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# First record of the tomato potato psyllid *Bactericera cockerelli* from South America

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## Abstract

The tomato potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera Psylloidea), is a major pest on potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) in North and Central America and, introduced, in New Zealand; it has recently been reported from Western Australia and the Norfolk Island. The TPP causes the plant disorder called "psyllid yellows" in addition to vector the devastating plant pathogen *Candidatus* Liberibacter solanacearum (LSO), which is the causal agent of the zebra chip (ZC) disease on potato. ZC is detrimental to a number of solanaceous crops in North and Central America as well as in New Zealand. Here we report for the first time the presence of *B. cockerelli* feeding on potatoes in South America (Ecuador). Molecular tests on a mitochondrial gene confirmed the population from Ecuador as central haplotype. This suggests that the psyllid populations found in Ecuador might come from North or Central America, probably along with agricultural products or other plant material. All Ecuadorian TPPs in our study were tested for LSO and they are free of the pathogen. Nonetheless, there is a risk of introduction of psyllid yellows and ZC into South America. Comprehensive field surveys of solanaceous plants and fine-scaled genomic studies should be considered to locate the source of the psyllid, which could help to prevent the introduction of the pathogen into South America.

**Key words:** tomato potato psyllid, haplotype, psyllid yellows, zebra chip, *Candidatus* Liberibacter solanacearum, Ecuador.

## Introduction

Rapid growth of global trade of agricultural products greatly benefits the human population around the world but also promotes, inevitably, the introduction and spread of pests and diseases. Examples of recent introductions are the brown marmorated stink bug, *Halyomorpha halys* (Stal), in North America and *Drosophila suzukii* (Matsumura) in southern Argentina (Rice *et al.*, 2014; Deprá *et al.*, 2014; Valentin *et al.*, 2017). For mitigating the risk of introduced pests and diseases, the very first step is to identify the pathogen/pest species and to detect the source of the introduction, which is an interest shared by law enforcement agencies, the scientific community and growers. This is particularly important in cases where insects act as vectors of plant pathogens that harm agricultural crops.

The tomato potato psyllid, *Bactericera cockerelli* (Sulc) (TPP), is a major pest on potato (*Solanum tuberosum*) and tomato (*Solanum lycorpersicum*) in most regions in North America where these crops are being produced (Munyaneza, 2015; Prager and Trumble, 2018; Vereijssen *et al.*, 2018). The species was first described by Šulc (1909) from specimens collected in Colorado, USA. This plant louse sucks phloem sap, causing yellowing and chlorosis on the foliage and, thereby, reducing potato tuber size. This disease is known as "psyllid yellows" (Pletsch, 1947; Wallis, 1955). Even more devastating is the capability of the TPP to vector a bacterial plant pathogen, *Candidatus* Liberibacter solanacearum (LSO), the causal agent of zebra chip (ZC) disease. In North and Central America and New Zealand, LSO is detrimental to solanaceous crops such as potatoes and tomatoes, in addition to infect Convolvulaceae such as sweet potato (*Ipomoea batatas*). LSO infected plants display curling and discolouration of the foliage, and proliferation of buds and branches. Infected potato tubers exhibit dark and light-yellow stripes when sliced and deep fried, thus the name "zebra chip", which largely reduces their market value (Munyaneza *et al.*, 2007; Crosslin *et al.*, 2010; Workneh *et al.*, 2018; Vereijssen *et al.*, 2018).

A series of devastating ZC outbreaks were documented since 1994, starting from northern Mexico moving northward in the western United States along corridors in the Rocky Mountains (Secor and Rivera-Varas, 2004; Crosslin *et al.*, 2010; Munyaneza, 2015). In 2017, several potato psyllids, collected in potato fields in southern Alberta, Canada, were tested to carry LSO (Johnson *et al.*, 2017). In Central America, ZC was reported from Guatemala, Honduras (Secor and Rivera-Varas, 2004; Munyaneza *et al.*, 2007; Espinoza, 2010; Rehman *et al.*, 2010) and Nicaragua (Nicaragua-MAGFOR, 2012). Across the Pacific Ocean, LSO and TPP were first documented from New Zealand in 2006, where they were accidently introduced. This had a serious economic impact on the local potato and tomato industry (Liefting *et al.*, 2008; 2009; Vereijssen *et al.*, 2018). The same species of LSO but different haplotypes have been reported from Europe where they infect Apiaceae, including carrot, celery, parsley and parsnip, e.g. in Norway, Sweden, Germany, France and Spain (Munyaneza *et al.*, 2011; 2015, 2016; Alfaro-Fernández *et al.*, 2012; Hajri *et al.*, 2017). Recently, it was also reported from Greece, Israel, Morocco and Tunisia (Tahzima *et al.*, 2014; 2017; Ben Othmen *et al.*, 2018a; 2018b). In the Palaearctic region, LSO is vectored by two species of carrot psyllid, *Trioza apicalis* Foerster and *Bactericera trigonica* Hodkinson (Nissinen *et al.*, 2014; Teresani *et al.*, 2014; Munyazena *et al.*, 2016; Antolinez *et al.*,

2017a; 2017b). Conversely, the TPP, the LSO vector in North and Central America as well as in New Zealand, is currently absent from Europe (Munyaneza, 2010; Munyaneza *et al*., 2016; Ouvrard, 2018). There are no Apiacae confirmed as psyllid hosts in North America and New Zealand, and it seems unlikely for the TPP to transmit LSO to carrots (Munyazena *et al*., 2016). The psyllid fauna of the Nearctic is only incompletely known. *Trioza stugma* Tuthill from California, a close relative of *T. apicalis*, probably develops on Apiaceae (Burckhardt, 1986). It is noteworthy that, in Brazil, carrot has been recently confirmed as host of the neotropical *Russelliana solanicola* Tuthill, a polyphagous species (Kuhn *et al*., 2016).

Despite their co-presence in Central and North America, neither the TPP nor LSO have been reported from South America yet. In South America, species of following psyllid genera develop on Solanaceae: *Lanthanaphalara*, *Leuronota*, *Russelliana* and *Schedoneolithus* (Tuthill, 1959; Burckhardt, 1987; Burckhardt and Queiroz, 2012; Serbina and Burckhardt, 2017). Of particular interest is the potato pest *R. solanicola*, which originates from the Andes but has been introduced into eastern Argentina, Uruguay and southern Brazil (Burckhardt, 1987; Serbina *et al*., 2015; Syfert *et al*., 2017). The Ecuadorian highlands are part of the northern Andes, ranging from 1600 m above sea level (asl) at the valleys to 3400 m asl at the mountain slopes. The primary staple crop in this region is potato. As one of the countries where potatoes originate, Ecuador hosts approximately 500 native potato varieties as well as other cultivated Solanaceae, such as tamarillo (*Solanum betaceum*), naranjilla (*Solanum quitoense*) and uvilla (*Physalis peruviana*) (Spooner and Sytsma, 1992; Hijmans and Spooner, 2001; Van den Eynden *et al*., 2003; Carrillo-Perdomo *et al*., 2015).

Here we report, for the first time, the occurrence of the TPP in South America based on specimens collected in Ecuador. On the other hand, LSO could not be detected yet in the country (Castillo Carrillo *et al*., 2018). We discuss the distribution and possible scenarios of introduction of the TPP in Ecuador.

## Materials and methods

### Psyllid collections and morphological identification

Specimens of *B. cockerelli* were collected in two localities in Ecuador: province of Pichincha, Quito, Camino al Belen, -0.365500 -78.555583, 3050 m asl, September 2017, on *S. tuberosum* (leg. C. Castillo); and province of Pichincha, Checa, Alberto Osorio, -0.130611 -78.311500, 2570 m asl, September 2017, on *S. tuberosum* (leg. C. Castillo). The specimens are preserved in 90% ethanol. Voucher specimens are deposited in the collections of the Naturhistorisches Museum Basel, Switzerland.

The specimens were identified morphologically using colour and structural characters of the head, antenna, forewing and, in particular, male and female terminalia (see also Yen and Burckhardt, 2012).

### DNA extraction

DNA extraction was performed following the procedure described by Crosslin *et al*. (2013). For this, 75 µl of 100 mM Tris-HCl (pH 8.0), 5 mM sodium ethylenediaminetetraacetic acid (EDTA) and 0.5% (v/v) Tween 20 were added to a microcentrifuge tube containing a single TPP. Then, 2 µl of Proteinase K (20 mg/ml) (Life Technologies, Carlsbad, CA) were added to the mixture. Psyllids were macerated with a sterile pipette tip, and tubes were incubated in a thermo bath (FinePCR, Korea) for 15 minutes at 37 °C, then 10 minutes at 100 °C, and lastly held at 10 °C. After centrifugation for 10 seconds at 1,600 g, the samples were either used immediately in PCR tests or stored at −20 °C for later use.

### Molecular identification and LSO detection

PCR was performed following the procedure described by Crosslin *et al*. (2011). Primers used in the PCR were COIF3 and COIR3, which target 500 bp of the mitochondrial cytochrome oxidase subunit I gene (COI) of *B. cockerelli*. The programme for PCR was 94 °C for 2 minutes, then 35 cycles of 94 °C for 15 seconds, 52 °C for 60 seconds and 72 °C for 60 seconds, followed by a final incubation at 72 °C for 5 minutes, in a thermal cycler (Applied Systems, SimpliAmp, India). PCR products were visualized on 2% agarose gels. Samples that displayed the product of the predicted size (500 bp) were selected. In order to accurately locate variant sites of the COI gene of our samples and compare to the previous study (Swisher *et al*., 2012), we conducted Sanger sequencing of 10 psyllid samples, using the same primers as in the PCR amplification described above. Sequences were cleaned and aligned with known COI gene sequences using programme MUSCLE (Edgar, 2004), the psyllid COI-haplotype was determined by comparing psyllid sequences from our study to the NCBI database using the programme BLAST (Altschul *et al*., 1990). Presence of LSO pathogen was detected using conventional PCR with primers OA2/OI2c by following the protocol by Crosslin *et al*. (2011). The programme for the PCR was 94 °C for 2 minutes, then 40 cycles of 94 °C for 30 seconds, 62 °C for 30 seconds and 72 °C for 60 seconds, followed by a final extension at 72 °C for 5 minutes, in a thermal cycler (Applied Systems, SimpliAmp, India). DNA from psyllids that was infected with LSO was kindly provided by J. S. Munyaneza (USDA-ARS, Wapato, WA), and was used as positive control in the PCR procedure to assure the detection worked properly. PCR products were visualized on 2% agarose gels.

## Results

Here we report for the first time the occurrence of *B. cockerelli* in South America. In September 2017, the TPP, represented by all developmental stages was found on potato plants in two localities in the vicinity of Quito (Ecuador, province of Pichincha, figure 1). Specimens were identified using structural and colour characteristics. The head lacks genal processes; the forewing is broadest in apical fifth, has a long sinuate vein Rs, so

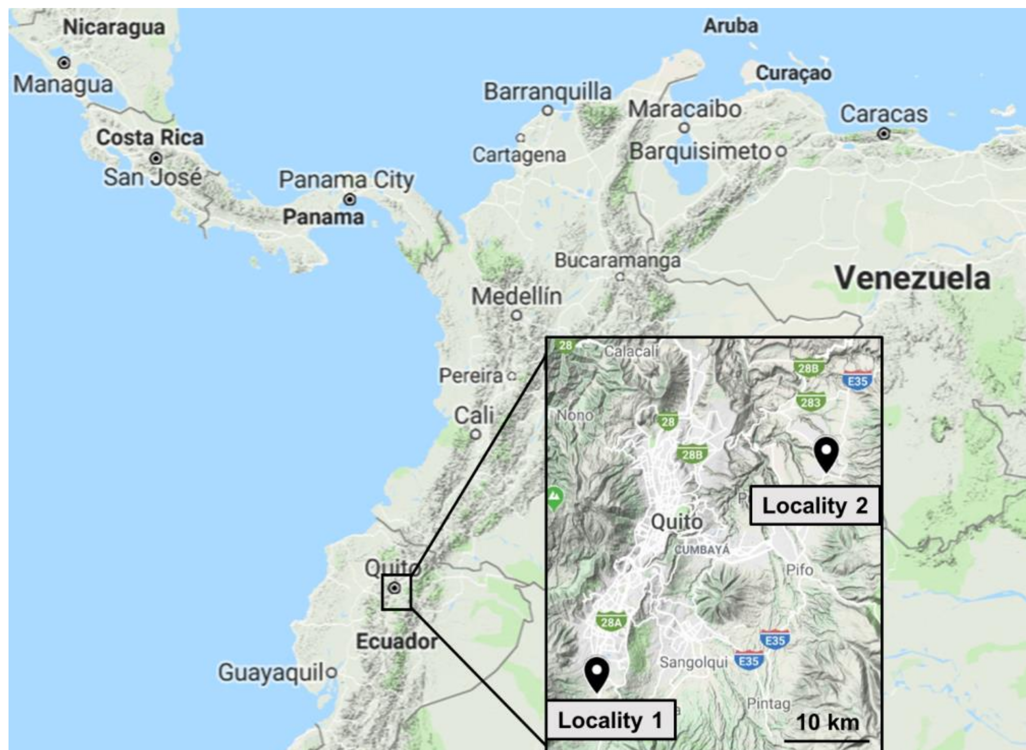


**Figure 1.** Map showing the localities at which *B. cockerelli* were found in Ecuador (map generated using Google Maps: https://maps.google.com/).

that the bifurcation of vein M lies near the line connecting apices of vein Rs and $Cu_{1a}$, and lacks surface spinules in apical half of wing; the male proctiger has triangular posterior lobes, the parameres are lamellar with a sclerotized, forward directed point apically; the female terminalia are short and cuneate. The body of mature adults is almost black with a whitish margin around the vertex, with a transverse whitish stripe in the middle, and yellowish basal two thirds of antennal segments 3-8; white lines are also present on the first and last abdominal segments, the latter bearing, the characteristic inverted 'V'-shaped white mark (Šulc, 1909; Pletsch, 1947; Wallis, 1955; Yen and Burckhardt, 2012).

**Table 1.** Sequences of cytochrome c oxidase subunit I of *B. cockerelli* from Ecuador.

| Sample | Length | Identity (%) to the top BLAST hit | Accession of the top BLAST hit |
|---|---|---|---|
| 1 | 460 | 100% | JQ708094 |
| 2 | 472 | 100% | JQ708094 |
| 3 | 471 | 100% | JQ708094 |
| 4* | 468 | 99% | JQ708094 |
| 5 | 473 | 100% | JQ708094 |
| 6# | 427 | 98% | JQ708094 |
| 7 | 473 | 100% | JQ708094 |
| 8* | 470 | 100% | JQ708094 |
| 9* | 475 | 99% | JQ708094 |
| 10 | 473 | 100% | JQ708094 |

# sample was removed from the analysis given poor sequence quality; *samples were shown in multiple sequence alignment in figure 2.

We sequenced 10 samples and characterized variant sites between our samples and known *B. cockerelli* COI gene sequences. The length of sequences of samples ranged from 427 bp to 473 bp. Among those, one sample yielded a low-quality sequence (sample 6) and was excluded from the alignment and further analysis. The rest of the nine sequences exhibited 99-100% identity to the central haplotype of *B. cockerelli* (GenBank accession JQ708094, table 1). Seven samples exhibit 100% identity to psyllids of the central haplotype (JQ708094, table 1 and figure 2). Sample 4 and sample 9 were 99% identical to JQ708094, where sample 4 had two deletion sites comparing to JQ708094 and sample 9 exhibited an ambiguous base "N". The sequence of sample 8 was deposited in NCBI GenBank with access No. MK054304. None of the samples tested were positive for presence of the LSO pathogen. The positive control sample exhibited expected 1168 bp band in the gel electrophoresis.

## Discussion

This is the first report of *B. cockerelli* from South America. The TPP is widely distributed in western North America, including Mexico, USA and Canada, and has been reported from Central America, e.g. Guatemala, Honduras and Nicaragua (Secor and Rivera-Varas, 2004; Munyaneza *et al*., 2007; Espinoza, 2010; Rehman *et al*., 2010; Nicaragua-MAGFOR, 2012; Johnson *et al*., 2017). The occurrence of the TPP in New Zealand is the result of an accidental introduction, possibly due to misidentification despite the strictly imple-

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Sample4     1 ---TCGGAATTCTAGGATTCATTGTTTGAGCACATCATATATTTACAGTAGGTATAGATGTTGATTCTCGTGCCTATTTC
Sample9     1 CAATCGGAATTCTAGGATTCATTGTTTGAGCACATCATATATTTACAGTAGGTATAGATGTTGATTCTCGTGCCTATTTC
Sample8     1 ---TCGGAATTCTAGGATTCATTGTTTGAGCACATCATATATTTACAGTAGGTATAGATGTTGATTCTCGTGCCTATTTC
JQ708094   14 CAATCGGAATTCTAGGATTCATTGTTTGAGCACATCATATATTTACAGTAGGTATAGATGTTGATTCTCGTGCCTATTTC

Sample4    78 ACTTCCGCAACTATAATTATTGCTGTCCCTACAGGAATTAAAATTTTTAGTTGATTAGCAACTATTTATGGGATAAAAAT
Sample9    81 ACTTCCGCAACTATAATTATTGCTGTCCCTACAGGAATTAAAATTTTTAGTTGATTAGCAACTATTTATGGGATAAAAAT
Sample8    78 ACTTCCGCAACTATAATTATTGCTGTCCCTACAGGAATTAAAATTTTTAGTTGATTAGCAACTATTTATGGGATAAAAAT
JQ708094   94 ACTTCCGCAACTATAATTATTGCTGTCCCTACAGGAATTAAAATTTTTAGTTGATTAGCAACTATTTATGGGATAAAAAT

Sample4   158 ATATTTTTCTCCAAGTATTATTTGATCTCTAGGATTCATTTTCCTGTTTACACTGGGAGGTTTAACAGGTGTAATTTTAG
Sample9   161 ATATTTTTCTCCAAGTATTATTTGATCTCTAGGATTCATTTTCCTGTTTACACTGGGAGGTTTAACAGGTGTAATTTTAG
Sample8   158 ATATTTTTCTCCAAGTATTATTTGATCTCTAGGATTCATTTTCCTGTTTACACTGGGAGGTTTAACAGGTGTAATTTTAG
JQ708094  174 ATATTTTTCTCCAAGTATTATTTGATCTCTAGGATTCATTTTCCTGTTTACACTGGGAGGTTTAACAGGTGTAATTTTAG

Sample4   238 CAAATTCTTCAATTGACATTATTTTACATGACACATACTATGTAGTAGCACATTTCCATTATGTTCTATCTATAGGGGCT
Sample9   241 CAAATTCTTCAATTGACATTATTTTACATGACACATACTATGTAGTAGCACATTTCCATTATGTTCTATCTATAGGGGCT
Sample8   238 CAAATTCTTCAATTGACATTATTTTACATGACACATACTATGTAGTAGCACATTTCCATTATGTTCTATCTATAGGGGCT
JQ708094  254 CAAATTCTTCAATTGACATTATTTTACATGACACATACTATGTAGTAGCACATTTCCATTATGTTCTATCTATAGGGGCT

Sample4   318 GTATTTGCAATTATTGCTAGATTTATTAATTGATACCCTTTAATAACAGGAGTAATTATAAATAAAACTTTATTAAAAAC
Sample9   321 GTATTTGCAATTATTGCTAGATTTATTAATTGATACCCTTTAATAACAGGAGTAATTATAAATAAAACTTTATTAAAAAC
Sample8   318 GTATTTGCAATTATTGCTAGATTTATTAATTGATACCCTTTAATAACAGGAGTAATTATAAATAAAACTTTATTAAAAAC
JQ708094  334 GTATTTGCAATTATTGCTAGATTTATTAATTGATACCCTTTAATAACAGGAGTAATTATAAATAAAACTTTATTAAAAAC

Sample4   398 ACA-ATTTATTAGTACTTTTATTGGTGTTAACCTTACTTTTTTCCCCC-ACATTTCTTAGGACTCATAGG-ATA
Sample9   401 ACANATTTATTAGTACTTTTATTGGTGTTAACCTTACTTTTTTCCCCCAACATTTCTTAGGACTCATAGGAATA
Sample8   398 ACA-ATTTATTAGTACTTTTATTGGTGTTAACCTTACTTTTTTCCCCCAACATTTCTTAGGACTCATAGGAATA
JQ708094  414 ACA-ATTTATTAGTACTTTTATTGGTGTTAACCTTACTTTTTTCCCCCAACATTTCTTAGGACTCATAGGAATA
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**Figure 2.** Multiple sequence alignment of representative TPP samples from Ecuador and of central haplotype (GenBank accession: JQ708094).

mented inspection and quarantine regulations in New Zealand (Liefting *et al*., 2008; 2009). It is believed that the wide host range, e.g. Solanaceae, Convolvulaceae and Lamiaceae (Wallis, 1955) increased the success of incursion of the TPP into New Zealand (Teulon *et al*., 2009). Now it constitutes one of the most devasting pests of solanaceous crops (Vereijssen *et al*., 2018).

The introduction pathway of the TPP into Ecuador remains unknown. The haplotype test showed that the psyllids in our study (central haplotype) is genetically close to TPPs that were found in Texas, Mexico (Workneh *et al*., 2018) and Central America (Swisher *et al*., 2012; 2013). It is interesting to note that *B. cockerelli* has not been reported from Costa Rica, Panama and Colombia, three adjacent countries connecting Central and South America. It seems quite likely that the TTP was introduced into Ecuador along with agricultural goods, given the highly active potato commerce between countries in northern South America (CCB, 2015). Other explanations imply the passive transport by winds or active migration. In the older literature (reviewed by Prager and Trumble, 2018), it was assumed that TPPs migrate from overwintering sites at lower altitudes in Arizona and Mexico to more northern sites at higher altitudes where they spend the summer. These migrations are explained by the seasonally different availability of suitable host plants. However, recent studies show that TPPs of the northwestern haplotype overwinter in the Northwest of the USA, whereas other haplotypes migrate (Swisher *et al*., 2014). The presence of TPP in Ecuador cannot be explained by this type of seasonal migrations. Similarly, there is no evidence for a passive drift by winds as there are no prevailing winds or jet streams from North to South America. Even though the TPPs in Ecuador are genetically close to the ones from North and Central America (central haplotype), it is likely that these psyllids differ in their genomic variation. It was shown that there is fine-scale genomic variation among the TPPs that are placed in the same haplotype group (Fu *et al*., 2017). The geographical origin of the TPP remains unknown; it might originate from north and central America (Prager and Trumble, 2018), which is different than the origins of potato crops: South America. Lastly, it is also possible that TPPs are present in other South American countries. TPP is a minute insect which can be easily overlooked or misidentified if handled by untrained personnel without adequate knowledge in psyllid taxonomy. Further sampling of potential host plants is required to determine the geographical extent of invasion of TPP in South America.

In view of its wide host range, the presence of the TPP in South America is particularly alarming due to the availability of a large number of cultivated and wild Solanaceae plants (Pletsch, 1947; Wallis, 1955; Prager and Trumble, 2018). Even though LSO has not been found in our current study, the presence of the vector makes the risk of its arrival and spread higher. It is imperative to conduct comprehensive surveys on different solanaceous and convolvulaceous crops to examine which of these are used by *B. cockerelli* and whether the LSO pathogen can be detected in any these host plants. It is equally essential to survey solanaceous crops in other South American countries, e.g. Colombia and Pe-

ru, for the presence of *B. cockerelli.* The study of the genomic variation of TPPs at a finer scale will be useful to examine the relationships to other TPP populations and, thereby, reconstruct the provenience of the Ecuadorian TPP (Fu *et al*., 2017). Ultimately, any outbreak of zebra chip in outside North America and New Zealand should be prevented.

## Conclusion

Our study presents the first report of the TPP from South America (Ecuador). No LSO pathogen was detected in the psyllids. Sequencing of the COI gene confirmed all psyllid samples were central haplotype. This suggests that the TPPs from Ecuador are genetically related to those from Texas (USA) and Central America (Swisher *et al*., 2012; 2013), where they may originate from. It is most likely that the TPPs discovered in Ecuador were introduced along with agricultural goods, but more work is needed to determine their origin. The discovery of TPP in Ecuador is alarming. Little is known about the South American populations regarding host range, population dynamics and potential to transmit LSO to solanaceous and convolvulaceous crops, including wild relative species. Therefore, a comprehensive survey is required to determine which solanaceous and convolvulaceous crops as well as their wild relatives support psyllid populations and to understand better their interactions with the TPP and LSO pathogen. This will help to develop strategies to control the TPP and make recommendations for local potato growers.

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