, consistent with LsoB found in psyllids this same season. In 2018, five Lso-positive tubers of the six tested were carrying LsoA, which was different from LsoB found in psyllids.

## Discussion

After the ZC outbreak in the states of Washington and Oregon and its first report in Idaho in 2011 (Crosslin et al. 2012a, b; Rondon 2012), efforts were made to manage and study the psyllid populations colonizing the potato fields of the Pacific Northwest, Idaho included (Munyaneza et al. 2009b; Rondon 2012; Wenninger et al. 2014). Data were collected about psyllids and their Lso status as well as respective haplotypes of the pathogen and its vector from 2012 to 2015 in Idaho (Dahan et al. 2017; Wenninger et al. 2017), and this monitoring was also performed for the 2016 to 2018 seasons as reported here. In addition to this testing, we gathered data on Lso presence in symptomatic potato tubers collected after harvest in Idaho from 2012 to 2018.

During the 2016 potato-growing season, a high number of psyllids were collected on sticky cards in Idaho fields, mainly in August and September, totaling >6,500 insects (Table 1). This number was exceptionally high compared with the numbers of psyllids collected from 2013 to 2015 and also between 2017 and 2018 using a similar collection setup, which ranged from 170 to 1,603 individuals collected (Wenninger et al. 2017) (this study). In 2012, however, the population of psyllids captured was notably higher than in the subsequent years, taking into account the much restricted setup used with a smaller number of fields covered (Wenninger et al. 2017). Given this discrepancy, it is expected that the extent of infestation in 2012 was not fully assessed and that it was probably closer to the 2016 season in terms of psyllid numbers. Interestingly, 2012 also featured the highest percentage of Lso-carrying psyllids detected in the Idaho monitoring program (26%). The percentage of positive psyllids in 2016 was much lower and similar to the levels recorded from 2013 to 2015 and from 2017 to 2018, reaching only 1.4% (Dahan et al. 2017) (this study). However, looking at per-month distribution in 2016, around 10% of the psyllids collected in July carried the pathogen (Table 1). This number may be viewed as high enough to qualify as an outbreak of the psyllid population and concomitantly, ZC disease, similar to 2012 (Wenninger et al. 2014). As can be seen here, this assertion is supported by the higher than usual number of symptomatic tubers tested and found positive that same year (Table 3). The timing of the peak in Lso-positive psyllids in July could be linked to that higher number of symptomatic tubers at the end of the season. Indeed, it has been shown that timing of infection during the growing season determines severity of tuber symptoms at harvest, with 80 to 95% of tubers showing symptoms when the plant was infected ≥4 weeks before harvest (Rashed et al. 2014). It is also interesting to note that most of the Lso-positive psyllids in 2017 and 2018 were collected at the end of June and in July as well (Table 1).

Origins of Lso-carrying psyllids found in potato fields in Idaho are still undetermined. Although the first appearance of Lso in the Pacific Northwest could be owing to introduction of psyllids coming from infected plants from southern states, the continued presence of Lso in potato in Idaho at a very low rate since then is puzzling, because no local source of the bacterium could be identified to the best of our knowledge, although infected psyllids might overwinter on alternative hosts in the Pacific Northwest, including the potato-growing regions of Idaho (Swisher et al. 2013b; Thinakaran et al. 2017;

**Table 2.** Geographical origin of psyllids collected from 2016 to 2018

| Area | 2016[w] | 2017 | 2018 |
|---|---|---|---|
| Magic Valley[x] | 781 (13) | 565 (3) | 114 |
| Treasure Valley[y] | 5,712 (78) | 266 (1) | 373 (3) |
| Others[z] | 26 | 52 (1) | 3 |

[w] Numbers of collected psyllids are given for each year and geographical area considered, and numbers of 'Liberibacter solanacearum'-positive psyllids are indicated in parentheses.
[x] Magic Valley covers the following counties in southcentral Idaho: Blaine, Cassia, Gooding, Jerome, Minidoka, and Twin Falls.
[y] Treasure Valley covers the following counties in southwest Idaho: Ada, Canyon, Elmore, Gem, Owyhee, and Payette.
[z] Others includes the counties of Bannock, Bingham, Oneida, and Power in southeast Idaho.

**Table 3.** Numbers of total and 'Liberibacter solanacearum' (Lso)-positive tubers tested grown from the 2012 through the 2018 seasons and Lso haplotyping data

| Year | No. of tested symptomatic tubers | No. of Lso-positive tubers (haplotyped) | Haplotyping | | |
|---|---|---|---|---|---|
| | | | A | B | Mixed[z] |
| 2012 | 176 | 48 (23) | 23 | – | – |
| 2013 | 1 | 1 (1) | – | 1 | – |
| 2014 | 3 | 1 (1) | – | 1 | – |
| 2015 | 4 | 3 (3) | 2 | 1 | – |
| 2016 | 79 | 44 (44) | 22 | 18 | 4 |
| 2017 | 3 | 2 (2) | – | 2 | – |
| 2018 | 6 | 5 (5) | 5 | – | – |

[z] This includes tubers infected with both haplotypes (two samples in 2016) and different Lso-positive tubers from the same sample harboring different haplotypes (two samples in 2016). Dashes indicate no sample.

Wenninger et al. 2019), providing a limited local source. However, such a mechanism has not been demonstrated for Lso so far, and furthermore, haplotyping of Lso present in the 2016 Lso-positive insects gave unexpected results, with all samples that could be haplotyped being LsoB, as well as for the two subsequent seasons (Table 1). This contrasted with the results from the previous years where, except for in 2012 (all haplotyped samples were LsoA), both haplotypes could be found (Dahan et al. 2017). Interestingly, the proportion of LsoB in haplotyped psyllid samples kept increasing since the first appearance of LsoB in Idaho field during the 2013 season. No data are available that could help understand that result, because origins of positive Lso psyllids in the area are still undetermined.

Based on the testing of symptomatic tubers, we reported here the presence of the bacterium Lso, responsible for the ZC disease, in southern Idaho potato fields during the 2012 and 2018 seasons. Although no outbreak of the ZC disease was reported in Idaho for the 2011 potato season as opposed to Washington and Oregon states, the pathogenic bacterium could be detected in a total of 83 potato tubers exhibiting typical symptoms of ZC found in an Idaho packing facility and during storage (Crosslin et al. 2012a; Wen et al. 2013). In 2012, Lso infestation was very obvious in potato-growing areas along the Snake River and was confirmed by the psyllid monitoring set up that year (Dahan et al. 2017). Symptomatic plants were observed in potato fields, and although most of the fields were clear of ZC disease, in some areas the infection level reached up to 15 to 20% (Wenninger et al. 2014, 2017). Accordingly, 27% of the 176 tested symptomatic tubers were positive for Lso (Table 3). Numbers of positive psyllids were also exceptionally high overall, with >26% of the tested psyllids found carrying the bacterium (Dahan et al. 2017). Given the extremely high numbers of psyllids caught in 2016, this season can also be tentatively labeled an outbreak year for both the number of psyllids (Table 1) and the number of ZC-positive tubers collected during this season (Table 3).

Lso haplotyping of the positive tubers showed contrasting results for the two outbreak years 2012 and 2016, and an unexpected discrepancy was observed between Lso haplotype prevalence found in psyllid and potato samples. Specifically, all haplotyped tubers were infected with LsoA in 2012 (Table 3). This was fully consistent with the Lso haplotype that could be identified in psyllids collected on sticky cards in Idaho potato fields that same year, in 2012, which was only LsoA (Dahan et al. 2017), and with what was already reported for tubers from 2011 and 2012 in Idaho (Wen et al. 2013). Starting in 2013, LsoB could be found in Lso-positive tubers, and it was the only haplotype detected in potato tubers in the years 2013, 2014, and 2017 (Table 3), whereas psyllids harbored both LsoA and LsoB haplotypes in 2013 through 2015 (Dahan et al. 2017). Starting in 2016 and through the 2018 season, LsoB became the only haplotype of the bacterium found in psyllids caught in southern Idaho (Dahan et al. 2017) (this work). This contrasted with the presence of LsoA found in tuber samples in 2015, 2016, and 2018 (Table 3). In 2015, 2017, and 2018, the availability of tubers for testing was low, and the discrepancy in prevalence of LsoA and LsoB Lso in tubers and psyllids could be explained by these low tuber numbers. However, in 2016, the numbers of Lso-positive psyllids and tubers were higher, and a trend became visible, with LsoB being the only haplotype found in psyllids and an almost equal mixture of LsoA and LsoB found in tubers (Tables 1 and 3).

Clearly, there were at least two separate inoculum sources for the Lso found in potato tubers in 2016, resulting in either LsoA- or LsoB-infected potato plants. Given the strict dependence of the bacterium on psyllids for transmission, several explanations may be discussed. First, it is possible that LsoA psyllids were present in the fields in 2016 but were not caught or were not haplotyped, because a preponderance of positive psyllids could not be haplotyped owing to technical reasons (~61%) (Table 3). This explanation assumes that the psyllid monitoring network deployed in southern Idaho (Wenninger et al. 2017) somehow excluded areas inhabited by LsoA-carrying psyllids, especially after 2015. Second, so far, no correlation was described between the Lso haplotypes present in psyllids and the haplotypes found in the plants and/or tubers. It is generally thought that ZC-infected plants acquire the disease exclusively through transmission by the vector, but another possibility could be that the LsoA-harboring tubers are progeny of infected seed tubers. Although having a significantly reduced sprouting rate and growth, ZC-infected seed tubers could be grown and generate progeny tubers (Henne et al. 2010). Third, another explanation could be that, although the tested tubers were for the most part selected during preparation for storage, they might also come from growing areas outside Idaho, where LsoA may be more abundant than in Idaho. Two tubers carrying both LsoA and LsoB were also found in 2016 (Table 3). Albeit exceptional, this is not unusual and has already been described in Texas samples (Wen et al. 2013). Psyllids also can carry both haplotypes at the same time, but no data are available on the efficiency of transmission of one haplotype over the other, although it has been shown that LsoB reduced nymphal survival rate (Yao et al. 2016).

The data reported here complement our knowledge of psyllid and Lso infestation in the potato fields of Idaho. We could demonstrate that, although Lso was present at a basal level in psyllids during 2013 to 2018, infected tubers could still be found during the same period, indicating a constant disease pressure in the fields that can result in more or less severe outbreaks on occasion as reported for 2012 and 2016. The lack of correlations between Lso haplotypes detected in tubers and psyllids over the same seasons, although surprising, raises concerns about transmission and infectivity of one haplotype over the other and calls for more studies on the vector and the bacterium to better understand the pathogenicity of Lso and epidemiology of the ZC disease.

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